# STUDIES ON NEWFOUNDLAND *AEDES* MOSQUITOES WITH REFERENCE TO THEIR REPRODUCTIVE AND VECTOR POTENTIALS

JOSEPH MOKRY[1]

Research Unit on Vector Pathology, Memorial University of Newfoundland, St. John's, Newfoundland, Canada A1C 5S7

ABSTRACT. The four major mosquito species of insular Newfoundland were studied in terms of their seasonal succession and reproductive capacities. Fecundity was positively correlated with wing length in all species. The mean number of ovarioles per female was greatest in *Aedes canadensis* (173±48), followed by *Ae. punctor* (138±29), *Ae. abserratus* (121±27) and *Ae. communis* (107±36). Season-long sampling of the adult population levels and physiological age-grading suggested that no more than about 25% of adults (10% in *Ae. abserratus*) return for a second blood-meal and that <5% return for a third. The epidemiological significance of this to the transmission of California encephalitis viruses is discussed.

## INTRODUCTION

Northern *Aedes* mosquitoes have received attention over the years due to their fierce and massive feeding habits which have been a major obstacle to resource exploitation in certain areas, and due to a general fascination of their biology. Ecological studies have concentrated on larval development, species succession and, more recently, their association with particular vegetative habitats. While surveys have determined the identities of culicids attacking humans in Canada (Helson et al. 1980, Baldwin and Chant 1975, Corbet and Danks 1973, Belloncik et al. 1982), there are no published reports of the changing age-structure of adult mosquito populations nor how this relates to vector potential or reproductive capacity. This paper reports the results of examinations of *Aedes* mosquitoes in Newfoundland throughout the summer months in an effort to provide this information.

## MATERIALS AND METHODS

Collections of blood-seeking females were begun on May 30, 1982 and on May 28, 1983 and continued until the end of August of each year at the Little Powers Pond study site located approximately 8 km from St. John's, Newfoundland. This area has been under heavy surveillance since the discovery in 1980 of a mosquito pool infected with Jamestown Canyon virus (California encephalitis group) (Mokry et al. unpublished data). The breeding site was an extensive bog, often overgrown and filled by snow-melt along its edges, but containing numerous open pools. Females were collected using dry-ice-baited CDC-miniature light traps or fan traps fitted onto pyramid-shaped box traps, and by netting. Two collectors usually netted attacking females twice/week for 2 hr during the evening. The dry-ice-baited traps were cleared and replenished daily at 1000 and 1500 hr. Specimens were transported to the laboratory for identification and dissection.

Wing lengths were measured from the tip to the base of the cubitus vein with the aid of an eye-micrometer and a Wild M7 dissecting microscope. Ovarian dissections were done in a physiological saline solution (Spencer 1979). The number of ovarioles/female indicated the potential fecundity and dilatations on the ovariole stalk were determined to assess physiological age according to the methods of Detinova (1962).

Seven samples were taken weekly or biweekly during the summer beginning on June 12 to monitor the change in physiological age. For convenience an index called the 'mean sample age' was initiated and calculated on the basis of numerical values of 0 for nullipars, 1 for 1-pars, 2 for 2-pars, and so on. The total numerical value for each species in the sample was divided by the number of individuals to give the mean sample age. For example, in a sample consisting of 12 nullipars, 6 1-pars and 1 2-par, the mean sample age is 0.42 ($12 \times 0 + 6 \times 1 + 1 \times 2 = 8/19 = 0.42$).

Some females of each species were allowed to blood-feed on a restrained, anesthetized, guinea pig or a human collector and then maintained in individual plastic containers supplied with 10% sucrose solution at 22°C and 85% R.H. Ten days after blood-feeding, each container was partially flooded with water for oviposition and a record was kept of the day on which oviposition commenced. The degree-lab/days for ovariole maturation in the lab were compared to field data through the use of Canadian Meteorological Service published

[1] Present address: Vector Control Consulting, P.O. Box 966, St. John's, Newfoundland, Canada A1C 6M3.

monthly records of minimum, maximum and mean daily temperatures.

## RESULTS

Although 12 species of culicids were caught at the Little Powers Pond site over 2 yr, four species, *Aedes punctor* (Kirby) (56%), *Ae. communis* (De Geer) (27%), *Ae. abserratus* (Felt and Young) (12%) and *Ae. canadensis* (Theobald) (5%), together comprised over 99% of the collections and only these species are reported here (Table 1). There were differences in both the relative species composition and the general level of biting activity over the 2 yr, but as the temporal distribution for each species was similar over the 2 yr the catches were totaled.

Table 1. Collections of adult mosquitoes from Little Powers Pond, Newfoundland, 1982–83.

| Species | Year | | Total |
|---|---|---|---|
| | 1982 | 1983 | |
| *Aedes punctor* | 2721 | 4435 | 7156 |
| *Ae. abserratus* | 1064 | 526 | 1590 |
| *Ae. communis* | 1766 | 1716 | 3482 |
| *Ae. canadensis* | 252 | 367 | 619 |
| Total | 5803 | 7044 | 12,847 |

*Aedes communis* was the earliest emerging aedine in the St. John's area (no later than the third week in May) and normally preceded both *Ae. punctor* and *Ae. abserratus* by about 2 wk. The latter two species usually began emerging simultaneously and along with *Ae. communis* reached a peak abundance during the last week in June and the first week in July. *Aedes canadensis,* however, did not reach peak abundance until mid-July.

Fecundity was positively correlated with wing length among individuals of each species (Table 2). However, the larger species, *Ae. punctor,* was considerably less fecund than the smaller *Ae. canadensis* though somewhat more fecund than both *Ae. abserratus* and *Ae. communis.*

Table 2. Relationship between wing length and fecundity in Newfoundland mosquitoes, 1982–83.

| Species | No. | Wing length (mm) ± S.D. | Ovarioles | R* |
|---|---|---|---|---|
| *Aedes punctor* | 27 | 4.5 ± 0.23 | 138 ± 29 | 0.73 |
| *Ae. abserratus* | 26 | 4.4 ± 0.29 | 121 ± 27 | 0.73 |
| *Ae. canadensis* | 23 | 4.1 ± 0.41 | 173 ± 48 | 0.81 |
| *Ae. communis* | 19 | 4.1 ± 0.3 | 107 ± 36 | 0.93 |

R* = correlation coefficient.

Ovarian dissections outlined the seasonal aging process in Newfoundland's univoltine mosquitoes. Table 3 summarizes these results in terms of gonotrophic cycles completed at the time of capture. As gonotrophic age was naturally very strongly correlated with calendar age (*Ae. punctor,* r=0.98; *Ae. communis,* r=0.99; *Ae. abserratus,* r=0.98; *Ae. canadensis,* r=0.96), it was possible later to estimate by regression relatively precisely when the mean sample ages of 1.0 and 2.0 occurred. In general very few individual females survived to return for a second or third blood-meal. No 4-par *Ae. canadensis* or *Ae. abserratus* females were ever confirmed and only four *Ae. punctor* and two *Ae. communis* of this age were caught. Figures 1–6 illustrate ovarian dissections for determination of physiological age.

Nearly all females of all species showed at least some dwarf ovarioles and in the case of one *Ae. punctor* female more than ⅓ were such. It was also frequently noted that some females showed ovaries of different ages, e.g., one ovary nullipar and the other 1-par. Even individual ovaries were often of mixed age as in an *Ae. abserratus* female with both ovaries demonstrating ovarioles ⅓ nullipar and ⅔ 1-par. Retained eggs were common in all species but no records were kept of the incidence.

The relationship between the changing age structure of the population and the cumulative percentage of females caught during the season (cf. Corbet and Danks 1973) is shown in Fig. 7. For *Ae. communis,* the figure demonstrates that in 1983 the mean population age of 1.0 was reached in the first week in July and implies that only 26% of females survived to seek a second blood-meal. The 2.0 mean age was reached just over 3 wk later in the last week of July when approximately only 4% of the population remained. This, then, suggests a mortality rate of 74% from emergence to the return for the second blood-meal and a further 85% decrease between then and the return for the third blood-meal. Similar results were obtained for *Ae. punctor* except that the 1.0 and 2.0 mean age levels were reached approximately 1 wk later. It would appear that survival is poor in *Ae. abserratus* with no more than 10% of females returning for a second blood-meal. This confirms observations over the past four summers of a dramatic drop in the number of this species caught after mid-July. Survival results for *Ae. canadensis* were very similar to those obtained for both *Ae. communis* and *Ae. punctor.*

Three species (*Ae. abserratus* excepted) showed an approximate 3–3.5 wk span between the 1.0 and 2.0 mean age levels, implying that this was the time required to complete one gonotrophic cycle in the field (from one blood-meal to the next). According to Canadian

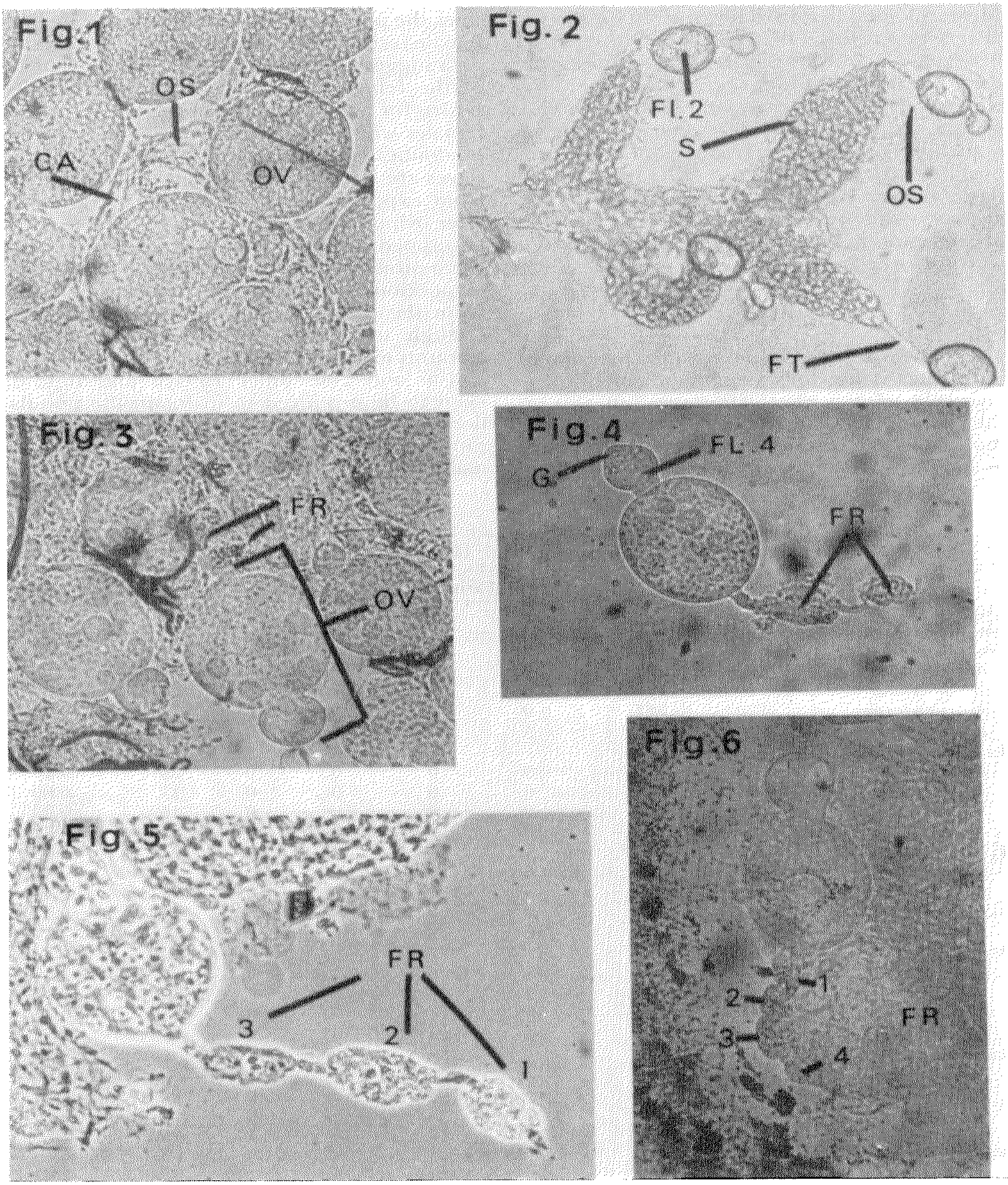
Meteorological Service daily profiles for the period between July 11–Aug. 2, 1983 (the period between 1.0 and 2.0 for *Ae. punctor*), these 23 days totaled 380 degree-days above 0°C, whereas laboratory trials with 72 blood-fed *Ae. punctor, Ae. abserratus, Ae. communis* and *Ae. canadensis* gave similar results for all species, viz., 337±31 degree-days at 22°C (mean, 15.5 days; range, 12–20 days). Field data for *Ae. abserratus* did not so closely approximate the above results as the time implied from 1.0 to 2.0 was just over 2 wk.

## DISCUSSION

The growth potential of any insect species in its habitat is not only dependent on the survivorship of its various life-stages but also on the reproductive capacity of the females. Heavy mortalities can be tolerated in the larval stages as long as sufficient eggs are produced to restock the population. This is dependent on both female fecundity and survivorship. The present data suggest that no more than about a quarter of the females (less in *Ae. abserratus*) studied return for a second blood-meal. The figure for females which actually oviposit at least once is no doubt higher, as oviposition attempts themselves are a source of mortality among females from exposure to predators during and just after oviposition as well as the risk of becoming entrapped on the water's surface. However, if we assume a 25–30% range of successful ovipositions, then of 100 females emerging from the pupal stage 25–30 would live long enough to oviposit. For *Aedes punctor* this amounts to about 3,500 to 4,100 eggs produced and as only 200 adults (100♀♀ and 100♂♂) need to be produced to maintain the population, a preimaginal mortality rate of 93–95% can be tolerated. Since a percentage of the females oviposit a second time, even higher mor-

Table 3. Summary of age-grading results of adult mosquitoes from Little Powers Pond, Newfoundland, 1982–83.

| | | Parity | | | | | |
|---|---|---|---|---|---|---|---|
| Time and species | Nullipars | 1 | 2 | 3 | 4 | No. | Mean age |
| Second week/June | | | | | | | |
| *Aedes punctor* | 21 | 0 | 0 | 0 | 0 | 21 | 0.00 |
| *Ae. abserratus* | 19 | 0 | 0 | 0 | 0 | 19 | 0.00 |
| *Ae. canadensis* | 14 | 0 | 0 | 0 | 0 | 14 | 0.00 |
| *Ae. communis* | 18 | 5 | 0 | 0 | 0 | 23 | 0.22 |
| Last week/June | | | | | | | |
| *Aedes punctor* | 15 | 5 | 0 | 0 | 0 | 20 | 0.20 |
| *Ae. abserratus* | 17 | 0 | 0 | 0 | 0 | 17 | 0.00 |
| *Ae. canadensis* | 15 | 0 | 0 | 0 | 0 | 15 | 0.00 |
| *Ae. communis* | 7 | 18 | 0 | 0 | 0 | 25 | 0.72 |
| First week/July | | | | | | | |
| *Aedes punctor* | 12 | 6 | 1 | 0 | 0 | 19 | 0.42 |
| *Ae. abserratus* | 13 | 8 | 0 | 0 | 0 | 21 | 0.38 |
| *Ae. canadensis* | 13 | 1 | 0 | 0 | 0 | 14 | 0.07 |
| *Ae, communis* | 0 | 16 | 2 | 0 | 0 | 18 | 1.11 |
| Mid-July | | | | | | | |
| *Aedes punctor* | 0 | 14 | 6 | 0 | 0 | 20 | 1.30 |
| *Ae. abserratus* | 0 | 12 | 1 | 0 | 0 | 13 | 1.08 |
| *Ae. canadensis* | 4 | 14 | 1 | 0 | 0 | 19 | 0.84 |
| *Ae. communis* | 0 | 11 | 13 | 0 | 0 | 24 | 1.54 |
| Last week/July | | | | | | | |
| *Aedes punctor* | 0 | 10 | 5 | 2 | 1 | 18 | 1.67 |
| *Ae. abserratus* | 0 | 14 | 6 | 2 | 0 | 22 | 1.45 |
| *Ae. canadensis* | 1 | 12 | 3 | 0 | 0 | 16 | 1.13 |
| *Ae. communis* | 0 | 2 | 17 | 5 | 1 | 25 | 2.20 |
| First week/August | | | | | | | |
| *Ae. punctor* | 0 | 0 | 11 | 5 | 1 | 17 | 2.41 |
| *Ae. abserratus* | 0 | 0 | 9 | 12 | 0 | 21 | 2.57 |
| *Ae. canadensis* | 0 | 4 | 23 | 0 | 0 | 27 | 1.85 |
| *Ae. communis* | 0 | 0 | 15 | 6 | 1 | 22 | 2.36 |
| Third week/August | | | | | | | |
| *Aedes punctor* | 0 | 0 | 4 | 8 | 2 | 14 | 2.86 |
| *Ae. abserratus* | 0 | 0 | 1 | 8 | 0 | 9 | 2.89 |
| *Ae. canadensis* | 0 | 0 | 9 | 4 | 0 | 13 | 2.31 |
| *Ae. communis* | 0 | 0 | 0 | 3 | 0 | 3 | 3.00 |




Figs. 1–6. Dissected ovaries of some Newfoundland mosquitoes. Fig. 1. Nulliparous *Aedes canadensis;* Fig. 2. *Ae.* sp. 36–48 h, post-oviposition; Fig. 3. 2-par, *Ae. punctor,* partially dissected; Fig. 4. 2-par, *Ae. punctor;* Fig. 5. 3-par, *Ae. abserratus*; Fig. 6. 4-par, *Ae. punctor*, partially dissected. CA- calyx, OV- ovariole, OS- ovariole sheath, S- sac, G- germarium, FL- follicle, FT- follicular tube, FR- follicular relic.

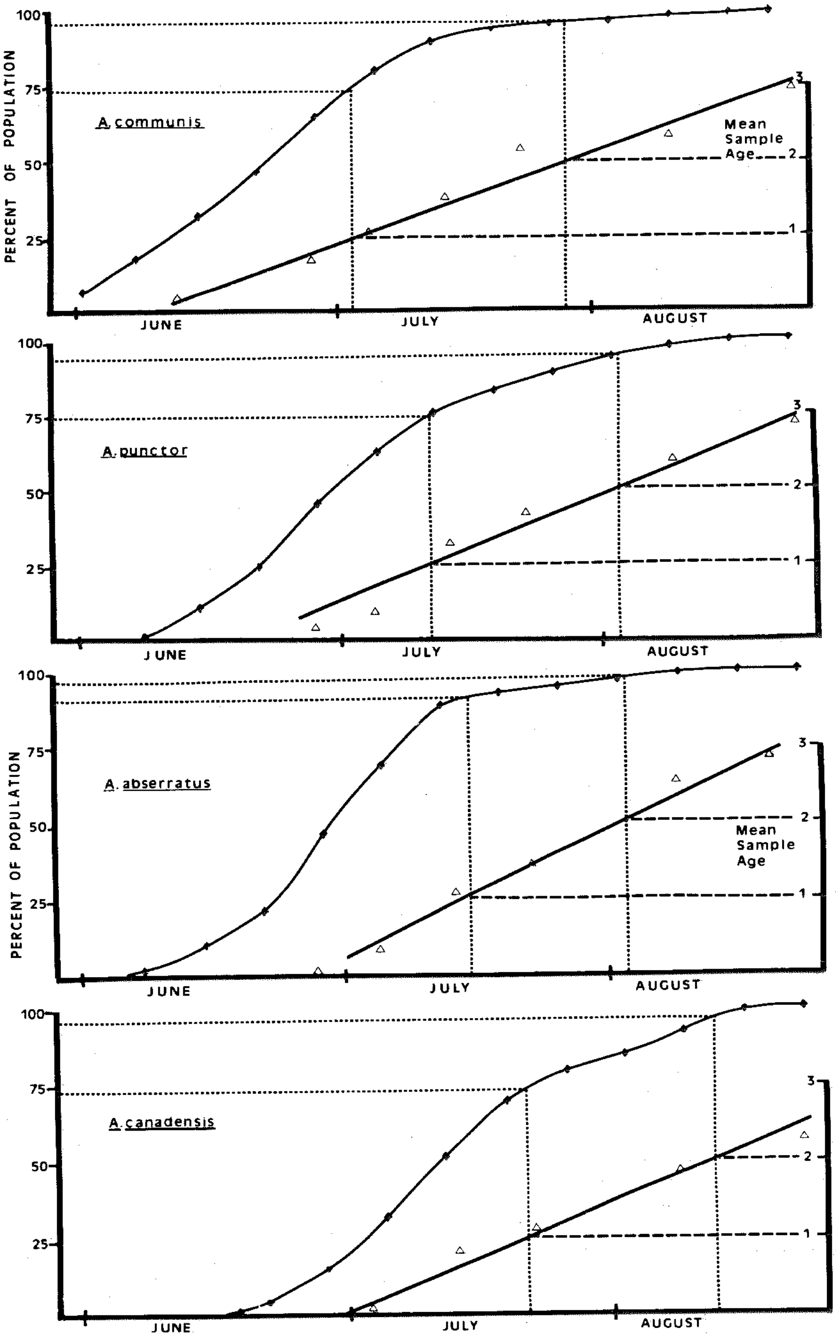



Fig. 7. Relationship between percent of mosquito population captured over the summer months and the mean sample age.

talities can be borne, though it is likely that fewer eggs are laid by females in their second and third cycles (Detinova 1949, Lavoipierre 1961, Andreadis and Hall 1980).

*Aedes punctor, Ae. canadensis* and *Ae. abserratus* are all widely distributed in Newfoundland (Pickavance et al. 1970, Nielsen and Mokry 1982), but *Ae. communis* has only recently been collected on the Avalon Peninsula. *Aedes punctor* clearly dominates the Newfoundland mosquito fauna being in many ways a pioneer species in that it occupies almost every available larval niche. This is often at the apparent expense of *Ae. abserratus* which tends to be confined to open bog pools. *Aedes communis* was second in abundance only to *Ae. punctor* which was unexpected as not a single adult or larva had been collected over the previous 4 yr (1978–81). Its early emergence and aggressive biting behavior set it apart from all other Newfoundland species except for the overwintering *Culiseta impatiens* (Walker). The local breeding sites of *Ae. communis* have not yet been identified. These data on the relative seasonal abundance of *Ae. punctor* and *Ae. communis* are very similar to those reported by Belloncik et al. (1982) and Baldwin and Chant (1975). The latter authors also demonstrated a late season hatch or second generation in *Ae. canadensis* in August but no evidence to support this was found in the present study.

Generally, it was clear that few females of the four species studied survived long enough to oviposit more than once, confirming the assertion of McLean (1975) for northern *Aedes* and the field data of DeFoliart (1983) for *Ae. triseriatus*. This is especially interesting considering how long, in terms of calendar age, many individuals of the population live. This is partly explained by the frequent periods of cool, rainy weather experienced in Newfoundland which, while inhibiting flight and biting activity, actually promotes survival times. The length of the gonotrophic cycle itself, even at 22°C, also works toward reducing the mean physiological age of the population. Given the generally cool temperatures prevalent on the island (the mean 24 hr temperature for the period July 11–Aug 2 noted in the results as the time between the mean sample ages of 1.0 and 2.0 for *Ae. punctor* was only 16.4°C), the gonotrophic cycle could easily be extended to 3 wk or more. The 380 degree-days estimated in the field for the length of the gonotrophic cycle vs the 337 degree-days recorded in the laboratory may indicate that daily temperature extremes dropped below certain minimal levels for ovariole maturation to continue as has been shown for *Culex* spp. in Ontario (Madder et al. 1983). At present we have little information on minimum temperature requirements for this process in most northern *Aedes*. Corbet and Danks (1973) state on the basis of sweep net and light trap collections that in the Arctic *Ae. impiger* (Walker) and *Ae. nigripes* (Zetterstedt) have a gonotrophic cycle of from 9–10 days and 7–11 days, respectively. It is interesting to speculate, given the much longer day length in the high Arctic and that these two species reportedly often choose resting places in direct sunlight, that females may actually achieve a much higher than ambient temperature thereby reducing the length of the gonotrophic cycle compared to the four species studied here. Also based on their collections of the varying numbers of gravids over the summer, the same authors detected at least three and perhaps four cycles for some females of each species. Attempts to confirm these results by laboratory feedings or ovariole dilatations were not reported.

Estimating the longevity of females is complicated in that emergence of the adults took place over a rather extended period of time, though perhaps this is less so in *Aedes communis*. In the case of *Ae. punctor,* all females caught in the second week of June were nulliparous. The presence of nullipars in the collections confirms that adults were continuing to emerge until mid-July, though by that time 2-par females were being collected. Also at that point over 75% of the population sample had been taken, suggesting that the majority of the population is not really very long-lived. If the 3 wk estimate of the length of the gonotrophic cycle under Newfoundland conditions is accurate, however, then 2-, 3- and 4-par females must be 6, 9 and 12 wk old, respectively. It follows that these older females, certainly the 4-par group, were also those emerging earliest, say, before mid-June. Those 2-par in the third week of August would have been amongst the last to emerge, appearing as the last of the nullipars in mid-July. Nevertheless, it is apparent that no more than about 25% of females live longer than 3–4 wk, including less than a week for mating, nectar-feeding and host-seeking.

It is of epidemiological significance that such a small proportion of Newfoundland mosquitoes return to take a second blood-meal. In the case where vectors first acquire a transmissible microorganism by feeding on an infected vertebrate host, the maintenance of the microorganism's cycle requires that the vector subsequently refeed to infect further hosts. Only about a quarter or less of the females of the species studied here would seem to live long enough to fill this role. The present data further imply that transmission of two California-group viruses, snowshoe hare (SSH) and Jamestown Canyon (JC) so far discovered here (Mokry et al., unpublished data), might not take place until mid-July or later. The most epidemiologically significant females, those returning for a third blood-meal according to the mean sample age, do not appear until August.

Transovarial transmission has been demonstrated for both SSH (McLean et al. 1975) and JC (Berry et al. 1977). Adults emerging in the spring from overwintering infected eggs are thus capable of transmitting these viruses after sufficient virus replication has taken place. Under these circumstances it is to be expected that early summer virus transmission would result and this was clearly demonstrated by Belloncik et al. (1982) who recorded numerous isolations of SSH in Quebec from *Ae. punctor* and *Ae. communis* in early summer and none past mid-July or August. Virus amplification in vertebrate hosts and infection of previously virus-free mosquitoes while biting in subsequent cycles therefore assume a lesser role in the maintenance of viruses in nature. Further work on these viruses may show similar results in Newfoundland.

## ACKNOWLEDGMENTS

This work formed a part of a study on the transmission of California serogroup viruses in Newfoundland and the support and encouragement of Health and Welfare Canada during this period are gratefully acknowledged. The author is also pleased to acknowledge the able and enthusiastic assistance provided by several summer students, particularly, Ms. Elaine Stanley, Mr. Geoff Thompson and Ms. Sarah Nettleton.

### *References Cited*

Andreadis, T. G. and D. W. Hall. 1980. Relationship between physiological age and fecundity in *Culex salinarius* (Diptera: Culicidae). J. Med. Entomol. 17:485–486.

Baldwin, W. F. and G. D. Chant. 1975. Seasonal succession of the mosquitoes (Diptera: Culicidae) of the Chalk River area. Can. Entomol. 107:947–952.

Belloncik, S., L. Poulin, A. Maire, A. Aubin, M. Fauvel and F. X. Jousset. 1982. Activity of California encephalitis group viruses in Entrelacs (Province of Quebec, Canada). Can. J. Microbiol. 28:572–579.

Berry, R. L., B. J. L. Weigert, C. H. Calisher, M. A. Parsons and G. T. Bear. 1977. Evidence for transovarial transmission of Jamestown Canyon virus in Ohio. Mosq. News 37:494–496.

Corbet, P. S. and H. V. Danks. 1973. Seasonal emergence and activity of mosquitoes (Diptera: Culicidae) in a high-arctic locality. Can. Entomol. 105:837–872.

DeFoliart, G. R. 1983. *Aedes triseriatus:* vector biology in relationship to the persistence of LaCrosse virus in endemic foci, pp. 89–104. In: Calisher, C. H. and W. H. Thompson (eds). California serogroup viruses. Alan Liss. New York.

Detinova, T. S. 1949. Physiological changes of ovaries in females of *A. maculipennis*. Med. Parazitol. (Mosk.). 18:410. (Read in translation).

Detinova, T. S. 1962. Age-grouping methods in Diptera of medical importance. W.H.O. Monogr. Ser. 47,216 pp.

Helson, B. V., G. A. Surgeoner and R. E. Wright. 1980. The seasonal distribution and species composition of mosquitoes (Diptera: Culicidae) collected during a St. Louis encephalitis surveillance program from 1976 to 1978 in southwestern Ontario. Can. Entomol. 112:865–874.

Lavoipierre, M. M. J. 1961. Blood-feeding, fecundity and aging in *Aedes aegypti* var. *queenslandensis*. Nature 191:575–576.

Madder, D. J., G. A. Surgeoner and B. V. Helson. 1983. Number of generations, egg production, and developmental time of *Culex pipiens* and *Culex restuans* (Diptera: Culicidae) in southern Ontario. J. Med. Entomol. 20:275–287.

McLean, D. M. 1975. Mosquito-borne arboviruses in arctic America. Med. Biol. 53:264–270.

McLean, D. M., S. K. A. Bergman, A. P. Gould, P. N. Grass, M. A. Miller and E. E. Spratt. 1975. California encephalitis virus prevalance throughout the Yukon Territory, 1971–1974. Am. J. Trop. Med. Hyg. 24:676–684.

Nielsen, L. T. and J. E. Mokry. 1982. Mosquitoes of Newfoundland—A report of new records and notes on the species. Mosq. Syst. 14:34–40.

Pickavance, J. R., G. F. Bennett and J. Phipps. 1970. Some mosquitoes and blackflies from Newfoundland. Can. J. Zool. 48:621–624.

Spencer, M. 1979. Age grouping of female *Anopheles farauti* populations (Diptera: Culicidae) in Papua New Guinea. J. Med. Entomol. 15:555–569.