LONGEVITY OF *AEDES AEGYPTI* (DIPTERA: CULICIDAE) COMPARED IN CAGES AND FIELD UNDER AMBIENT CONDITIONS IN RURAL THAILAND

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Abstract. *Aedes aegypti* (L.) were exposed to all of the physiological stresses of a natural environment, without mortality from predation or from the defensive behavior of bitten hosts. Each replicate consisted of four cages containing 50 male and 50 female, locally reared *Ae. aegypti.* The cages were placed in the bedroom and kitchen of a typical Thai house in the village of Hua Samrong, Chachoengsao Province. Replicates were repeated five times between October 1991 and June 1992. Mosquitoes had constant access to sugar and were offered blood meals every day. The number of dead mosquitoes and eggs were recorded daily for 30 days. Indoor maximum temperatures were high throughout the year, ranging from a mean of 32.8ºC in October/November to 37.6ºC in March-April, with an absolute maximum of 41ºC. Survival in cages was related to temperature, with the force of mortality lowest in November-December (0.002) and highest in May-June (0.043). The negative slope of cohort survival was also greatest in the warmest months. Egg laying rate was lower in the cooler months (minimum 16.4 eggs/female/day in November-December), but did not vary greatly in absolute value (maximum 22.7 eggs/female/day in March-April). Statistically, survival of females in cages was much greater than survival calculated from mark-release-recapture studies conducted by other authors in Hua Samrong. The difference in survival for mosquitoes released in the field and those confined to cages suggests that predation or defensive behavior may be important in regulating adult populations of this vector.

INTRODUCTION

Aedes (Stegomyia) aegypti (L.) is the most extensively studied species of mosquito in the world, not only because of its status as the principal vector of dengue (Rosen *et al,* 1985), yellow fever (Whitman, 1951; Aitken *et al,* 1977), and Chikungunya viruses (Turell *et al,* 1992), but also because of the ease with which it is reared in the laboratory (Christophers, 1960). One of the most basic questions that can be asked about any species is how long it lives, a point constantly brought to the attention of mosquito biologists answering questions from the public. Therefore, the longevity of *Ae. aegypti* has been the topic of numerous studies, dating back at least to 1933 (Christophers, 1960). The conduct of these studies has ranged from observations on caged mosquitoes under carefully controlled conditions (Muir and Kay, 1998) to mark-release-recapture studies in the field (Harrington *et al*, 2001). Interpretation of the data has become considerably more sophisticated than older studies, which simply reported the mean and range of longevity. A large number of factors in the laboratory or field can cause variation in longevity. Therefore, it is important to keep in mind that the various calculated statistics are only as important as the comparisons to which they are applied.

The study described in this paper had the specific purpose of comparing longevity of female *Ae. aegypti* in cages to longevity of females released in the field, using a study site known to be a focus of dengue transmission (Strickman *et al,* 2000). Fortunately, other investigators had conducted mark-release-recapture studies only

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a few kilometers away from the site of these experiments (Day *et al,* 1994), also using first generation mosquitoes reared from locally collected stock. Subsequent mark-release-recapture studies conducted in 1998 (Harrington *et al*, 2001) provided even more data for comparison. The current study produced data strongly suggesting that mortality factors outside the caged environment accounted for most of the deaths of wild *Ae. aegypti* in this rural Thai setting.

MATERIALS AND METHODS

The location for this study was a wooden home (House No. 104) in Village 2, Ban Hua Samrong, Plaeng Yao District, Chachoengsao Province, Thailand. This house was close to the area of the village with small businesses, Hua Samrong Primary School, and the public health office (in Thai, *anamai*) (Strickman and Kittayapong, 2001). Typical of Thai houses in the area, this home was largely open to the outdoors, with permanently open windows and semi-enclosed rooms. The house consisted of three main parts: a living room with a cement floor, a kitchen area open on one side to the outdoors and with a wooden floor, and a bedroom with a wooden floor.

Mosquitoes for the experiment were the first generation produced from adult *Ae. aegypti* collected at Hua Samrong. The wild-caught adults were transported to the laboratory in Bangkok, where they were offered daily human blood meals and constant access to 10% sucrose solution. Prior to an experiment, 600 first-instars were hatched under partial vacuum (Barbosa and Peters, 1969) and taken to the study house in Hua Samrong to complete rearing. In Hua Samrong, the larvae were distributed among covered pans, each containing 60 larvae and 1.5 liters of well water. The larvae were fed 0.1 g of finely ground fish food (Tetramin Baby Fish Food "E" for Egglayers, Ulrich Baensch GmbH, Germany) on the day they were first distributed among the pans (day 0), 0.1 g on day 2, and 0.1 g on day 4. All pupae were placed in a dish of water in a cage (30 x 30 x 30 cm) with 10% sucrose solution available. The pupae were left in place for 48 hours, allowing complete emergence of adults. This rearing procedure (Sumanochitrapon *et al*,1998) synchronized development and produced large adults (female wing length approximately 2.96 mm). Although female *Ae. aegypti* in the region of the study may not take sugar meals very often (Edman *et al*, 1992), sugar was offered to adult mosquitoes in most of this experiment. The reasoning was that the males would require sugar and that offering mosquitoes the choice was a closer approximation to conditions in the field.

For each experiment, 50 female and 50 male mosquitoes were placed in each of four cages containing a black oviposition container. Two cages were located in the bedroom on a table near the wall and two cages in the kitchen on a table near the middle of the room. Each day for 30 days, mosquitoes were offered a blood meal from a human hand. The number of eggs and dead females were counted daily. The experiment was replicated five times during the year (5 October - 4 November 1991; 23 November - 23 December 1991; 26 January - 25 February 1992; 16 March - 15 April 1992; and 5 May - 4 June 1992). Each replication was limited to 30 days because it was known that a very small percentage of mosquitoes in the wild survived that long. All cages had 10% sucrose solution available, except for one bedroom cage and one kitchen cage in the last two experiments (in an effort to determine whether the sugar meal was influencing longevity or fecundity). Indoor temperature was recorded daily on a maximum/minimum thermometer in the public health office for the first two experiments and in the study house for the last three experiments. Practical considerations made it necessary to change the location of the thermometers, but the two locations were less than 200 m apart.

Life table statistics were calculated according to Carey and Liedo (1999). Cohort survival (l_x) was the proportion of individuals alive on day x compared to the starting day. The number of mosquitoes on the starting day (the denominator) was adjusted downward for those few mosquitoes that escaped from the cage during the experiment. The statistical significance of differences between cohort survivals was tested using confidence limits of percentages according to the binomial distribution (Steel and Torrie, 1960). Following validation that location of a cage within the house did not influence cohort survival, data were pooled for all cages in order to examine seasonal effects. Cages without sugar were eliminated from the seasonal comparisons because significant differences existed on some days between cages with and without sugar in the last two experiments. Another useful life table statistic was the force of mortality (μ_x), calculated as the negative of the natural log of the fraction of individuals at age x surviving to age x + 1 $\{-\ln[1+(l_{x+1}/ l_x)]\}$ (one added in order to avoid taking the ln of zero). In practice, the mean force of mortality for days 0 - 29 was the closest estimate of the daily mortality rate.

Three published studies of longevity of *Ae. aegypti* were very relevant because they were conducted in Village 6, about 4 km from the site of this study. One study (Scott *et al*, 1997) used pupae from the village as a source of female mosquitoes. The main purpose of this study was to compare longevity and fecundity of mosquitoes fed either blood alone or blood and sugar. The experiment was carried out from 11 January through 24 February with 18 mosquitoes in each treatment. Mosquitoes were held in individual cages in a village home in an effort to expose them to ambient temperature conditions (mean maximum 31.7ºC, mean minimum 25.0ºC). Since the data were only presented graphically, l_x was estimated from each of the first 30 days of their study in order to calculate μ_x for the group fed blood and sugar. The other two studies were performed in Village 6 by marking, releasing, and recapturing mosquitoes reared in the laboratory from stock collected in the village. The first study (Day *et al*, 1994) presented data graphically for the 8 days that marked mosquitoes (1,000 released) were recaptured. Finally, Harrington and others (Harrington *et al*, 2001) presented carefully analyzed data on a mark-release-recapture study in Village 6 performed during November with groups of 492 to 498 mosquitoes. These authors released mosquitoes at multiple sites in the village in proportion to populations measured by aspiration off the walls of homes. An additional refinement was release of adults that were either 3 or 13 days old. For the purpose of comparison, their report of daily survival rate was converted to an estimate of the mean μ_x for the 12 days they recaptured mosquitoes.

Fecundity was calculated as eggs per surviving female on each day. The statistical significance of differences between means of eggs per surviving female was tested by analysis of variance (SPSS for Windows, Version 6.1.2). Preliminary comparisons showed that there were no differences between locations and when sugar was or was not available. The data were pooled for all cages in a monthly experiment in order to make comparisons between seasons.

RESULTS

The five experiments with caged *Ae. aegypti* mosquitoes were conducted from the end of the rainy season (October) through the hot season (June), representing a wide range in temperature conditions for the location (Table 1). Daily maximum indoor temperatures varied from a lowest value of 29ºC in October to the highest value of 41ºC in May. The minimum temperatures ranged from 21ºC in February to 30ºC in March - May.

Table 1
Indoor temperatures (ºC) during observation of caged cohorts in Hua Samrong, Thailand, 1991-1992.

Dates	n	Daily maximum (range)	Daily minimum (range)
5 Oct - 4 Nov	30	32.8 (29-35)	24.7 (22-27)
23 Nov - 23 Dec	25	33.5 (32-36)	23.6 (21-26)
26 Jan - 25 Feb	30	34.0 (31-36)	24.5 (22-26)
16 Mar - 15 Apr	30	37.6 (37-40)	27.4 (26-30)
5 May - 4 Jun	30	36.6 (33-41)	28.6 (27-30)

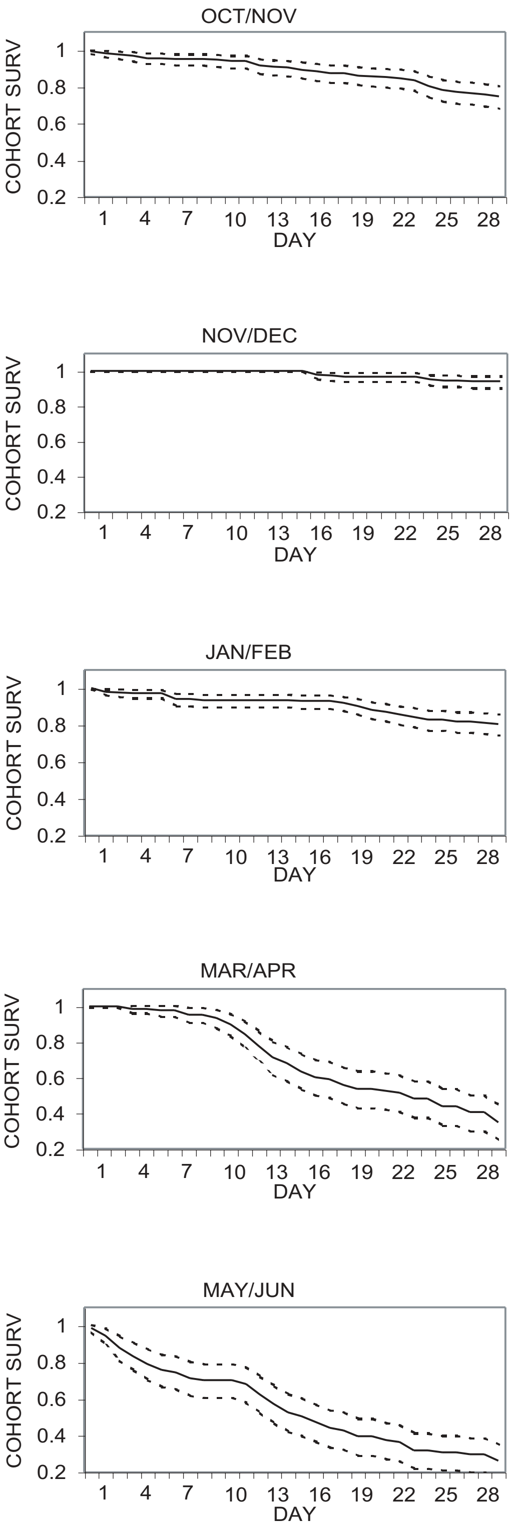

Fig 1–Comparison of cohort survival (l_x) during entire 30 days period of five experiments in cages. Dashed lines represent 95% confidence interval.

Table 2
Eggs deposited per female per day from day 3 to day 30 in four cages (results pooled) located at ambient temperature in a Thai home, repeated during five months.

Month	No. of females Day 3	No. of females Day 30	Eggs per female
Oct/Nov	186	142	20.9±8.44[ac]
Nov/Dec	192	181	16.4±7.55[b]
Jan/Feb	184	152	18.7±11.00[bc]
Mar/Apr	179	61	22.7±11.27[a]
May/Jun	147	29	19.1±18.49[ac]

Mean ± SD not followed by the same superscripted letter significantly different at the 95% level following ANOVA and Duncan's multiple range test (F = 4.41; df = 4, 555; p = 0.0016).

The actual range of mean eggs/female/day was small (Table 2), although some of the differences between months were statistically significant. The lowest number was 16.4 eggs/female/day in November - December, corresponding to the month with the lowest mean daily minimum temperature indoors. The number of eggs was also low in January - February, another relatively cool period. The greatest number of eggs per female per day (22.7) was observed in March - April, the month with the highest mean daily maximum temperature. The number of eggs was less in May - June and October - November, but not statistically different from the maximum.

Cohort survival was very high for the caged mosquitoes in the three cooler months (Fig 1), with more than 75% of females alive after 30 days. Survival was dramatically reduced in the two warm months. Although greater than 60% of the females survived past day 13, less than 40% remained by day 30. The mean force of mortality for days 1-8 was similar to the values for days 1-29 (Table 3), indicating that the rate of mortality was fairly constant in the caged trials. The force of mortality for the warmer months was greater than for the cooler months. Mosquitoes in the May - June cohort suffered a rate of mortality roughly 10 times greater than in the other months.

Table 3
Five experiments with caged *Aedes aegypti* (only those with access to sugar) compared to published studies in Hua Samrong.

Source of data	Days 0-8	Days 0-12	Days 0-29	No. on Day 0
Oct/Nov	0.0054	0.0043	0.0099	190
Nov/Dec	0	0	0.0020	192
Jan/Feb	0.0082	0.0060	0.0073	188
Mar/Apr	0.0056	0.015	0.036	91
May/Jun	0.043	0.034	0.046	91
Scott[1]			-0.085	18
Day[2]	0.56			1,000
Harrington[3]				
3 d old		0.33		494
3 d old		0.25		498
13 d old		0.17		493
13 d old		0.16		492

Mean Force of Mortality (μ_x)
[1]Scott *et al*, 1997: 18 caged mosquitoes in January-February.
[2]Day *et al*, 1994: 1,000 marked mosquitoes of which some were recaptured during 8 days.
[3]Harrington *et al*, 2001: Groups of 492-498 mosquitoes released at 3 or 13 days of age of which some were recaptured during 12 days.

DISCUSSION

Counts of the number of eggs per gonotrophic cycle have been the most common measurement of *Ae. aegypti* fecundity, making comparison to this study difficult. Assuming 2 days for development of eggs and one for deposition (Strickman and Kittayapong, 1993), the equivalent values from the trials in this study would range from 49 eggs/cycle in November - December to 68 eggs/cycle in March - April. These values were comparable to reports of 86 (Christophers, 1960), 73 (Nayar and Sauerman, 1975), and 58 eggs/cycle (Dye, 1984) from laboratory studies that included access to sugar solution.

The study by Naksathit and Scott (1998) probably provides the best comparison of fecundity. Using mosquitoes from Puerto Rico fed on human blood and held in individual cages, they found that *Ae. aegypti* reared to a large size (wing length 2.95 mm, similar to the size in this study) deposited 13 eggs/day if given sugar and 18 eggs/day if not given sugar. These values were considerably lower than our observations and showed a significant increase in fecundity when sugar was withheld. Possibly the large cages in our study provided conditions more conducive to egg production, but less sensitive to differences caused by sugar feeding. The contrast between the studies shows that data from laboratory studies of fecundity must be applied cautiously to quantitative models of mosquitoes in the field.

Longevity of the mosquitoes in cages as measured by cohort survival was much lower in March - April and May - June, the hottest time of the year. Indoor temperatures during these months approached or reached the thermal death point of 41ºC for a one-hour exposure (Christophers, 1960), suggesting that some mosquitoes were killed directly by elevated indoor temperatures in March - June. Confining the mosquitoes to cages prevented them from seeking cooler temperatures elsewhere, either indoors our outdoors. The artificial confinement in these experiments makes it difficult to evaluate whether the lifespan of naturally occurring *Ae. aegypti* is shortened during the hottest months in Thailand.

The mean force of mortality was remarkably constant whether 8, 12, or 29 days were used in the calculation. This suggests that for

30 days the common assumption of constant daily survival rate is practical, at least for general comparisons. Mortality in our study was much lower than in other studies in Hua Samrong (Table 3). Using small cages with individual mosquitoes placed indoors in January - February, Scott and others (1997) produced data supporting an estimate of the force of mortality almost 12 times greater than our measurement for the same month. The discrepancy might be the result of their small sample size or an unfavorable aspect of the small cages. In mark-release-recapture studies, the force of mortality observed by Day and others (1994) was 13 times greater than the greatest value observed in our study. The study conducted by Harrington and others (2001) in November produced estimates of the force of mortality between 37 and 77 times greater than the value observed for caged mosquitoes. Mark-release-recapture studies in other parts of the world produced daily survival rates [Bangkok = 0.81 (Sheppard *et al*, 1969), Tanzania = 0.66 (Conway *et al*, 1973), Kenya = 0.89 (McDonald, 1977), Bangkok = 0.63 - 0.88 (Dye, 1984), Kenya = 0.85 (Trpis and Hausermann, 1986), Australia = 0.86 or 0.9 (Muir and Kay, 1998)] suggesting much higher values for the force of mortality than in our caged trials.

Longevity of *Ae. aegypti* in our study of caged mosquitoes was much greater than the longevity calculated from various mark-release-recapture studies of mosquitoes in the field, suggesting the need for additional research in a number of areas. First, the direct effect of temperature on survival of field populations is not clear because of the difficulty in studying the cumulative temperature exposure of wild mosquitoes that fly from place to place. Second, there are very few studies examining the effect of larval conditions on subsequent adult longevity. The study by Harrington and others (2001) of the effect of size on daily survival rate addressed the effect of larval nutrition indirectly, but other influences on larvae [*eg*, toxicants (Vasuki, 1992)] may also affect adult longevity. Finally, predators (Nandi and Raut, 1985; Sulaiman *et al*, 1990; Strickman *et al*, 1997; Fox, 1998) and defensive behavior (Edman and Spielman, 1988) may cause a great deal of mortality in wild populations of adult *Ae. aegypti*. If predators are a significant source of mortality, household practices and mosquito control measures that reduce predator populations could increase the longevity of *Ae. aegypti* populations. In general, any influence on the vector that increases female longevity will increase the likelihood of survival through the extrinsic incubation period of the virus (Watts *et al*, 1987) and the risk of dengue transmission. Logically, community practices that tend to extend longevity of *Ae. aegypti* should be modified so that natural forces of mortality are expressed to the maximum extent.

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