9 Physiological Age Grading, Parity and Natural Survival of *Oc. niveus*

9.1 Summary

A total of 3625 Oc. niveus mosquitoes were collected from man landing collections in Teressa Island during the year long study period. The parous biting density was found to range between 0.4 during the month of December and 1.9 in February. The density of nulliparous females was found to show four peaks. The overall probability of daily survival of Oc. niveus through one gonotrophic cycle was 0.75, two gonotrophic cycles was 0.70 and three gonotrophic cycles was 0.64. The pattern in probability of daily survival rates was not different between the seasons. Survivorship analysis indicates that mortality increases with age. The proportion of mosquitoes belonging to nulliparous, 1-D, 2-D and 3-D was 76.33%, 18.17%, 5% and 1% respectively. Almost similar proportions were observed between the seasons. The epidemiological significance of these findings is discussed in this chapter.

9.2 Introduction

The survival rate of field populations of mosquito vectors of disease is an important determinant of their disease transmission capability. The parous rate and age grading are sensitive indices that readily indicate the changes in the population structure of mosquitoes (De Meillon and Khan 1976b). Bancroftian filariasis and its vectors in India have been the subject of several studies (Sharma 1976; Das 1976; Gubler and Bhattacharya 1974 and Rozeboom *et al.* 1968) on vector survival.

Studies carried out in Samoa (Samarawickrema *et al.* 1987) suggest that *Ae. polynesiensis* taking a blood meal after 3 ovipositions would be potentially infective. However, the number of infected *Ae. samoanus* age graded was too small to observe a definite correlation. Earlier studies (Kalra 1974; Russel *et al.* 1975 and Tewari *et al.* 1995) undertaken in the Nicobar group of islands have shown that *Oc. niveus* biting the native aborigine tribes in sufficient densities and that transmission exists. But no attempt was made to age grade the vector population. In the present chapter, the age composition, parity status, abundance in parous and nulliparous population and daily survival rates of *Oc. niveus* are discussed.

9.3 Material and Methods

The data obtained from man landing collections in the three mf zones were used for the analysis

9.3.1 Determination of Age of the Vector

Perry (1912) used the degree of wear and tear or rubbing on the wings as the criteria to differentiate the old mosquitoes from young ones. Corbet (1960, 1962) observed that external wear of the abdominal sternites was a reliable method to differentiate the nulliparous mosquitoes. Mer (1932) found that the size of the ampulla at the anterior end of the common oviduct increases in size during oviposition. Detinova (1945) used the presence or absence of ovariole "skeins" to distinguish the nulliparous mosquitoes from parous ones. From all the above methods it will be possible only to determine whether the mosquitoes were parous or nulliparous. But, the method of Polovodova (1949), which is based on the number of dilatations formed in a follicular tube of the ovariole following the egg laying is the most important, and using this method the physiological age of the mosquito can accurately be determined. Hence, the Polovodova's method was used in the present study. After removing the legs and wings, the mosquito was placed in a drop of saline on a clean glass slide; the ovary was removed using fine entomological needles and separated into ovarioles. As many ovarioles as possible were examined under high power magnification of a compound microscope before determining the number of dilatations in each mosquito. The mosquitoes that have completed one gonotrophic cycle (i.e. with one dilatation) were designated as 1-parous, two dilatations as 2-parous etc. The parous rate was determined using the following formula

$$\text{Parous Rate (\%)} = \frac{\text{No. of parous mosquitoes}}{\text{No. dissected}} \times 100$$

9.3.2 Abundance of Vector Population in Relation to Age

The parous biting density of the vector population was calculated by multiplying the biting density (No. females biting per man/hour) by the proportion of parous females. In a similar way nulliparous density was also calculated.

9.3.3 Vector Survival

The vector survival was estimated from the ratio of female mosquitoes with L3 to those with early stage larvae (Laurence 1963). Since L1 are commonly found in nulliparous gravid females and L3 are found more frequently in gravid females which have completed 2 gonotrophic cycles (Samarawickrema 1967), the ratio between mosquitoes with L3 and those with L1 stage gives an estimation of survival over two gonotrophic cycles. The survival over one gonotrophic cycle was estimated as the square root of L3: L1 ratio.

The probability of daily survival of *Oc. niveus* was calculated from the physiological age grading, by assuming the gonotrophic cycle duration of *Ae. aegypti* which is 5-3-3 (Christophers 1969). The estimates of the probability of survival through each gonotrophic cycle was calculated as shown by Macdonald (1957), Laurence (1963) and Samarawickrema (1967).

Thus, the probability of survival through one day (p)= $\sqrt[n]{P}$

where,

n = the length of gonotrophic cycle (in days)

P = proportion of mosquitoes surviving to that gonotrophic cycle

The estimates of survival for the whole life were calculated by regressing the number of mosquitoes in each age group transformed into $\log_e$ (y +1) as a function of the duration of the gonotrophic cycle in days (Gillies 1961; Reisen and Aslam Khan 1979; Reisen *et al.* 1980). The probability of survival p was estimated by the expression p = e^b where, e is the base of natural logarithm and b is the slope of the fitted regression equation. Then, the survivorship curves were drawn by plotting a linear regression of the number of mosquitoes in different age groups on the age of the mosquitoes.

The month-wise parity and survival rates were analyzed for biting *Oc. niveus* population collected during the study period.

9.3.4 Statistical Analysis

Standardized normal Z test was applied to examine the variation with season in the parous to nulliparous ratio and the proportion of L1 developed to L2 and Student's *t* test to assess the average number of L3 larvae per infective female.

9.4 Results

9.4.1 Parous Rate and Age Composition

The parous rates in different months of the year are presented in the Table 15. The parous rate was not uniform during the different months of the year. A definite pattern could be seen in the parity status of the man landing population of *Oc. niveus* (Fig 22). The parous rate starts to decline from January (26.67%) until July (15.2%) and thereafter the rates begin to raise from August and attains a peak during the month of September (36.42%) and thereafter shows a steady decline during the subsequent months. A higher survival is noticed during the late monsoon months (August to October).

The age composition of man landing *Oc. niveus* is depicted in Fig 23. The proportion of mosquitoes belonging to nulliparous, 1-D, 2-D and 3-D was 76.33% (2767/3625), 18.17% (659/3625), 5% (172/3625) and 1% (27/3625) respectively. Almost similar proportions were observed between the seasons.

9.4.2 Abundance of Vector Population in Relation to Age

The biting density of parous and nulliparous *Oc. niveus* during each month of the study period is depicted in Fig 24. The parous biting density was found to range between 0.4 during the month of December and 1.9 in February. The biting density of parous females was also the lowest in December–January then rise in February and thereafter showed small fluctuations towards a declining trend until March-July and thereafter gradually shows a raising trend from August until November and then dips down in December (Fig 24). The density of nulliparous females was found to show four peaks, the first peak in February, second in April, third in June and the fourth in November and showed no marked monthly fluctuations between July-October in comparison to the density of parous females.

Table 15. Month Wise Parous Rates in Man Landing Population of Oc. niveus

Month	No. dissected	Parous	
		Numbers	Rate (%)
January	195	52	26.67
February	549	129	23.50
March	376	77	20.48
April	419	83	19.81
May	290	45	15.52
June	385	56	14.55
July	171	26	15.20
August	287	91	31.71
September	302	110	36.42
October	172	58	33.72
November	369	105	28.46
December	110	26	23.64
TOTAL	**3625**	**858**	**23.67**

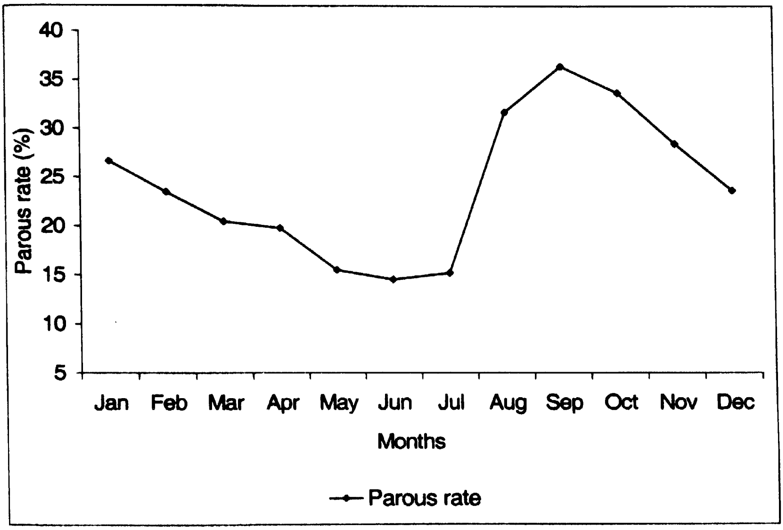

Fig 22. Month Wise Parous Rates in Man Landing Population of Oc. niveus

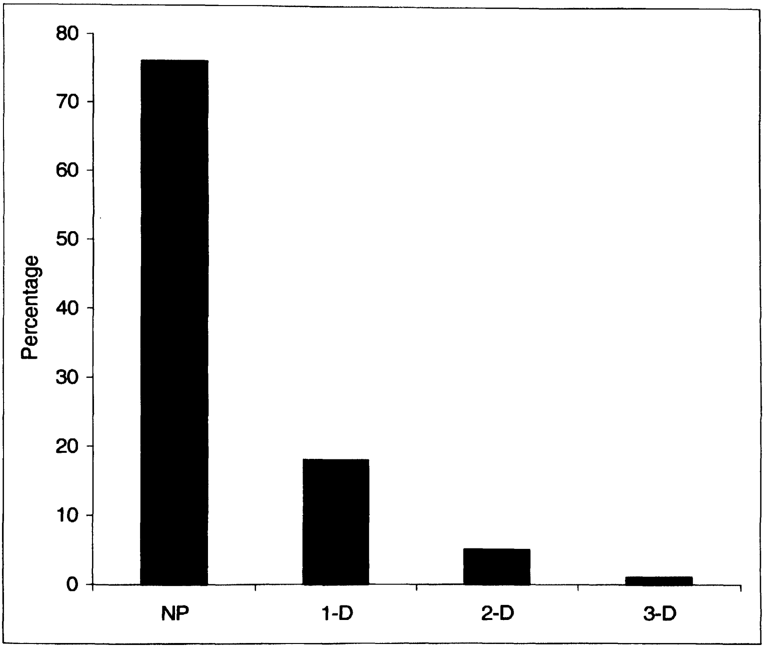

Fig 23: Age Composition of Man Landing Population of Oc. niveus

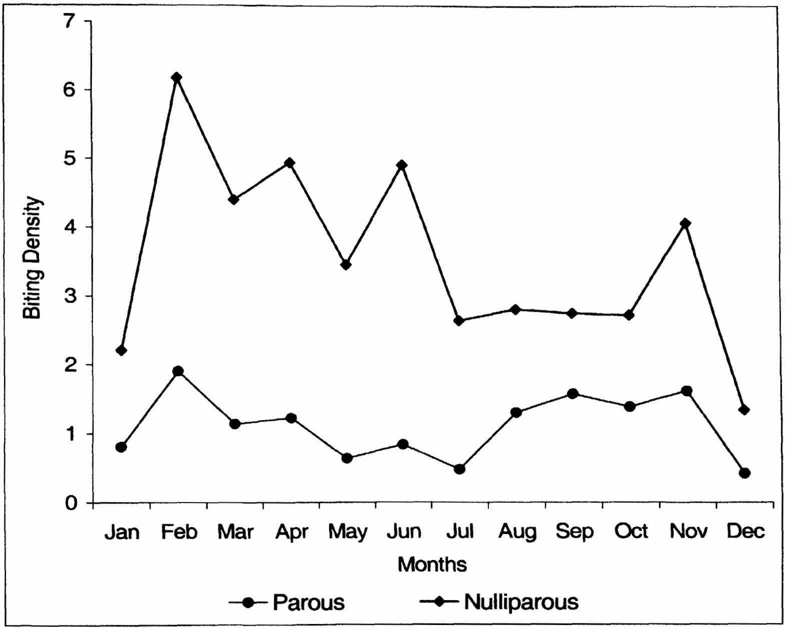

Fig: 24 Abundance of Parous and Nulliparous population of Oc. niveus During Different Months

9.4.3 Vector Survival

Survival of *Oc. niveus* also varied as indicated by monthly/seasonal changes in the proportion of parous to nulliparous females. The number of nulliparous females exceeded parous in all the months of the year. The ratio of parous to nulliparous was the highest during September and October (Table 16). The proportion of parous mosquitoes was significantly different between seasons (χ^2=8.12, p=0.017217, df=2). Females that had completed 3 gonotrophic cycles could be seen in all the months. The proportion of each of the parous age group was slightly higher during the winter and monsoon seasons than in the summer season (Table 17)

The probability of daily survival of man landing population of *Oc. niveus* during different months are presented in the Table 18 The daily survival through first gonotrophic cycle during the study period ranged from 0.68 in June to 0.82 in September. The total probability of daily survival of *Oc. niveus* through one gonotrophic cycle was 0.75, two gonotrophic cycles was 0.70 and three gonotrophic cycles was 0.64 respectively.

The survivorship curves drawn for man landing *Oc. niveus* indicate that the mortality increases significantly with age (Fig 25).

Table 16: Number Dissected, Proportion of Various Age Groups and Parous to Nulliparous Ratio of man landing Oc. niveus During Different Months

Month	No. dissected	No. NP (prop)	Number parous (Prop)			Total	Ratio (P/NP)
			1 D	2 D	3 D		
Jan	195	143 (0.73)	40 (0.21)	10 (0.05)	2 (0.010)	52	0.36
Feb	549	420 (0.77)	95 (0.17)	31 (0.06)	3 (0.005)	129	0.31
Mar	376	299 (0.80)	56 (0.15)	20 (0.05)	1 (0.002)	77	0.26
Apr	419	336 (0.80)	75 (0.18)	7 (0.02)	1 (0.002)	83	0.25
May	290	245 (0.84)	40 (0.14)	4 (0.01)	1 (0.003)	45	0.18
Jun	385	329 (0.85)	41 (0.11)	11 (0.03)	4 (0.010)	56	0.17
Jul	171	145 (0.85)	19 (0.11)	6 (0.04)	1 (0.005)	26	0.18
Aug	287	196 (0.68)	70 (0.24)	18 (0.06)	3 (0.010)	91	0.46
Sep	302	192 (0.64)	81 (0.27)	23 (0.08)	6 (0.019)	110	0.57
Oct	172	114 (0.66)	41 (0.24)	15 (0.09)	2 (0.011)	58	0.51
Nov	369	264 (0.72)	80 (0.22)	23 (0.06)	2 (0.005)	105	0.4
Dec	110	84 (0.76)	21 (0.19)	4 (0.04)	1 (0.009)	26	0.31

Table 17: Seasonal Variation in Different Age Groups and the Survival of Man Landing Oc. niveus, Development of Parasite and Transmission Potential.

Season	Total dissected	No. NP	No. of Parous			Total parous	(P/NP)	No. with		L3:L1	Sqt. L1:L3	TP
			1 D	2 D	3 D			L1	L3			
Winter (Nov-Jan)	674	491	141	37	5	183	0.3727	7	6	0.857	0.926	55.0
Summer (Feb–Apr)	1344	1055	226	58	5	289	0.2739	11	6	0.545	0.739	63.2
Monsoon (May-Oct)	1607	1221	292	77	17	386	0.3161	11	6	0.545	0.739	54.9
Overall	**3625**	**2767**	**659**	**172**	**27**	**858**	**0.3101**	**29**	**18**	**0.621**	**0.788**	**173.1**

Table 18. Probability of Daily Survival Through the Duration of Different Gonotrophic Cycles in Man Landing Oc. niveus

MONTHS	DAILY SURVIVAL		
	Duration		
	1- parous (5 Days)	2–parous (8 Days)	3–parous (11 Days)
January	0.77	0.71	0.66
February	0.75	0.71	0.62
March	0.73	0.70	0.58
April	0.72	0.61	0.58
May	0.69	0.60	0.60
June	0.68	0.67	0.66
July	0.69	0.67	0.63
August	0.79	0.72	0.66
September	0.82	0.75	0.70
October	0.80	0.75	0.67
November	0.78	0.71	0.62
December	0.75	0.68	0.65
Overall	**0.75**	**0.70**	**0.64**

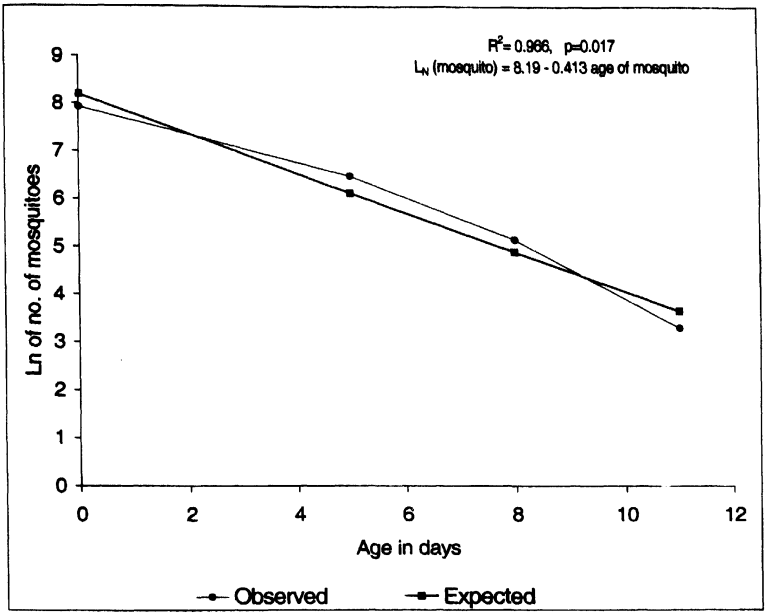

Fig 25. Survivorship Curve for Man Landing Population of Oc. niveus

9.5 Discussion

The lower parous rate, which means an increase in the nulliparous proportion of the population, observed during the study period is probably due to the addition of newly emerged mosquitoes. Similar observations have been made by other workers for *Cx. quinquefasciatus* - *W. bancrofti* vector parasite combination (De Meillon and Khan 1967b; Self *et al.* 1971; Graham and Bradley 1972).

Since the duration required for the development of mf and L1 into L3 stage is 10–12 and 8-10 days respectively (Samarawickrema 1967), the potential for transmission comes from the females that survive a minimum of 2 gonotrophic cycles. The decreased survival rate in the summer and monsoon season is reflected in the comparatively lower L3: L1 ratio, parous to nulliparous ratio (Table 17) the low parous biting density (Fig 26) and low proportion of 3-D mosquitoes. Hence the number surviving long enough to survive the development of the parasite to the infective stage is expected to be less during the summer season. Though no difference was observed in the proportion of L1 developed to L3 (Table 18) the average number of L3 per infective female varied with season, being higher in summer, the reasons for which are difficult to comprehend.

A mosquito must survive, assuming that it picks up microfilariae during the first blood meal itself, for a minimum period of 10 days in order to transmit the parasite. Assuming a gonotrophic cycle duration of *Oc. niveus* to be 5-3-3, the 2-D mosquitoes with gravid condition would be 10-11 days older. So, a proportion of 2-D mosquitoes and other higher age groups are the real transmitters of the parasite. In the present study the 2-D and 3-D constituted about 5.5% in the biting population. Tewari *et al.* (1995) observed that it takes 13 days for mf to become L3 in *Oc. niveus.* Similar observations have been made for another day biting vector, *Ae. polynesiensis* in South Pacific Islands (Samarawickrema *et al.* 1987). However, given the probability that only a proportion of this potential vector population (Hitchcock 1970) picks up and transmit the infection, it is really amazing that only a small proportion of the vector population is responsible for the maintenance of the infection. However, this disadvantageous phenomenon could be offset by the occurrence of high vector densities and parous rates.