10   *Parasite Distribution and Loss in Oc. niveus*

10.1 Summary

This chapter uses entomological data from Teressa Island to examine the evidence for the operation of density dependence on parasite transmission. Patterns of parasite distribution in Oc. niveus were studied. The microfilariae were found to be overdispersed in Oc. niveus. The later stages of the parasites in the vector population were randomly distributed. The distribution curves of various filarial larval stages suggest that loss of parasite or death of mosquito occurred as development progressed and it was maximal between first and second larval stages. Further it was found that both the prevalence of infection and the degree of parasite aggregation in the vector population falls significantly with parasite stage, implying the operation of density dependence, perhaps via parasite induced vector mortality or parasite loss.

10.2 Introduction

The central question in the epidemiology of vector borne diseases is whether nonlinear (density dependent) processes operate on parasite transmission from the vector population (Dietz 1988; Dye 1992). Generally speaking, this can manifest itself as facilitation (an increase in infection rate over some ranges of parasite density) or limitation (a density dependent constraint on transmission). The main mechanisms proposed to drive limitation are excess mortality of the parasites and parasite induced vector mortality at high parasite densities (Brengues and Bain 1972; Pichon *et al.* 1980a; Dye 1992; Subramanian *et al.* 1994; Das *et al.* 1995; Dye and Williams 1995).

Experimental (Crans 1973; Failloux *et al.* 1995; Subramanian *et al.* 1998) and field studies (Samarawickrema and Laurence 1978; Subramanian *et al.* 1994) have shown excess mortality of *Cx. quiquefasciatus* heavily infected with *W. bancrofti*. However, the rate of parasite loss in *Oc. niveus* and the parasite inflicted mortality of the vector population is not available.

The distribution of parasite counts in the host population can indicate the processes exerting regulatory influence on the host (Anderson and Gordon 1982). The distribution pattern of *W. bancrofti* larvae in Cx. *quinquefasciatus* has been used to see if there is any evidence for the operation of density dependence in regulating parasite numbers for *Oc. niveus*.

Samarawickrema and Laurence (1978) analyzed the distribution of *W. bancrofti* larvae in a natural population of the *Cx. quinquefasciatus* in an endemic region for bancroftian filariasis in Sri Lanka. They equated the successive stages of parasite larvae (mf, L1, L2, L3) with duration of infection in the vector, and examined the frequency distribution by fitting a log normal distribution. Having shown that the distribution of mf in freshly blood fed mosquitoes did not differ significantly from that in blood samples from the human population, they established that the distribution of L3 larvae in infected vectors had a significantly shorter tail than for the earlier parasite stages. They then

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used the results from experimental infections to interpret this relative reduction in the proportion of vectors with large burdens of older parasites in terms of the operation of parasite-induced host mortality. Similar observations have been made in Pondicherry, an endemic region for nocturnally periodic bancroftian filariasis transmitted by *Cx. quinquefasciatus* (Das *et al.* 1995). Patterns of the parasite distribution in *Oc. niveus*, vector of diurnally subperiodic filariasis is not known so far.

Therefore, an attempt was made to examine the role of density dependence in the process of *W. bancrofti* larval development in *Oc. niveus*. The results are dealt in this chapter.

10.3 Material and Methods

Entomological data collected from the three zones during the study period were pooled and used for analysis. The mosquito sampling procedure and the method of data collection are described in chapter 5 and the dissection technique in chapter 6. The probability of daily survival and the estimates of probability of survival through each gonotrophic cycle are described in detail in chapter 9.

Since the larval stage-wise distribution of parasite in different age classes of mosquito reflects the host dependent parasite mortality and the parasite dependent host mortality, distribution of four larval stages of parasite viz., mf, L1, L2 and L3 was also analyzed.

10.3.1 *Statistical Analysis*

To see the significant difference in vector infection and infectivity rates between mosquito age, chi-square test was carried out. To assess the distribution pattern of parasites, frequency distribution of parasite counts was constructed for each parasite stage. The trend of aggregation in parasite density was measured in terms of variance to mean ratio for different mosquito and

parasite stages. The probability of survival through one day was calculated based on 'nth' root of stage, where 'n' is the total development period from mf to the particular stage.

10.4 Results

10.4.1 Prevalence of Infection in Relation to Mosquito Age and Parasite Stage

The sample sizes and the prevalence of infection according to mosquito age and parasite stage are depicted in Table 19. The overall infection rate is significantly increasing with the age of mosquitoes (χ^2= 241.7; P=0.000). Similarly, the infective rate showed a significantly (χ^2= 1091; P=0.000) increasing trend with mosquito age.

10.4.2 Distribution of Parasite in Vector

The distribution pattern of various filarial stages is presented in the Fig 26. The distribution pattern of infective stage larvae considerably narrows down indicating the operation of density dependent factor while developing into later stages of the parasite.

10.4.3 Pattern of Parasite Aggregation in the Vector

The means and variance/mean ratio of infection intensity in the vector population, classified according to parasite stage and vector age are presented in Table 20. The overall intensity of parasites decreases with vector age. Aggregation of parasite, which is indicated by variance to mean ratio ranged from 4.1 in nulliparous to 7.5 in 1- parous mosquitoes. The parasite distributions tend to become less aggregated with larval stage in 1-parous mosquitoes. However, due to small samples this is not clear with increasing age of the vector.

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Table 19. Prevalence of Infection for Different Mosquito and Parasite Stages

Parasite stage	Mosquito age									
	NP		1P		2P		3P		Overall	
	(2767)	%	(659)	%	(172)	%	(27)	%	3625	%
MF	23	0.83	0	0.00	0	0	0	0.00	23	0.634
L1	12	0.43	14	2.12	1	0.581	2	7.41	29	0.800
L2	0	0.00	24	3.64	10	5.814	4	14.81	38	1.048
L3	0	0.00	1	0.15	5	2.907	12	44.44	18	0.496
Overall	35	1.26	35	5.31	14	8.140	12	44.44	108	2.654

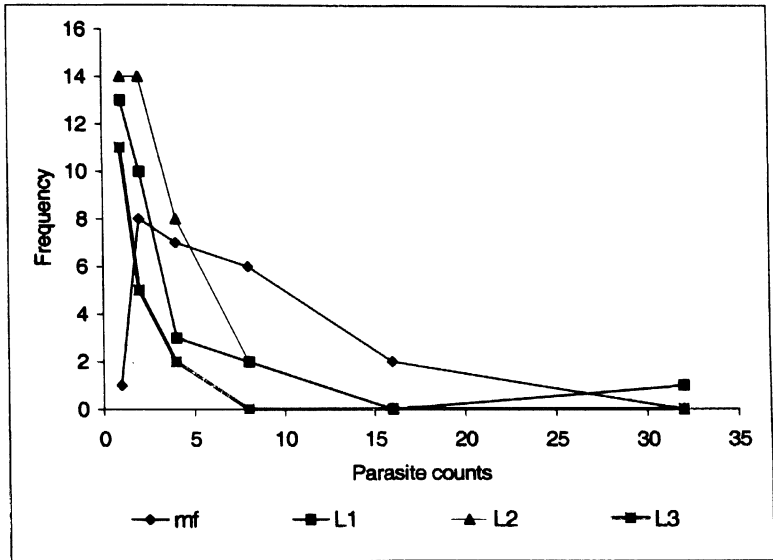Figures in parenthesis are sample sizes

Fig 26: Distribution pattern of mf, L1, L2 and L3 larval stages

Table 20. Parasite Intensity and Variance to Mean Ratio for Different Mosquito and Parasite Stages

Parasite Stage	Parity															
	NP				1P				2P				3P			
	No. of stages	Mean	Var	Vmr*	No. of stages	Mean	Var	Vmr*	No. of stages	Mean	Var	Vmr*	No. of stages	Mean	Var	Vmr*
MF	84	0.030	0.142	4.73	0	0	0		0	0	0		0	0.00	0.00	
L1	23	0.008	0.021	2.63	48	0.073	0.736	10.10	1	0.00581	0.006	1.03	2	0.07	0.07	0.96
L2	0	0.00	0.00	0.00	44	0.067	0.144	2.16	26	0.15116	0.515	3.41	7	0.26	0.43	1.66
L3	0	0.00	0.00	0.00	2	0.003	0.006	1.98	5	0.02907	0.028	0.96	21	0.78	1.18	1.52
Overall	107	0.039	0.162	4.15	94	0.140	1.074	7.51	32	0.18600	0.573	3.08	30	1.11	3.26	2.930

*VMR Variance /Mean Ratio

10.4.4 Loss of Parasite in the Vector

The loss of parasites in vector was estimated by comparing the distribution pattern of different larval stages of parasite. The length of the curve and its nature are the best methods of estimating the parasite mortality in vector (Adjei *et al.* 1986). The loss of parasites due to the mortality of heavily infected vectors was evident from the distribution pattern of different stages of parasites and drastically reduced tail of the curve of infective stage larvae (Fig 26). Wide gap in between L1 and L2 curve in Fig 26 indicates that the loss rate of para- sites in mosquitoes is the highest between L1 and L2 stages.

10.4.5 Parasite Induced Mortality in the Vector Population

The average number of parasites in different age groups of mosquitoes showed that the number increases as the mosquito age increases (Fig 27).

10.4.6 Survival Probabilities of the Parasite

To study the seasonal effects on the survival of parasites and vectors, survival probabilities were calculated season wise and are shown in Table 21. It is clear from this table that the survival probability of parasites is gradually decreasing from mf to L3 and was minimum in L3. Seasonal survival probabilities for mf, L1 and L2 do not show any specific pattern. But the survival probability of L3 is much higher in winter when compared to other seasons. The probability of daily survival did not differ much between the L2 and L3, whereas a difference in the probability of daily survival was noticed between L1 and L2.
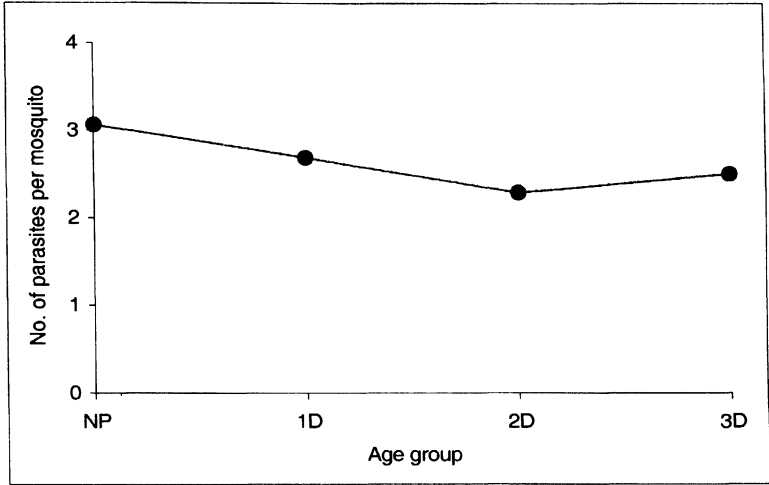
Fig 27: Average Parasite Load by Age in Man Landing Oc. niveus

Table 21. Season Wise Probability of Survival of mf, L1, L2 and L3 Stage Larvae in Oc. niveus

Seasons	Number of parasites					Probability of survival					
						Total			Daily		
	Mf	L1	L2	L3	Total	L1	L2	L3	L1	L2	L3
Winter (Nov-Jan)	7	14	13	9	43	0.33	0.30	0.21	0.57	0.83	0.87
Summer (Feb-Apr)	40	15	41	11	107	0.14	0.38	0.1	0.37	0.86	0.81
Monsoon (May-Oct)	37	45	23	8	113	0.40	0.20	0.07	0.63	0.78	0.79
Total	84	74	77	28	263	0.28	0.29	0.11	0.53	0.83	0.82

10.5 Discussion

The prevalence and intensity of infection declines with increasing age of the parasite but increases with age of the vector. Similar observations were also reported for *W. bancrofti - Cx. quinquefasciatus* combination (Das *et al.* 1995; Subramanian *et al.* 1994). Since the parasite cannot either multiply or be transferred from one mosquito to another, the increase in both prevalence and intensity is probably due to accumulation of infection as the vector age increases. The reduction in prevalence of infection with increasing age of the parasite could be either due to density dependent parasite loss or mosquito mortality or a combination of both. However, due to complex host parasite inter- actions it is difficult to estimate the survival rate of the mosquitoes based on the parity alone (Subramanian *et al.* 1994).

Further, examination of the degree of parasite aggregation in the vector population indicates that it falls significantly with parasite stage as evidenced from variance to mean ratio. In particular, L3 distributions are markedly less aggregated than earlier larval distributions as reported elsewhere Samarawick- rema *et al.* 1978; Das *et al.* 1995). This implies the operation of density depend- ence, perhaps via parasite induced vector mortality.

The over dispersion pattern of mf in vector mosquitoes indicates that the mf are over dispersed in humans also. However, the later stages of the parasite showed random distribution phenomenon in the vector. So the over dispersion of mf in human host cannot be attributed to distribution pattern of infective stage larvae in mosquitoes. The distribution curves of various filarial larval stages suggest that loss of parasite occurred as development progressed and it was maximal between first and second larval stages.

Conflicting observations were made on the mortality in vectors due to parasite density. Rosen (1955) and Bryan and Southgate (1976) reported that infection of mosquito with parasite do not cause recognizable mortality. However, a few studies (Symes 1960; Zielke 1977) suggested that heavy

infection of the vector mosquitoes can cause mortality and this mortality is more when the larvae reach the infective stage. The results of the present study also indicate that infection do cause mortality in vectors.

Survival probability of parasites suggests a gradually decrease from mf to L3. Seasonal effects on survival probabilities for mf, L1 and L2 apparently do not show any specific pattern. However, the survival probability for L3 was much higher in winter when compared to other seasons. The results suggest that probability of daily survival did not differ much between the L2 and L3, whereas a difference in the probability of daily survival was noticed between L1 and L2. Similar observations have been made by Ramaiah (1990) in Pondicherry for *Cx. quinquefasciatus*.

The dynamics of infection in the vector mosquito is complex, because both the acquisition and loss of infection are continuous processes as the vector can lose or gain infection during subsequent blood feeding. The relationship is further complicated by the different rates of survival of both parasite and mosquito. The successful development of the ingested mf to become an infective larvae (parasite yield) is an essential component of the transmission success of this parasite. Quantification of the effects of parasitism in the vector host needs modeling of these complexities.