Nutrition and Overcrowding Effects on Larval Development and Fecundity of Female Aedes albopictus (Skuse)

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***Abstract*- Two studies were conducted to determine the effect of nutrition and overcrowding on larval growth and fecundity of the female *Aedes albopictus*. In the first study, different larval densities (100, 200, 300, 400 and 500 larvae) were tested with optimal and sub-optimal amounts of larval food under laboratory conditions. In the second study, the effect of nutrition and overcrowding at larval stage on the fecundity of female *Ae. albopictus* mosquitoes was observed. Results showed that larval growth of sub-optimally fed larvae, especially those from higher density, was significantly affected. Furthermore, the fecundity of female *Ae. albopictus* was found to be greatly affected by nutrition and overcrowding. Overall, nutrition and overcrowding resulted in increase of larval development time, increase in larval mortality and reduced fecundity of female mosquitoes.**

Keywords- Aedes albopictus; nutrition; overcrowding

I. INTRODUCTION

Population levels of mosquito larvae are determined by both extrinsic and intrinsic factors (Anderson & May, 1991). Extrinsic factors such as weather, climate and geographical features that affect larval and adult abundances may be obtained through ecological survey of mosquito population while intrinsic factors (density-dependents) are obtained through laboratory experiments (Shaman *et al*. 2002). According to Rogers *et al*. (2002), intrinsic or density-dependent factor is a very important element, which is often ignored, in models of mosquito-transmitted diseases in either vector or host populations.

Factors involved in determining mosquito population abundance are not well characterized. Though some authors associate mosquito population with availability of larval habitat (Patz *et al*., 1998; Shaman *et al*., 2002), the ability of adult mosquitoes in selecting larval habitat with sufficient nutrient for oviposition remains a mystery. Nutrition is an important factor that influences larval development (Reisen, 1975; Telang & Wells, 2004) and the reproductive capacity of female mosquitoes (Clements, 1992). This implies that deprivation of nutrition in both larval and adult stages could affect the potential reproduction of a female mosquito (Clements, 1992).

Many studies have shown that the duration of larval development are influenced by both biotic and abiotic factors, such as temperature, food supply (Ho et al., 1972; Wijeyaratne et al. 1974; Hien, 1975; Estrada-Franco & Craig, 1995), sex (Haddow et al., 1959; Estrada-Franco and Craig, 1995), crowding (Estrada-Franco & Craig, 1995), depth of water and salinity (Clements, 1992). In the current study, two parameters were measured to examine the effect of nutrition and overcrowding. This was carried out in two separate studies. The aim of the first study was to observe the larval development and survival rate of field strain *Aedes albopictus* larvae following increase in larval density (overcrowding) between controlled food and space under laboratory conditions. In the second study, the effect of nutrition and overcrowding at larval stage on the fecundity of female *Ae. albopictus* mosquitoes was observed. In order to ensure that the food availability is the only factor, field strain *Ae. albopictus* mosquitoes were reared under laboratory conditions. In addition to the findings reported by Estrada-Franco & Craig (1995) on *Ae. albopictus*, it is hoped that this paper will provide information on its survival under a stressful condition and at the same time allow us to evaluate its fitness capability.

II. MATERIALS AND METHODS

A. Larval collection and preparation

Field strain *Ae. albopictus* mosquitoes were obtained with ovitraps. Only F1 generation of field strain *Ae. albopictus* were used in this study. Eggs were hatched by force-hatching or vacuum hatch technique (Sivanathan, 2006) to ensure uniform larval age. Emerged first instar larvae were separated to respective densitites (see below) into plastic trays (height= 5 cm, width= 12 cm and length= 17 cm) filled with 400 ml of seasoned water.

The study was conducted in an insectarium located in Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia. Studies were conducted under controlled conditions (temperature: 26 ± 2℃, relative humidity: 60 ±10%, photoperiod: 12 h light,12 h dark).

B. Effect of overcrowding and starvation on larval growth of Ae. albopictus larvae

Two groups of larvae were prepared according to the designated density for *Ae. albopictus.* The density of test larvae was 100, 200, 300, 400 and 500 larvae per tray. The first group of larvae was fed with 100 mg (optimal) of larval food and the second group was given 1 mg (sub-optimal) of larval food (dog biscuit, sun-dried beef liver, yeast, milk powder in a ratio of 2:1:1:1, ground into a powder). The test was replicated three times. The medium was changed everyday throughout the experimental period to avoid scum formation. The numbers of pupae and mortality in each tray were recorded every day at a fixed time.

C. Effect of starvation at larval stage on the fecundity of female Ae. albopictus mosquitoes

In order to ensure standardization of the test, median larval population which gave acceptable number of emergence were selected for the second test. Female *Ae. albopictus* mosquitoes which emerge from 300 larvae per tray were selected for 2 reasons. First, the number of mortality for sub-optimal group is 50%; second, the number of females emerged from sub-optimal group in a day is higher compared to other higher densities.

Ae. albopictus was divided into two groups; the first group was fed with 1 mg of larval food and the second fed was fed with 100 mg of larval food. Each group consisted of six trays (height= 5 cm, width= 12 cm and length= 17 cm) filled with 400 ml of seasoned water and sprinkled with larval food according to the test groups. Water was changed every day.

Adult mosquitoes that emerged from each group of larvae were kept in separate cages (approximately 200 adult or 100 pairs per cage) and were given 10% sucrose. Female *Ae. albopictus* mosquitoes were given a blood meal provided by a rat confined in a tight fitting fine wire mesh cage on the fifth day (from 1800h to 2100h) after emergence. Two days after the blood meals were given, 60 gravid female mosquitoes from each group were transferred into individual glass cups for oviposition. The females were left in the oviposition cup for seven days and 10% sucrose was supplied every day. Filter paper (Whatman No. 1, folded into cone and moistened, served as the oviposition substrate) was changed every day until no eggs were found on the filter paper. Upon completion of the test, female mosquitoes were returned to their respective cages. The filter papers that were removed from each glass cup were air-dried before the number of eggs was counted and recorded every day.

D. Statistical analysis

For the effect of nutrition and overcrowding on larval growth, data were analysed using Mann Whitney *U*-test. As for the effect of nutrition and overcrowding on the fecundity of female *Aedes* mosquitoes, normality of the data was determined using Kolmogorov-Smirnov tests. The data were further tested with Mann Whitney *U*-test. Statistical test was analysed using SPSS version 11.0 and considered significant at $P = 0.05$.

III. RESULTS

A. Effect of nutrition and overcrowding on larval growth of Ae. albopictus larvae

Mean development rate of *Ae. albopictus* larvae from optimally and sub-optimally fed group is between 6-7 days (Table 1) and 6-9 days (Table 2), respectively. Development rate were significantly different in first pupation between optimally and sub-optimally fed group at larval densities 400 (Mann-Whitney U test, *P*= 0.034, Table 1) and 500 (Mann-Whitney U test, *P*= 0.034, Table 1). Mean larval growth between 2 different nutrition was found to be significantly different at every larval density for final pupation (Mann-Whitney U test, $P < 0.05$, Table 2). Overcrowding and shortage of food has significantly prolonged the larval growth up to 36 days (Table 2).

TABLE I NUMBER OF DAYS TAKEN BY FIRST AE. ALBOPICTUS LARVA/ LARVAE REARED AT DIFFERENT LARVAL DENSITIES AND NUTRITION TO REACH PUPAL STAGE (FIRST PUPATION)

Larval densities per tray	**Optimal larval food (n=3) Mean (SD)#**	**Sub-optimal larval food (n=3) Mean (SD)#**	**Z-statistic**	**P value@**
100	6 (0)	6.67 (0.58)	-1.581	0.114
200	6.67 (0.58)	7 (0)	-1.000	0.317
300	7 (0)	7.67(0.58)	-1.581	0.114
400	7 (0)	9.33(0.58)	-2.121	0.034*
500	7 (0)	9.33(0.58)	-2.121	0.034*

@ Mann Whitney *U* - test
# number of day taken by first larva to reach pupal stage
* P <0.05

TABLE II NUMBER OF DAYS TAKEN BY LAST *AE. ALBOPICTUS* LARVA/ LARVAE REARED AT DIFFERENT LARVAL DENSITIES AND NUTRITION TO REACH PUPAL STAGE (FINAL PUPATION)

Larval densities per tray	Optimal larval food (n=3) Mean (SD)#	Sub-optimal larval food (n=3) Mean (SD)#	Z-statistic	P value@
100	8.33 (0.58)	20.0 (1.73)	-2.023	0.043*
200	13.0 (1.73)	31.7 (0.58)	-2.023	0.043*
300	13.3 (0.58)	36.0(3.46)	-2.023	0.043*
400	13.7 (0.58)	34.7(1.53)	-1.993	0.046*
500	14 (0)	35.7(0.58)	-2.121	0.034*

@Mann Whitney *U* - test
\# number of day taken by last larvae to reach pupal stage
* P <0.05

Significant pupation rate between optimally and sub-optimally fed larval group was observed at larval densities 200 to 500 (Mann-Whitney U test, *P* = 0.05, Table 3). The mortality rate of sub-optimally fed larval group at highest density (500 larvae per tray) was more than 70% of the tested larvae population (Table 4).

TABLE III NUMBER OF *AE. ALBOPICTUS* PUPAE PUPATED REARED AT DIFFERENT LARVAL DENSITIES AND NUTRITION

Larval densities per tray	Optimal larval food (n=3) Mean (SD)#	Sub-optimal larval food (n=3) Mean (SD)#	Z-statistic	P value@
100	94.3 (3.06)	90.3 (2.08)	-1.328	0.184
200	181.7 (9.07)	137.3 (15.8)	-1.964	0.050*
300	279.3 (9.71)	145.7(20.6)	-1.964	0.050*
400	386.0 (4.36)	140.7(3.79)	-1.964	0.050*
500	468.0 (14.0)	126.3(15.2)	-1.964	0.050*

@Mann Whitney *U* - test
\# number of larvae reaches pupal stage
* P <0.05

TABLE IV MORTALITY RATE OF *AE. ALBOPICTUS* LARVAE REARED AT DIFFERENT LARVAL DENSITIES AND NUTRITION

Larval densities per tray	Optimal larval food (n=3) Mean (SD)#	Sub-optimal larval food (n=3) Mean (SD)#	Z-statistic	P value@
100	5.67 (3.06)	9.67 (2.08)	-1.328	0.184
200	9.17 (4.54)	31.3 (7.91)	-1.964	0.050*
300	6.89 (3.23)	51.4 (6.85)	-1.964	0.050*
400	3.5 (1.09)	64.8 (0.95)	-1.964	0.050*
500	6.4 (2.80)	74.7 (3.04)	-1.964	0.050*

@ Mann Whitney *U* - test
\#mortality rate (%)
* P <0.05

B. *Effect of nutrition and overcrowding on the fecundity of female Ae. albopictus mosquitoes*

The differences in sizes of female mosquitoes were noted between adult mosquitoes emerging from optimal and sub-optimally reared larval group. Adult female mosquitoes which emerge from optimally fed group were found to be large as compared to female mosquitoes from sub-optimally fed group which is smaller in size. A significant number of eggs were produced by female *Ae. albopictus* mosquitoes from optimally fed group as compared to females from sub-optimally fed group (Mann Whitney *U*-Test, $P < 0.01$, Table 5). Duration of first gonotrophic cycle of female *Ae. albopictus* from optimally and sub-optimally fed group is between 6 -14 days and 6 -17 days (Table 5), respectively.

TABLE V DURATION OF FIRST GONOTROPHIC CYCLE AND NUMBER OF EGGS LAID BY FEMALE *AE. ALBOPICTUS* MOSQUITOES REARED ON DIFFERENT NUTRITION AT LARVAL STAGE.

***Aedes sp*. (n=60)**	**Optimal larval food (n=60)**	**Sub-optimal larval food Value***	**P**
Duration of first gonotrophic cycle (day) Min-Max (SD)	6 - 14 (1.84)	6 - 17 (2.39)	-
Number of eggs Laid Mean (SD)	67.5 (33.9)	23.3 (17.4)	<0.01
No of females laid eggs (%)	51 (85)	47 (78.3)	

* Mann Whitney *U* - test

IV. DISCUSSION

This experiment showed that overcrowding and starvation of larvae had a detrimental effect on larval development, and subsequently on the fecundity of adult female *Aedes* mosquitoes. Our findings were similar to other studies that had found evidence of extended larval development (Beach et al., 1977; Mori, 1979), increased mortalty (Hien, 1975; Mori & Wada, 1978) and low fecundity (Bar Zeev, 1959).

For larvae that were reared under sub-optimal larval food condition, the development time was significantly prolonged. For instance, development time for the last group of larvae from higher larval density (200 to 500 larvae per tray) to reach pupal stage was extended over 30 days with increasing mortality rate. This observation was supported by several authors, who claimed that the larval development will be stopped (Clements, 1992) or prolonged (Brust, 1968; Beach *et al.,* 1977; Mori, 1979) if the amount of food decreased.

Cannibalism was detected during the study. This behaviour was present within the group of underfed larvae. Healthy and large larvae were found feeding ferociously on small larvae. Competition for food causes these larvae to behave aggressively which in turn caused the depletion of larval population. Similar findings were also reported by Seifert *et al*. (1981) and Goma (1966). Both authors reported evidence of cannibalism among non-predatory larvae in some species when the larval densities were high and food was not available or scarce. In addition, the larvae and pupae from the underfed group appeared weak and showed slow reaction towards disturbance of the water surface.

It was noted that during blood feeding session, larger female mosquitoes from optimally fed larval group were seen attacking the blood source rather aggressively as soon as blood source were introduced into the cages. Our observation on smaller size female mosquitoes from sub-optimally reared larval group found that the females did not attack the blood meal immediately but took more than 60 minutes before they showed any interest towards blood source.

Nutrition and overcrowding affect not only the physical condition of the female *Ae. albopictus* mosquitoes but also its blood feeding behaviour. The differences in blood feeding pattern between females from optimal and sub-optimal reared group can be seen in by egg laying activity. The gonotrophic cycle last longer for females from sub-optimally fed group which produce low number of eggs as compared to females from optimally fed group. Larger and healthier females are found to be fitter, capable to hunt for blood source and engaged in multiple blood feeding which increase its reproduction.

V. CONCLUSION

In conclusion, nutrition and overcrowding of *Ae. albopictus* larvae resulted in increase in larval development time, increase in larval mortality and presence of cannibalism among larvae. Furthermore, the effect of nutrition and overcrowding is reflected on female *Ae. albopictus*'s physical condition, blood feeding pattern and as well as reduced fecundity.

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