THE EFFECTS OF INTRASPECIFIC COMPETITION FOR FOOD AND SPACE ON THE LARVAL DEVELOPMENT OF *CULEX QUINQUEFASCIATUS*

M. SULEMAN[1]

Zoology Department, University of Peshawar, Peshawar, Pakistan.

ABSTRACT. Individual and combined effects of larval nutrition and density on developmental mortality, rate of development, adult body size and sex ratio of *Culex quinquefasciatus* were examined in the laboratory using 4–5 levels of larval food and density. At uniform densities of 1 larva per ml of rearing water or cm^2 surface area and with varying amounts of food per larva, the developmental mortality decreased while rate of development and sex ratio (male/total) increased, as a linear function of the amount of food per larva. With a constant concentration of food the developmental mortality increased and sex ratio decreased progressively with increasing density and/or decreasing food per larva, but development rate and body size did not show a significant linear change. When larval density was varied with a constant amount of food per larva, the development rate varied as a linear function of the larval density, but developmental mortality, body size and sex ratio, remained unaffected. A bioassay of larval rearing water using conspecific first instar larvae showed that no growth retardant factor (GRF) was produced at larval densities ranging from 1 to 5 per ml or cm^2 surface area, when food per larva was not limited. Instead, a growth promoting factor seemed to be produced at higher densities. The results indicate that larval competition for food is more important than competition for rearing space as a population regulating factor in *Cx. quinquefasciatus*.

INTRODUCTION

A number of studies have indicated that larval crowding in mosquitoes elicits a growth retardant factor (GRF) which may be involved in population regulation in nature. GRF has been demonstrated in *Aedes aegypti* (Linn.) (Moore and Fischer 1969, Peters et al. 1969), *Anopheles stephensi* Liston (Reisen and Emory 1977), *Culex tritaeniorhynchus* Giles (Siddiqui et al. 1976) and *Culex quinquefasciatus* Say (Ikeshoji and Mulla 1970). GRF functions as an autotoxin which can retard development, increase mortality and result in the production of small-sized adults. Production of GRF, in the foregoing studies, was observed when larvae were reared under crowded conditions and food was limited, so the effects of space and food were not determined separately. Moore and Whitacre (1972), however, studied the effects of food and space separately and concluded that larval nutrition, not larval density, affected the production of GRF by *Ae. aegypti*.

Rajagopalan et al. (1976) studied the development and survival rate of immature stages of *Cx. quinquefasciatus* at different density levels, both in experimental containers and natural breeding sites (wells) in rural areas near Delhi. They found that density dependent factors play an important role in regulating population size, but the underlying density dependent factors and their mechanism of action were not identified.

This study investigated the effects of larval nutrition and rearing density, individually and in combination, on selected developmental and adult attributes of *Cx. quinquefasciatus* to identify which factor is more important in population regulation.

MATERIALS AND METHODS

First instar larvae from field collected eggs were pipetted into pans within 6 hours of eclosion and reared under controlled food regimens and density levels.

[1] Present Address: Environment and Policy Institute, East-West Center, Box 1446, 1777 East-West Road, Univeristy of Hawaii, Honolulu, Hawaii 96848.

Larvae were fed milk powder commercially known as "Farex" and raised in 150 ml of deionized water in plastic 15 × 10 cm lunch boxes (pans). Measured quantities of food were given on alternate days, and water loss due to evaporation was replenished daily. The number of larvae pupating and adults emerging were recorded daily. Median values for larval period (time from eclosion to pupation) and total developmental period (time from eclusion to adult emergence) were calculated, separately for each replicate, from frequency distributions using standard method of linear interpolation. These replicate median values were averaged to get a mean and SE. The SE values obtained in this way might appear to be small as they do not reflect the variations within replicates. This procedure was adopted for simplicity and uniformity since other attributes measured as proportions (e.g. developmental mortality and sex ratio) were dealt with in this way. Wing-lengths were measured at the anterior margin with an ocular micrometer using adults which emerged approximately in the middle of the total emergence cycle. All experiments except the bioassay test were conducted at room temperature.

EXPERIMENT 1—EFFECTS OF INTRASPECIFIC COMPETITION FOR LARVAL FOOD. One hundred and fifty first instar larvae were pipetted into each of the 4 rearing pans (150 cm^2 surface area) yielding a density of 1 larva/ml or 1 larva/cm^2. One group was given the basic food ration which was:

Day 0 - 0.025 mg,
Day 2 - 0.050 mg,
Day 4 - 0.100 mg,
Day 6 - 0.150 mg,
Day 8, 10, ... - 0.125 mg.

The food supply was continued until the last larva pupated or died. The other 3 groups were given food rations equivalent to 2, 4 and 6 times the basic ration, respectively. The experiment was replicated 3 times. Room temperature averaged 33.9 ± 0.3°C with a daily mean maximum 35.9°C (31.7 – 38.8) and mean minimum 31.9°C (28.3 – 35.6).

EXPERIMENT 2 – COMBINED EFFECTS OF LARVAL NUTRITION AND REARING DENSITY. Larvae numbering 150, 300, 450, 600 and 750 were added to each of the 5 pans yielding larval densities of 1 larva, 2 larvae, 3 larvae, 4 larvae and 5 larvae per ml or cm^2. A food ration equivalent to 4 times the basic ration was given to all groups irrespective of the densities with the result that the food per larva was different at different densities (4X, 2X, 1.3X, 1X and 0.8X at 1 through 5 larvae/ml, respectively). Observation was replicated 3 times. The experiment was performed at a mean temperature of 27.6 ± 0.2°C with a daily mean maximum and mean minimum of 28.7°C (26.1 – 32.8) and 26.5°C (23.9–30.6), respectively.

EXPERIMENT 3 – EFFECTS OF LARVAL REARING DENSITY. Larvae numbering 300, 450, 600 and 750 were added to each of the 4 pans yielding larval densities of 2 larvae, 3 larvae, 4 larvae and 5 larvae per ml or cm^2. A food ration equivalent to 1.5 times the basic ration per larva was given to all groups. (In an earlier attempt when ration scale equivalent to 3 times the basic ration was tried, it caused scum formation rendering the medium unsuitable for normal development.) The experiment was replicated 3 times at average temperature of 26.9 ± 0.1°C with a daily mean maximum of 27.7°C (26.1 – 32.2) and mean minimum of 26.2°C (23.9 – 27.8).

BIOASSAY TEST. After the completion of pupation in experiment 3, the rearing water was filtered through a Whatman No. 2 filter to remove large particulate matter and replicates were pooled for various densities. Bioassay tests were performed using 25 first instar larvae in 50 ml of rearing water from each density level. Larvae were reared in an incubator set at 29°C on the same ration as given in experiment No. 3. The experiment was replicated 4 times. The rate of development and rearing success were used to judge the presence of any growth-influencing factor in the rearing water.

STATISTICAL ANALYSIS. Data were sub-

jected to ANOVA and regression analysis, using replicate observations, leading to simultaneous tests of linearity and deviation from linearity, as recommended by Armitage (1971). Where linear regression was not significant but ANOVA was significant, a studentized range test was used for multiple comparison of treatment means, and relationship was checked for nonlinear regression using an appropriate linearizing transformation (log transformations of independent variable (X) were applied to linearize relationship; where independent variable (X) contained a quantity equal to unity, transformation applied was log (X)+1 or log (X)+2 since log of 1 being 0 cannot be used).

RESULTS

EXPERIMENT 1—EFFECTS OF INTRASPECIFIC COMPETITION FOR LARVAL FOOD. The total developmental mortality (larval + pupal mortality) decreased with increasing amount of food per larva, although individually larval and pupal mortalities were not linearly related to the nutritional level (Table 1). The rate of development as judged from the total developmental period and period up to first pupation varied as a linear function of the amount of food per larva. These observations agree with Siddiqui et al. (1976) who found significant decrease in P_{50} (median pupation period) and increase in larval mortality at higher densities (i.e., lower amount of food per larva) in *Cx. tritaeniorhynchus*. No significant difference in body size (as indicated by wing-length) was demonstrated among individuals of either sex which developed from larvae raised at different food regimens. As expected, males were smaller and developed faster than the females throughout. The sex ratio differed as a function of larval food with proportionately fewer males completing development at the lower ration.

Considering developmental and adult attributes together, the ration equivalent to 4 times the basic ration was judged best for rearing and thus was used in experiment 2.

EXPERIMENT 2—COMBINED EFFECTS OF LARVAL NUTRITION AND REARING DENSITY. Developmental mortality increased as a function of increasing density and/or decreasing the amount of food per larva (Table 2). Though the rate of development was highest at a density of 1 larva/ml, there was not a linear relationship with density or the amount of food per larva across the range of experimental densities. (That is, deviations from linearity were also significant.) The lack of a linear relationship between rate of development and amount of food per larva, in contrast with the results in experiment 1, indicates an interaction between food per larva and rearing density. It appears that the available food was used more effectively at higher densities, perhaps because of increased phagostimulation, as reported by Dadd and Kleinjahn (1974) in *Cx. pipiens* Linn., or a growth promoting factor which was produced at higher (but not overcrowded) densities. In addition, the differences in food rations (ranging from 0.8X to 4.0X in experiment 2 and 1X to 6X in experiment 1) as well as in experimental temperatures (26.5° to 28.7°C in experiment 2 and 31.9° to 35.9°C in experiment 1) might also have contributed to the lack of comparability of relationship between rate of development and amount of food per larva in the two experiments. Variations in body size (as indicated by wing-lengths) among various densities or food levels was not significant but sex ratio varied significantly showing a linear pattern. The sex ratio favored the males at lower densities (higher amount of food per larva) and females at higher densities (lower amount of food per larva) and females at higher densities (lower amounts of food per larva), as in experiment 1.

When the larval mortality from Tables 1 and 2 are examined graphically in terms of food ration (rather than larval density in the case of Table 2, and disregarding the highest food level of 6X in Table 1 for which there is no comparable food level

Table 1. Effects of larval nutrition on various developmental and adult attributes in *Cx quinquefasciatus*: mean ± SE for 3 replicates.

Attributes	Ration scale per larva (X = basic ration)				ANOVA	Regression	
	1X	2X	4X	6X		Linear	Non-linear
Larval mortality (%)	78.67 ± 6.31[a]	48.44 ± 6.25[b]	33.11 ± 6.89[b]	48.22 ± 7.73[b]	*(0.05)	ns(0.05)	*(0.05)
Pupal mortality (%)	45.83 ± 7.68	24.86 ± 5.99	32.13 ± 5.33	13.63 ± 4.51	ns(0.1)		
Total developmental mortality (%)	88.45 ± 6.71	62.22 ± 4.24	54.44 ± 6.90	55.33 ± 6.70	*(0.05)	*(0.05)	
Total developmental period (days)							
Females	23.90 ± 0.57	19.30 ± 0.45	15.93 ± 0.23	12.80 ± 0.17	*(0.025)	*(0.005)	
Males	23.28 ± 0.74	18.37 ± 0.51	14.34 ± 0.28	11.60 ± 0.18	*(0.05)	*(0.005)	
Period (days) up to							
First pupation	13.00 ± 0.00	11.00 ± 0.94	9.33 ± 0.54	7.66 ± 0.98	*(0.05)	*(0.01)	
Last pupation	23.33 ± 0.98	20.33 ± 2.59	18.00 ± 1.63	14.00 ± 1.25	ns(0.1)		
Wing length (mm)							
Females	2.50 ± 0.25	2.98 ± 0.06	2.93 ± 0.05	2.77 ± 0.26	ns(0.05)		
Males	2.40 ± 0.13	2.45 ± 0.10	2.57 ± 0.15	2.59 ± 0.13	ns(0.05)		
Sex ratio (males/total)	0.33 ± 0.09	0.42 ± 0.03	0.48 ± 0.12	0.53 ± 0.14	*(0.05)	*(0.05)	

* = significant; ns = not significant; figures in parentheses indicate alpha level; different letter postscripts indicate significant difference ($P<0.05$) among treatments.

Table 2. Combined effects of larval nutrition and rearing density on various developmental and adult attributes in *Cx. quinquefasciatus;* mean ± SE for 3 replicates (larval food 4 times the basic ration per ml.)

Attributes	Density (larvae/ml or cm²)						Regression	
	1	2	3	4	5	ANOVA	Linear	Non-linear
Larval mortality (%)	3.56 ± 1.43	19.67 ± 2.45	42.59 ± 2.11	63.94 ± 2.18	75.73 ± 0.82	*(0.001)	*(0.001)	
Pupal mortality (%)	9.44 ± 0.27	11.95 ± 8.16	15.74 ± 0.31	15.49 ± 7.59	12.25 ± 5.71	ns(0.75)		
Total developmental mortality (%)	12.67 ± 1.13	28.67 ± 8.46	52.74 ± 1.98	68.45 ± 5.19	78.53 ± 1.15	*(0.001)	*(0.001)	
Total developmental period (days)								
Females	29.40 ± 0.27[a]	36.37 ± 0.36[b]	38.42 ± 0.39[b]	38.20 ± 0.32[b]	36.28 ± 0.29[b]	*(0.05)	ns(0.05)	ns(0.05)
Males	25.39 ± 0.31[a]	32.30 ± 0.43[bc]	36.69 ± 0.43[b]	35.00 ± 0.59[bc]	34.32 ± 0.38[c]	*(0.05)	ns(0.05)	ns(0.05)
Period (days) up to								
First pupation	14.66 ± 1.21	13.67 ± 0.67	17.00 ± 1.00	19.00 ± 1.00	18.33 ± 2.67	ns(0.1)		
Last pupation	29.67 ± 2.61[a]	46.33 ± 0.88[bc]	53.00 ± 3.46[b]	47.33 ± 1.20[bc]	42.33 ± 1.67[c]	*(0.005)	ns(0.005)	ns(0.005)
Wing length (mm)								
Females	2.86 ± 0.16	2.69 ± 0.06	2.68 ± 0.05	2.66 ± 0.06	2.71 ± 0.06	ns(0.05)		
Males	2.35 ± 0.07	2.38 ± 0.09	2.28 ± 0.09	2.34 ± 0.08	2.41 ± 0.06	ns(0.05)		
Sex ratio (males/total)	0.57 ± 0.02	0.48 ± 0.03	0.45 ± 0.01	0.47 ± 0.03	0.43 ± 0.02	*(0.05)	*(0.025)	

* = significant; ns = not significant; figures in parentheses denote alpha levels; different letter postscripts indicate significant difference ($P < 0.05$) among density levels.

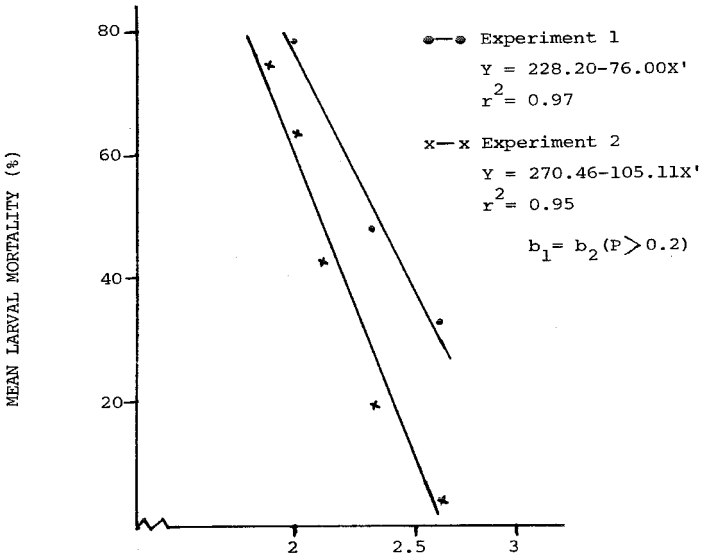

Fig. 1. Larval mortality in relation to food ration per larva in experiments 1 and 2, based on data derived from Tables 1 and 2 (Y = regression equation, b = slope, r^2 = coefficient of determination).

in Table 2), the 2 curves are very similar in slope (Fig. 1, $P > 0.2$). This implies clearly that under combined effects of larval nutrition and density as in experiment 2 it is the nutritional factor rather than density which is more directly associated with larval mortality. The higher overall mortality in experiment 1 may have been due to the rather high temperatures encountered.

EXPERIMENT 3—EFFECTS OF LARVAL REARING DENSITY. Developmental mortality, body size and sex ratio did not change significantly as a result of different larval rearing densities (Table 3). The rate of development (as reflected by total developmental period and period up to last pupation) increased significantly with increasing larval densities, indicating a mutual stimulation of development at higher densities. This stimulating effect may be due to a growth promoting factor (GPF) whose nature and mechanism of action is not known. It may work through phagostimulation and be produced at a density lower than that required for the production of a growth retardant factor (GRF). Phagostimulation at larval densities ranging from 1 to 10 larvae/ml has also been reported in *Cx. pipiens* (Dadd and Kleinjahn 1974). However, another likely hypothesis is that proposed by Reisen (1975), namely, that the growth promoting effect is the result of accumulated nutrients from the previous feeding cycle.

BIOASSAY TEST. The rate of development was higher in higher-density water as compared to lower-density waters and deionized water control, showing a linear

Table 3. Effects of larval rearing density on various developmental and adult attributes in *Cx. quinquefasciatus*; mean ± SE for 3 replicates; amount of food per larva uniform for all density levels.

Attributes	Density (larvae/ml or cm²)				ANOVA	Linear Regression
	2	3	4	5		
Larval mortality (%)	60.78 ± 2.29	53.85 ± 6.80	36.44 ± 11.59	72.80 ± 8.02	ns(0.1)	
Pupal mortality (%)	11.36 ± 3.45	17.56 ± 4.98	29.39 ± 4.54	27.27 ± 2.98	ns(0.1)	
Total developmental mortality (%)	65.00 ± 3.28	60.52 ± 7.15	55.56 ± 8.35	80.35 ± 5.78	ns(0.1)	
Total developmental period (days)						
Females	40.72 ± 0.71	37.00 ± 0.54	30.19 ± 0.31	27.20 ± 0.44	*(0.05)	*(0.025)
Males	33.99 ± 0.71	32.49 ± 0.57	26.89 ± 0.34	25.15 ± 0.32	*(0.025)	*(0.005)
Period (days) up to						
First pupation	18.00 ± 0.47	15.67 ± 0.72	14.33 ± 0.72	17.67 ± 1.09	ns(0.05)	
Last pupation	51.67 ± 0.72	45.67 ± 0.27	40.67 ± 2.88	43.33 ± 1.45	*(0.025)	*(0.01)
Wing-length (mm)						
Female	2.31 ± 0.09	2.67 ± 0.06	2.45 ± 0.07	2.95 ± 0.09	ns(0.05)	
Males	2.24 ± 0.08	2.41 ± 0.08	2.31 ± 0.06	2.31 ± 0.09	ns(0.05)	
Sex ratio (males/total)	0.52 ± 0.01	0.54 ± 0.01	0.48 ± 0.02	0.56 ± 0.00	ns(0.05)	

* = significant; ns = not significant; figures in parentheses denote alpha levels.

Table 4. Results of the bioassay test for the determination of a growth influencing factor produced by larvae reared at various densities as used in experiment 3; mean ± SE for 4 replicates.

Parameters used	Control[1]	Density (larvae/ml or cm²) 2	3	4	5	ANOVA	Regression Linear	Non-linear
Larval mortality (%)	55.56 ± 4.33[a]	52.62 ± 3.67[a]	43.98 ± 2.46[ab]	36.72 ± 2.33[b]	58.45±2.54	*(0.05)	ns(0.05)	ns(0.05)
Larval period (days)	23.25 ± 0.40	20.20 ± 0.60	20.40 ± 0.68	19.14 ± 0.52	18.00 ± 0.42	*(0.05)	*(0.05)	

[1] = deionized water;
* = significant; ns = not significant; figures in brackets denote alpha levels; different letter postscripts indicate significant difference ($P<0.05$) between density levels.

relationship with the density level (Table 4). Larval mortality in higher-density water was less than (or at least not significantly higher than) that in the control group. These results suggest that no autotoxin or GRF was produced at any of the densities used in experiment 3. Instead, they suggest the production of a growth promoting factor (GPF) at higher densities, reinforcing the results of experiment 3. These observations are consistent with those of Reisen (1975) who noted that larvae of *An. stephensi* raised in previously conditioned larval rearing water developed faster than those reared in distilled water, and those reared in water conditioned under uncrowded conditions developed the fastest and had lowest mortality. The growth promoting factor (GPF) in the conditioned media may be due to accumulation of various salts from food, larval metabolites, etc. as suggested by Reisen (1975).

DISCUSSION

A comparison of the results of the 3 experiments indicates that larval survival and development rate are more directly associated with larval nutrition rather than density *per se.* Within tolerable limits, both rearing success and rate of development increase with increasing amounts of food per larva.

It is competition for larval food and not larval rearing space which seems more important as a population regulating factor in nature. Unless food is limited, a density up to 5 larvae/ml shows no negative effect on the rate of larval development, and a density greater than this is not common in nature. Rajagopalan et al. (1975) recorded an average larval density equivalent to 3.066 larvae/cm² and 2.83 larvae/ml (upper range 3.1 larvae/ml) in an area selected for high degree of breeding near Delhi. This conclusion is consistent with Gilpin and Langford (1978) who reported evidence for larval food competition as an overriding factor governing field population of *Aedes sierrensis* (Ludlow).

It follows that the overcrowded larval densities of 20–27 larvae per cm^2 and more than 5 per ml used by Ikeshoji and Mulla (1970) to demonstrate the production of GRF would rarely be expected under natural conditions and thus, GRF would seem to have a minimal role in the regulation of natural populations, unless severe conditions of starvation prevail in nature. At moderate larval densities, when food is not a limiting factor, it appears that a growth promoting factor (GPF), instead of growth retardant factor is produced. Such a factor may function as an oviposition attractant whereby rearing water is preferred for oviposition (Suleman and Shirin 1981).

The effect of larval nutrition on the sex ratio at emergence (Tables 1 and 2) is compatible with the field observations of Hayes and Hsi (1975) and Hayes and Downs (1980) who noticed a seasonal shift in sex ratio in favor of females as a result of lower temperature. It appears that male larvae are less competitive and/or more sensitive to environmental stress such as food shortage and low temperature compared to female larvae, an effect which seems to be of adaptive value for the survival and maintenance of the species under adverse environmental conditions in nature. Given ample food, larval densities up to 5 larvae/ml showed no selective effect on sexes (Table 3), indicating that the primary limiting factor is larval food and not larval density. Without monitoring the food requirement of the larvae, no valid relationship can be expected between sex ratio and larval rearing density. It is probably for this reason that variable results have been reported regarding the sex ratio and density relationship in other mosquitoes such as *Ae. aegypti,* for which Hickey (1970) reported no appreciable density effect and Wielding (1929) found disproportionately more males and Barbosa et al. (1972) proportionately more females at higher larval densities.

The important role that larval food plays in the development, survival and sex ratio of *Cx. quinquefasciatus* explains how harvesting and threshing of wheat could lead to a rapid build up of mosquito density and how the carrying capacity of a breeding site (e.g., a well) changes seasonally as reported by Rajagopalan et al. (1976). Seasonal abundance of *Cx. quinquefasciatus* during spring and winter in rural agricultural villages of the Punjab as reported by Reisen (1978) also seems related to maturation and harvesting of crops and may be explained as follows:

Harvesting and threshing of *rabi* crops (wheat, barley, fodders, etc.) are carried out in April–May, generating large amounts of chopped hay and bran which get scattered all over the surrounding areas. This material being dry and light is easily carried by wind to neighboring breeding sites where it soon starts decaying under the warm spring temperature, and apparently provides ample larval food resulting in high mosquito density, until the hot-dry conditions during premonsoon season check the population. The population remains low during the monsoon season apparently due to a flushing effect of heavy rainfalls. Again, harvesting and threshing of *kharif* crops (rice, sugar cane, maize, fodders, etc.) in November, presumably sets a stage for a 2nd rise in the population during winter. The phenomenon of density-dependent regulation described by Rajagopalan et al. (1976) with its intensity changing seasonally even in the same breeding sites, also seems to be a manifestation of seasonal variation in the amount of larval food. Thus, larval food (in addition to climatic conditions) seems to play an important role in the population dynamics of *Cx. quinquefasciatus.*

ACKNOWLEDGMENT

Thanks are due to William K. Reisen, University of California Berkeley School of Public Health for helpful suggestions in preparing the manuscript and Dr. Gerald G. Marten, East-West Center Environment and Policy Institute for reviewing the manuscript. Dr. D. Elmo Hardy, Department of Entomology, Uni-

versity of Hawaii, kindly read the manuscript.

References Cited

Armitage, P. 1971. Statistical methods in medical research. London. Blackwell. 504 pp.

Barbosa, P., T. M. Peters and N. C. Greenough. 1972. Overcrowding of mosquito populations: response of larval *Aedes aegypti* to stress. Environ. Entomol. 1:89–93.

Dadd, R. H. and J. E. Kleinjahn. 1974. Autophagostimulant from *Culex pipiens* larvae: distinction from other mosquito larval factors. Environ. Entomol. 3:21–28.

Gilpin, M. E. and R. P. Langford. 1978. Evidence for density dependent growth regulation among larval *Aedes sierrensis* mediated by food competition. Proc. Calif. Mosq. Vector Control Assoc. 46:42–45.

Hayes, J. and T. D. Downs. 1980. Seasonal changes in an isolated population of *Culex pipiens quinquefasciatus* (Diptera: Culicidae): a time series analysis. J. Med. Entomol. 17:63–69.

Haves, J. and B. P. Hsi. 1975. Interrelationships between selected meteorologic phenomena and the immature stages of *Culex pipiens quinquefasciatus* Say: a study of an isolated population. J. Med. Entomol. 12:299–308.

Hickey, W. A. 1970. Factors influencing the distribution of sex ratio in *Aedes aegypti*. J. Med. Entomol. 7:727–735.

Ikeshoji, T. and M. S. Mulla. 1970. Overcrowding factors of mosquito larvae. J. Econ. Entomol. 63:90–96.

Moore, C. G. and B. R. Fisher. 1969. Competition in mosquitoes. Density and species ratio effects on growth, mortality, fecundity and production of growth retardant. Ann. Entomol. Soc. Am. 62:1325–1331.

Moore, C. G. and D. M. Whitacre. 1972. Competition in mosquitoes. 2. Production of *Aedes aegypti* larval growth retardant at various densities and nutrition levels. Ann. Entomol. Soc. Am. 65:915–918.

Peters, T. M., B. I. Chevone, N. C. Greenough, R. A. Callahan and P. Barbosa. 1969. Intraspecific competition in *Aedes aegypti*(L.) larvae: 1. Equipment, techniques and methodology. Mosq. News 29:667–674.

Rajagopalan, P. K., M. Yasuno and P. K. B. Menon. 1976. Density effect on survival of immature stages of *Culex pipiens fatigans* in breeding sites in Delhi villages. Indian J. Med. Res. 64:689–708.

Rajagopalan, P. K., P. K. B. Menon, T. R. Mani and G. D. Brooks. 1975. Measurement of density of immatures of *Culex pipiens fatigans* in effluent drains. J. Commun. Dis. 7:327–337.

Reisen, W. K. 1975. Intraspecific competition in *Anopheles stephensi* Liston. Mosq. News 35:473–482.

Reisen, W. K. 1978. A quantitative mosquito survey of 7 villages in Punjab Province, Pakistan with notes on bionomics, sampling methodology and the effects of insecticides. Southeast Asian J. Trop. Med. Public Health 9:587–601.

Reisen, W. K., and R. W. Emory. 1977. Intraspecific competition in *Anopheles stephensi* (Diptera: Culicidae). II. The effects of more crowded densities and the addition of antibiotics. Can. Entomol. 109:1475–1480.

Siddiqui, T. F., Y. Aslam and W. K. Reisen. 1976. The effects of larval density on selected immature and adult attributes in *Culex tritaeniorhynchus*. Trop. Med. (Nagasaki). 18:195–200.

Suleman, M., and M. Shirin. 1981. Laboratory studies on oviposition behavior of *Culex quinquefasciatus* Say (Diptera: Culicidae): Choice of oviposition medium and oviposition cycle. Bull. Entomol. Res. 71:361–369.

Wielding, K. 1929. Die Beeinflussung Von Eirohrenzahl und grosse eiger Dipteran durch Hunger in Larvenstadium. Z. Angew. Entomol. 14:69–85.