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The Effects of Larval Density on Selected Immature and Adult Attributes in *Culex tritaeniorhynchus* Giles*

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ABSTRACT : When reared under crowded conditions, *Culex tritaeniorhynchus* larvae were found to take longer to develop and exhibited increased mortality. As expected, the amount of food needed to initiate and complete pupation increased with larval density; however, the efficiency of rearing (number of pupae per mg of food) was curvilinear with the optimum density at 1.0 larvae/ml under the present dietary regimes. The adult sex ratio at emergence did not vary with density. The wing length of the first 5 males and females emerging decreased with increasing density, while those of the last 5 males and females remained unaltered. The wings of the males were consistently shorter than those of the females. Individuals emerging first had longer wings than those taking longer to develop. These phenomena were attributed to the effects of intraspecific competition for food and space. Water conditioned by rearing larvae at crowded densities was found by bioassay to increase mortality without significantly altering the rate of development.

Recent studies of competition among mosquitoes have suggested that crowding elicits the production of larval autotoxins, growth retardant factors (G.R.F.) (Moore and Fisher, 1969), in at least three species of mosquitoes, *Aedes aegypti* (L.) (Moore and Fisher, 1969 ; Barbosa, *et al.*, 1972), *Anopheles stephensi* Liston (Reisen, 1975), and *Culex pipiens quinquefasciatus* (Say) (=*fatigans* Wied.) (Ikeshoji and Mulla, 1971). These autotoxins typically retard the duration of larval development, increase mortality, and result in the production of smaller sized adults. In addition to this interference competition, exploitative coactions for available food supplies may produce similar results nutritionally (Nayar, 1969), although Moore and Whitacre (1972) suggested that these effects may be inseparable. With the exception of Nayar and Sauerman (1970), most available information on larval crowding has dealt with competition among domestic, artificial container breeding species. The purpose of this present investigation was to describe the effects of larval competition in *Culex tritaeniorhynchus* Giles, a ground pool breeding species, emphasizing the effects of competition for food and space on rearing success and the induction of larval inhibitory compounds.

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METHODS AND MATERIALS

Egg rafts of the Lahore strain of *C. tritaeniorhynchus* were isolated in test tubes containing dilute straw-water infusion. After 24 hours, the number of eggs hatching per raft were counted, and the 1st instar larvae pooled so that 32, 158, 315, 945, 1890 and 2835 larvae were added to 21 × 15 cm (315 cm^2) enamel pans filled with 315 ml of well water yielding densities of 0.1, 0.5, 1.0, 3.0, 6.0 and 9.0 larvae/cm^2 of surface area or ml of rearing media (L/cm), respectively. In the present experiment, 0.1 L/ml was considered undercrowded, 0.5 and 1.0 L/ml near optimum as indicated by the range of densities most often observed in nature, 3.0 and 6.0 L/ml crowded, and 9.0 L/ml overcrowded using the criteria of Ikeshoji and Mulla (1970) for *C. p. quinquefasciatus*. Each density was replicated thrice and fed a ration of liver powder sifted through a 40 mesh screen which was calculated on the basis of surface area or volume as suggested by Peters *et al.* (1969) (Table 1). After the first pupae appeared, the number pupating were counted twice daily (0700 hours and 1700 hours) into plastic jars where the adults emerged after 24 hours. The wings of the first and last 5 males and females emerging per density were measured from the proximal insertion at the mesothorax to the apex of the anterior distal margin excluding the fringe scales using an ocular micrometer.

After all larvae had pupated, the rearing water at each density was pooled, and then bioassayed for G.R.F. activity. Three replicates of 25 1st instar larvae per density were placed with 50 ml of the rearing water or well water into a 250 ml flask yielding the optimum density of 0.5 L/ml. Larval development time and the percent rearing success from 1st instar through adult were used as a criteria of G.R.F. activity (Reisen, 1975). The rearing water was then evaluated for turbidity, suspended solids, ammonia concentration and pH (Hach, 1973).

Mean (± standard error of the mean) insectary conditions were: temperature = 28.9 ± 0.2°C, relative humidity = 57.7 ± 0.3%, and photoperiod = 15:9 LL:DD with an 80 minute simulated crepuscular period.

Median pupation (P_{50}) and emergence (E_{50}) times were estimated from eclosion using the graphic techniques of Litchfield (1949) as applied by Moore and Fisher (1969). Among group comparisons were made using appropriate least squares analyses as described in Sokal and Rohlf (1969).

Table 1. Daily larval ration in mg/larvae of finely sifted liver-powder

Day	Ration mg/cm^2 or ml	Density (L/cm^2)					
		0.1	0.5	1.0	3.0	6.0	9.0
1	0.1	1.00	0.20	0.10	0.03	0.02	0.01
2	0.2	1.97	0.40	0.20	0.07	0.03	0.02
3	0.3	2.95	0.60	0.30	0.10	0.05	0.03
4	0.3	2.95	0.60	0.30	0.10	0.05	0.03
5	0.4	3.94	0.80	0.40	0.13	0.07	0.04
6 to end	0.5	4.92	1.00	0.50	0.17	0.08	0.06

RESULTS AND DISCUSSION

The rate of larval development, as indexed by the P_{50} values, was significantly retarded and larval mortality significantly increased as rearing densities, and presumably intraspecific competition, increased (Table 2). Most mortality seemed to occur among the early instar larvae which has been observed for other mosquito species (Ikeshoji and Mulla, 1970; Reisen, 1975). As expected, crowded larvae required more food to complete pupation; however, the efficiency of food utilization was not significantly linear with fewer pupae produced per mg of food at the overcrowded density (Table 2). Under the present food regimes, 1.0 L/ml was the most efficient rearing density producing the largest number of pupae per mg of food. Thus the rate as well as the total amount of food added was important in initiating pupation.

The E_{50} values for males and females exhibited a significant positive linear regresson with increasing rearing density (Table 2). There was no significant relationship ($P>0.05$) between the number of pupae successfully emerging and larval density as emergence success remained near 88% with the exception of the 0.1 L/ml group (Table 2). The overall E_{50} value for males was significantly ($P<0.05$) less than that of females agreeing with the frequently observed earlier emergence of male mosquitoes (Nayar, 1969; Reisen, 1975). The sex ratio (males/total) remained consistently greater than 0.5 and did not vary significantly as a function of density ($P<0.05$). The sex ratio of 0.487 observed in the 0.1 L/ml group was the result of a 0.333 value in one of the three replicates; the remaining ratios exceeded 0.5. This discrepancy was attributed to a chance selection of more females when the larvae were initially being counted into the pans. These results were in good agreement with Jones (1960) and Hickey (1970) who found no density-related sex ratio distortion, but disagreed with Reisen (1975) who found less males, and Weilding (1929) who found more males produced under crowded densities.

The wing lengths of the first 5 males and females emerging exhibitd a negative linear relationship with increasing rearing density, and the wing lengths of the males were significantly shorter ($P<0.05$) than those of the females (Table 2). Similar results have been obtained for other mosquitoes (Nayar and Sauerman, 1970). The last 5 males and females emerging were only successfully separated for those groups having E_{50} values greater than 250 hours. At lower densities more synchronous emergence prevented the separation of the last group. The wing lengths of these last emerging adults did not vary with increasing density, although the females remained larger than the males (Table 2). In the analysis of variance, mosquitoes emerging first were found to have significantly ($P<0.05$) larger wings than those taking longer to mature (Table 2) disagreeing with the findings of Barbosa *et al.* (1972) who found that *Ae. aegypti* pupating during the first quartile were consistently smaller than those pupating during the last quartile. Barbosa *et al.* (1972) attributed this discrepancy to the longer feeding period of the slower maturing larvae which agreed with the frequently observed larger size of adults reared at lower water temperatures (Clements, 1960). Apparently, in *C. tritaeniorhynchus* those genotypes which were able

Table 2. Some effects of increasing larval rearing density on selected immature and adult characteristics; mean ± standard error of mean presented

Characteristics	Larval Density (Larval/ml or cm^2) 0.1	0.5	1.0	3.0	6.0	9.0	Linear[1] Regression
P_{50} (hours)	149.0±3.8	156.7± 6.0	167.0± 9.6	228.3± 4.4	232.0± 8.5	250.0± 5.0	+L(0.01)
Food till lst pupation (mg/pan)	362.3±0.0	362.3± 0.0	362.3± 0.0	383.3±21.0	488.3± 0.0	530.3±42.0	+L(0.05)
Food till last pupation (mg/pan)	631.6±8.7	724.3±42.0	766.2±72.7	1354.0±42.0	1270.0±72.8	1438.0±84.0	+L(0.05)
Efficiency (pupae/mg food)	0.042±0.001	0.159±0.019	0.177±0.085	0.167±0.066	0.120±0.011	0.096±0.005	NSL(0.05)
Percent pupating	82.33±1.03	68.17±8.43	47.0 ±25.06	37.27±2.99	8.43 ±0.88	4.87 ±0.33	−L(0.001)
E_{50} (hours)							
Males	183.3±3.31	191.67±6.00	191.67±7.30	256.67±6.67	260.0±5.80	280.0±5.80	+L(0.005)
Females	188.3±6.00	198.30±4.40	201.67±11.70	266.67±3.33	263.33±6.70	283.33±3.33	+L(0.005)
Percent emergence	97.43±2.57	88.17±9.49	87.40±3.71	88.50±1.10	89.37±2.07	87.33±0.38	NSL(0.05)
Sex ratio (male/total)	0.487±0.079	0.528±0.060	0.535±0.064	0.557±0.022	0.541±0.034	0.517±0.019	NSL(0.05)
Adult wing length (mm)							
First males	2.54±0.03	2.71 ±0.06	2.58 ±0.05	2.40 ±0.04	2.41 ±0.02	2.35 ±0.03	−L(0.05)
First females	2.81±0.13	2.87 ±0.03	2.92 ±0.02	2.92 ±0.02	2.63 ±0.04	2.57 ±0.05	−L(0.05)
Last males	—	—	—	2.33 ±0.02	2.35 ±0.04	2.26 ±0.03	NSL(0.05)
Last females	—	—	—	2.42 ±0.01	2.46 ±0.02	2.35 ±0.05	NSL(0.05)

[1] NSL = no significant linear regression ($P>0.05$); L = significant linear regression ($P<\alpha$) and (+) or (−) indicates the slope, value in parenthesis is the level of significance (α).

Table 3. Bioassay of conditioned rearing water for possible G. R. F. activity. Mean of 3-replicates presented

Parameters	Well water (control)	Larval Density (L/ml) at which water was conditioned						Linear[1] Regression
		0.1	0.5	1.0	3.0	6.0	9.0	
E_{50}								
Males	192.5±3.59	190.0±2.89	188.33±1.67	182.67±3.71	170.00±5.00	181.67±1.67	213.33±13.33	NSL(0.05)
Females	200.0±0.00	193.33±6.67	200.0±0.00	188.33±1.67	182.50±2.50	186.67±3.33	206.67±12.02	NSL(0.05)
Pupation success (%)[2]	76.00±6.11[a]	73.33±3.53[a]	48.00±4.00	72.0 ±6.11[a]	12.0±6.93	37.33±2.67	16.0 ± 6.11	NSL(0.05)
Chemical properties of rearing water								
Turbidity (FTU)	5	400	700	350	800	750	700	NSL(0.05)
Suspended solids (mg/1)	0	140	300	100	300	350	260	NSL(0.05)
Conductivity (mohms/cm)	400	1200	1350	1150	1400	1600	1650	NSL(0.05)
Ammonia nitrogen (mg/1)	0.7	7.0	5.5	6.5	9.0	8.0	14.0	+L (0.01)
pH	7.3	7.7	7.9	7.6	7.9	8.6	8.0	+L (0.05)

1) designations follow Table 2.

2) groups followed by the same letter were not significantly different using an a-posteriori, least significant range test (P>0.05) on arc sine transformed means (Sokal and Rohlf, 1969).

to successfully compete for available foodstuffs grew to a larger size and emerged before the poorer competitors whose growth and development were retarded.

In the bioassay of the conditioned rearing water, the rate of development was not significantly inhibited by larval competitive compounds, and there was no significant difference ($P>0:05$) between larval development in conditioned rearing water and unconditioned well water (Table 3). The percentage of adults successfully emerging did not decrease linearly with increasing density, although there was significant among density statistical differences (Table 3). The significant increase ($P<0.05$) in mortality among the crowded and overcrowded groups was attributed to the presence of larval autotoxins using the assessment criteria of Ikeshoji and Mulla (1974). The activity of larval competitive compounds appears to vary among species as compounds elicited by *Ae. aegypti* (Moore and Fisher, 1969) and *An. stephensi* (Reisen, 1975) were found to retard development as well as increase mortality.

The conditioned rearing water had significantly higher ($P<0.05$) turbidity, suspended solids, ammonia and the pH than the unconditioned well water, although only ammonia and the pH increased linearly with rearing density (Table 3). Increased turbidity, suspended solids and conductivity were attributed in part, to increase bacteria feeding upon larval by-products and/or unconsumed foodstuffs. The fact that these parameters did not vary with rearing density may have been due to the action saprophytes utilizing excess food at the lower densities and increased larval by-products and dead larvae at the higher densities. The linear increase in ammonia concentration with larval density was considered proportional to the total number of larvae present, as mosquito larvae excrete free ammonia as do other aquatic insect larvae (Chapman, 1971). The increase in pH with density was attributed to the reaction of the free ammonia with water releasing 1 hydroxyl radical for each excreted ammoniaion.

In Lahore, *C. tritaeniorhynchus* larvae are the predominant mosquito species collected in ground pools during summer. Dessication and/or oviposition often results in larval densities simiar to those tested during the present experiment. That crowding has similar effects was suggested by the heterogeneous wing lengths of adults within the same collection. This variability was observed to increase during summer when population densities, and presumably the incidence of competition, were highest.

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LITERATURE CITED

1) Barbosa, P., Peters, T. M. and Greenough, N. C. (1972): Overcrowding of mosquito populations: Responses of larval *Aedes aegypti* to stress. Env. Ent., 1, 89-93.

2) Chapman, R. F. (1971): The Insects, Structure and Function. American Elsevier Pub.Co., Inc., New York, 819 pp.

3) Clements, A. N. (1963): The Physiology of Mosquitoes. Pergamon Press, London, 393 pp.

4) Hach (1973): DR-EL/2 Method Manual, Hach Chemical Co., Ames, Iowa.

5) Hickey, W. A. (1970): Factors influencing the distortion of sex ratio in *Aedes aegypti*. J. Med. Ent., 7, 727-735.

6) Ikeshoji, T. and Mulla, M. S. (1970): Overcrowding factors of mosquito larvae. J. Econ. Ent., 63, 90-96.

7) Ikeshoji, T. and Mulla, M. S. (1974): Overcrowding factors of mosquito larvae. 4. Activity of branched fatty acids against mosquito larvae. Env. Ent., 3, 487-491.

8) Jones, W. L. (1960): The effects of crowding on the larvae of *Aedes aegypti* (L.) when reared under aseptic and non-septic conditions. Ph. D. Diss., Ohio State Univ., Columbus, 72 pp.

9) Litchfield, J. T., Jr. (1949): A method for rapid graphic solution of time-percent effect curves. J. Pharmacol. Exp. Ther., 97, 399-408.

10) Moore, C. G. and Fisher, B. R. (1969): Competition in mosquitoes. Density and species ratio effects on growth, mortality, fecundity and the production of a growth retardant. Ann. Ent. Soc. Amer., 62, 1325-1331.

11) Moore, C. G. and Whitacre, D. M. (1972): Competition in mosquitoes. 2. Production of *Aedes aegypti* growth retardant at various densities and nutritional levels. *Ibid.*, 65, 915-918.

12) Nayar, J. K. (1969): Effects of larval and pupal environmental factors on biological status of adults at emergence in *Aedes taeniorhynchus* (Wied.). Bull. ent. Res., 58, 811-827.

13) Nayar, J. K. and Sauerman, D. M. (1970): A comparative study of growtn and development in Florida mosquitoes. 2. Effects of larval nurture on adult characteristics at emergence. J. Med. Ent., 7, 235-241.

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

15) Reisen, W. K. (1975): Intraspecific competition in *Anopheles stephensi* Liston. *Ibid.*, 35, 473-482.

16) Sokal, R. R. and Rohlf, F. J. (1969): Biometry. W. H. Freeman and Co., San Francisco, 776 pp.

17) Weilding, K. (1929): Die beeinflussung von eiröhrenzahl und grösse einiger Dipteran durch hunger im Larvenstadium. Z. angew. Ent., 14, 69-85.

コガタアカイエカの未成熟期及び成虫の属性に及ぼす幼虫密度の影響

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コガタアカイエカ幼虫を過密度の状態で飼育すると，幼虫の期間は長くなり死亡率は高くなった． 期待されたように，蛹化に必要な餌の量は幼虫密度にともなって増加した．しかし，餌 1mg 当りの蛹の数で表わした効率は，水 1ml 当りの幼虫数 1.0 の時に最高となった．成虫の性比は幼虫密度によって変化しなかった．最初に羽化した5個体の雄と雌の翅長は，高密度区で減少したが， 最後に羽化した5個体の雄と雌では，密度による変化は見られなかった．始めに羽化した個体は， おくれて羽化した個体よりも長い翅を持っていた． これらの現象は，餌と空間に対する種内競争の影響に帰することができた．過密度で幼虫を飼育した水は幼虫死亡率を増加させる作用のあることがわかった．

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