

## Comunicación breve

# Effect of food competition on *Culex pipiens quinquefasciatus* under laboratory conditions

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**Abstract.** *Food competition in the larval phase of *Culex pipiens quinquefasciatus* and its effect on the adult stage was experimentally studied under laboratory conditions. Cohorts with different densities of initial first instar larvae (from 50 to 4000 individuals per g of food) were followed until adult emergence, including egg laying. The number of individuals in the cohorts were counted every two days, until all adults emerged. Adult females were fed every four days. Surviving adults and eggs laid were counted daily. The proportion of adult females, adult weight, and female fertility decreased as larval density increased. Adult longevity and female fecundity did not show a density dependent change. The generation time increased and the realized rate of natural increase and net rate of increase showed a negative relationship with larval density.*

**Resumen.** *Se estudió experimentalmente el efecto de la competencia por alimento en la fase larval de *Culex pipiens quinquefasciatus* y su efecto sobre el estado adulto, bajo condiciones de laboratorio. Cohortes con diferente densidad inicial de larvas de primer estadio (desde 50 a 4000 individuos por gramo de comida) fueron estudiadas hasta la emergencia de adultos, incluyendo la postura de huevos. El número de individuos en las cohortes fue contado cada dos días, hasta que todos los adultos emergieron. Las hembras adultas fueron alimentadas cada cuatro días. Los adultos sobrevivientes y los huevos puestos fueron contados diariamente. La proporción de hembras adultas, el peso de adultos y la fertilidad de hembras disminuyeron a medida que la densidad larval aumentó. La longevidad de adultos y la fecundidad de hembras no mostró un cambio densodependiente. El tiempo generacional se incrementó y la tasa intrínseca de crecimiento natural y la tasa neta de incremento mostraron una relación negativa con la densidad larval.*

## Introduction

Mosquito biology offers an interesting system to study intraspecific competition for food because preadult stage occurs in a different niche from the adult phase. Clearly, no food competition among adults and preadults exists. In *Aedes sierrensis*, pupal size is more sensitive to larval density than larval mortality and larval development rate, although larval density does not affect pupation success (Hawley 1985). For *Wyeomia smithi*, Moeur (1980) reported different development time for 4th instar larvae and fertility at different larval density, but no effect on 3rd instar development rate. Nekrasova (1986) showed a density-dependent effect on body size, development time and wing size of *Ae. dorsalis*. Southwood et al. (1972), studying *Ae. aegypti*, concluded that the most important mortality occurred in 1st, 2nd and 4th instar larvae. They concluded that mortality in the age class (1st and 2nd) instar larvae is probably density dependent and that density regulation depended on density dependent mortality rather than fecundity changes. Fecundity is the total number of eggs laid by a female during

her lifetime, whereas fertility is the number of viable eggs (producing offsprings) during the same period (Southwood 1978).

Among adult females there would be food competition for blood because of host irritability (Murray 1987, Schofield 1982, Waage and Nondo 1982). Among larvae, food competition occurs in some species and density dependent variation in mosquito body size is widely documented. Therefore, direct effects on larvae because of food competition among larvae, and direct effects on adults because of food competition among adults could occur. There would also be an effect on adults because of food competition during the larval stage. As Hawley (1985) pointed out, the study of population density equilibrium and the degree to which preadult mortality is density dependent is very important to determine the epidemiological significance of density dependent adult survivorship in species involved in pathogen transmission.

In the present work, we studied food competition in the larval stage of *Culex pipiens quinquefasciatus* and its effect on the adult stage, under laboratory conditions. Our objective was to study the influence of larval density on a) larval age specific mortality, b) larval development rate, c) reproduction, d) population parameters, and e) adult sex ratio.

## Materials and Methods

### *Experimental procedure*

Data were obtained from cohorts beginning with the same number of first instar larvae, fed with powdered liver. Cohorts of 100 *Cx. p. fatigans* larvae were well developed with a food supply of 1000 mg (Gómez et al., 1977). Based on this experiment, the following quantity of food per cohort was used: 2000, 1000, 750, 500, 250, 100, 75, 50 and 25 mg. All cohorts began with 100 1st instar larvae, so the equivalent number of individuals per g of food (i/g) in each cohort was: 50, 100, 133, 200, 400, 1000, 1333, 2000 and 4000. Each treatment was replicated twice, except for 400, 1000, and 4000 i/g.

The food was weighted with an electric balance (accurate to 0.1 mg) and diluted in 5 ml of distilled water. These solutions were stored in a refrigerator, and 0.5 ml of each solution was provided daily to each replicate during the first 10 days of the experiment. The partition of the total quantity of food avoided the proliferation of algae and fungi in the cultures (Almirón, pers. com.). Larvae were developed in water containers of 30x 15x5 cm (length, width, depth) and 400 cm<sup>3</sup> of distilled water in controlled temperature chamber at 26 ± 2 °C.

The number of larvae in the different age classes was recorded daily. First and second instar larvae (L(1+2)) were considered together as one age class due to difficult discrimination. When pupae appeared, they were separated from the larvae culture in plastic vials until adult emergence. The emerged adults were transferred to special boxes, to allow for adult feeding, mating, and oviposition. Adult females were offered a blood meal every four days by introducing an immobilized quail within a nylon net-bag. The quail remained in the box from 1900 hr until 0800 hr next day. Adult males were fed with a sugar solution in a piece of cotton replaced every 48 hrs. Plastic cups with water were offered to females for oviposition. The number of egg masses, eggs per egg mass, and surviving adults were counted daily until death of the last female. After that, adults were dried at 50 °C to estimate their dry weight.

Age specific mortality and fecundity were used to calculate horizontal life tables, and to estimate the following population parameters: generation time (T), net reproductive rate (R<sub>0</sub>) and realized rate of natural increase (r). The estimate growth rate (r) is the realized or specific growth rate, and should be distinguished from r<sub>m</sub>, the maximum intrinsic rate of natural increase occurring in an unlimited environment, without density dependent effects (Southwood 1978).

**Table 1.** Age specific mortality, preadult development time (T) and percentage of adult emergence from larvae developed at different densities per unit of food. L(1 +2): first and second instar larvae; L3: third instar larvae; L4: fourth instar larvae. The values are averages of two replicates, standard deviation in brackets, except for treatments with 400, 1000 and 4000 i/g where one replicate was studied.

i/g	Mortality %					
	L1+2	L3	L4	Pupae	T (days)	Adult emergence
50	2.5 (0.71)	5.6 (6.58)	4.5 (3.39)	2.7 (2.12)	10.8 (0.14)	85.5 (7.78)
100	11.5 (6.36)	5.6 (0.35)	20.8 (11.74)	15.3 (7.78)	16.2 (1.34)	56.0 (7.07)
133	10.0 (0.00)	10.5 (0.78)	24.8 (8.63)	10.8 (2.33)	19.5 (0.42)	54.0 (7.07)
200	5.5 (4.95)	2.7 (2.40)	53.4 (14.85)	31.0 (12.73)	17.1 (2.83)	30.0 (12.73)
300	5.0 (1.41)	4.2 (2.90)	56.3 (12.52)	23.2 (2.16)	23.9 (0.21)	30.1 (9.19)
400	14.0	30.2	88.3	85.7	22.0	1.0
1000	37.0	52.4	96.7	100.0		0
1333	26.5	77.7	97.0	100.0	--	0
2000	43.5	65.6	100.0	--	--	0
4000	55.0	80.0	100.0	--	--	0

**Table 2.** Regression of development time (T, in days) on initial larval density (D) for each age class of *Cx. p. quinquefasciatus*.  $\rho$ : linear correlation coefficient; Sb: slope standard deviation; n: sample size.

Age class	$H_0: \rho=0$	Function	Sb	n
L (1 +2)	0.82 ( $p < 0.01$ )	$T = 3.673 + 0.00128 * D$	$2.29 * 10^{-4}$	17
L3	0.86 ( $p < 0.01$ )	$T = 2.519 + 0.00127 * D$	$1.93 * 10^{-4}$	17
L4	0.92 ( $p < 0.01$ )	$T = 1 / (0.00492 + 10.81 * D)$	1.51	17
Pupae	0.34 ( $p > 0.05$ )	$T = 3.359 + 94.2 / D$	82.90	12
L(1 +2) to Pupae	0.94 ( $p < 0.01$ )	$T = 1 / 0.03553 + 2.795 * D$	0.308	12

## Results

### Larval mortality

Mortality of L(1 +2) increased with density, from 2-3 % (for 50 i/g) to 55 % (for 2000 i/g); mortality of L3 increased from 5.6 % (for 50 i/g) to 80 % (for 4000 i/g) and mortality of L4 increased from 4.5 % for 50 i/g to 100 % for 2000 i/g (Table 1).

Development time for each instar larvae showed a high positive correlation with density (see Table 2). The number of emerged adults decreased with density. The proportion of emerged adults (relative to the initial number of L1) changed from 85% for 50 i/g to 0% for densities higher than 1000 i/g (Table 1).

### Population parameters

The realized rate of natural increase (r) showed a negative exponential decrease and high correlation with density (Table 3). The net rate of increase (Ro) (from the treatments where adults

**Table 3.** Realized rate of natural increase ( $r$ ), Net Replacement Rate ( $R_0$ ), and Generation Time ( $T$ ) of *Cx. p. quinquefasciatus* developed at different larval densities. Treatments with more than 300 i/g resulted in 100% preadult mortality (See Table 1).

Initial density	Replicate number	$r$	$T$	$R_0$
50	1	0.1121	46.26	61.43
100	1	0.0962	45.25	52.29
100	2	0.1088	43.12	61.05
133	1	0.0614	44.22	12.34
133	2	0.0600	43.38	11.45
200	1	0.0301	55.10	4.69
200	2	0.0536	49.38	10.34
300	1	0.0434	48.75	8.04
300	2	0.0307	47.25	8.22

**Table 4.** Female fecundity, adult sex ratio and adult dry weight, from larvae developed under different densities.

Initial density (i/g)	No. of eggs per fertile egg mass	No. of fertile eggs per female	Prop. of females	Prop. of fertile egg mass	Total fertility (%)	Female dry weight (mg)	Male dry weight (mg)
50	177.12	141.30	0.54	1.00	92.0	2.85	2.60
100	172.12	215.10	0.51	0.71	70.2	3.22	1.58
133	149.20	67.15	0.45	0.60	54.6	2.54	1.35
200	120.50	60.25	0.44	0.55	49.0	2.13	1.49
300	112.16	106.55	0.19	0.76	68.6	1.57	1.23

emerged) was strongly affected by density, showing a step-function-shape decrease, with the inflection point at 100 i/g (Table 3). Generation time ( $T$ ) showed a significant positive correlation with density.

#### Adult performance

The number of egg masses produced by females from different treatments did not change with density (Anova,  $P > 0.5$ ), but the proportion of fertile egg masses decreased with density ( $R^2 = 97.15\%$ ,  $p = 0.014$ ). Total fertility (hatched eggs / laid eggs) and the mean number of eggs per fertile egg mass were negatively correlated with density during larval development ( $R^2 = 96.92\%$ ,  $p = 0.015$  and  $R^2 = 88.17\%$ ,  $p = 0.018$  respectively). The general trend of the mean number of fertile eggs produced per female showed a negative relationship with larval density, although the value for 306 larvae per replicate showed a high bias from the expected value estimated by a regression line (Table 4). Adult sex ratio was also affected by density during larval development. From a 1:1 sex ratio in the cohorts with 50 or 100 individuals to 19 % of females in the cohorts with 300 individuals.

Adult female weights were higher than adult male weights (t-test,  $p = 0.0346$ ) and showed a linear negative correlation with larval density ( $r = -0.93$ ,  $p = 0.024$ ). Adult longevity was not affected by larval density in the cohorts (Anova,  $P > 0.05$ ). Female mean longevity (38 days) was longer than male mean longevity (14.2 days) (t-test,  $P < 0.01$ ).

## Discussion

The results showed that competition for food in the larval stage of *Cx. p. quinquefasciatus* occurs. Density dependent food competition occurred at 3rd and 4th instar larvae and affected the development and mortality rate of all age classes in the larval phase.

Food competition during the larval phase negatively affected adult weight, although females were always heavier than males. Apparently, as a female larvae needs more food than a male larvae to reach adulthood and competition is scramble, they suffer stronger mortality, leading to a decrease in the proportion of emerged females when food is scarce.

A density dependent effect on reproduction was shown by changes in fertility, although total fecundity did not change. This could be a clear indication that reserve accumulation during the preadult phase is important in *Cx. p. quinquefasciatus*. This effect has been reported in several other species, as well as its relevance for population dynamics (Prout and Mc Chesney 1985)

Food competition affected population parameters. There was an exponential negative decrease in the realized rate of natural increase ( $r$ ), a positive correlation with generation time ( $T$ ), and a step-function-shape decrease in the net rate of increase ( $R_0$ ). Analysis of the importance of the contribution of each age to  $r$  showed that, although female reproductive period ranged from 30 to 85 days, the value of  $r$  was mainly determined (95 %) by oviposition between 30 and 41 days of age (the first 11 days of reproductive period). Although only 43% (treatment 100 larvae per g) to 72% (treatment 300 larvae per g) of all eggs were laid during these 11 days, the remaining 57 to 28 % of the eggs contributed only 5 % to the value of  $r$ . All density treatments showed the same pattern.

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