A Laboratory Experiment on the Larval Development of *Aedes polynesiensis* under Different Rearing Conditions

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Abstract: Larvae of *Aedes polynesiensis* were reared from hatching to emergence in a laboratory under different conditions of the density, the amount of food and the space. Larval developmental period and mortality were clearly affected by the amount of food per larva. Larval aggregation effect was observed as shortening of larval period and decreasing mortality under the constant amount of food per larva.

Key words: Larval development, *Aedes polynesiensis*, Amount of food

INTRODUCTION

Larval develpment of aedine mosquitoes, which are small container breeders, is affected by various environmental conditions. Many works had been carried out to evaluate the effect of various factors on the development until around 1980 focusing to some medically important species. Especially in *Aedes aegypti*, after Surtees (1959) showed the influence of larval density on fluctuation of numbers in natural populations, Malcovitch (1960), Keirans and Fay (1968), and Peters *et al.* (1969) lightened the effect of food condition on the larval development. Wada (1965) made clear the density effect on the larval development through mutual interaction and resulting adult size. Barbosa *et al.* (1972) were ratheer interested in the biological contamination.

Aedes polynesiensis, which is very common and widely distributed in South Pacific regions, is also one of the medically important species, because this mosquito transmits subperiodic *Wuchereria bancrofti* in the regions. Ingram (1954) studied the bionomics of the species under laboratory conditions, and reported developmental delay in rearing containers with high density. Lowrie Jr. (1973a, b) stated that the species was always defeated by *Aedes albopictus* when these two species were reared together in a competition experiment, and suggested more stress in *Ae. polynesiensis* caused by mutual interaction. However, unlike the case of *Aedes aegypti*, the experiment with *Ae. polynesiensis* to highlighten factors affecting the development of immature population has not been carried out so far. We conducted a preliminary labaratory rearing experiment of immatures under different density, food and space conditions to clarify such factors.

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MATERIALS AND METHODS

The first instar larvae of the F2 generation of a natural population collected from Nukui village near Suva, Fiji were set up within 16 hours after hatching. The larvae were reared until adult emergence under certain combinations of rearing conditions as shown in Table 1. Temperature and photoperiod were not controlled but those were rather constant as they were 20−24℃ and 14 hours during the experiment. Although the initial feeding policy was that all larvae were fed the designed amount of fish meal only once when they were set up, it was found in the course of rearing that the amounts were too small to complete the normal larval development in many series. Therefore extra fish meal was added on the 13th or 14th day after hatching. Three types of experimental containers were used to examine the effect of the volume of water as the space for immatures. The smallest one was clear glass cylindrical tubes of 2.5 cm in diameter and 5.0 cm high. Vials of this type were used for a series of 15 cc water volume. As containers for 90 cc and 135 cc series, round plastic cups with 6.2 cm in diameter and 5.0 cm high were used. For 1,350 cc and 2,250 cc series, rectangular plastic jars of 20.0×27.0 cm and 5.0 cm high were prepared. Depth was kept constant. Tapped water was used, and the water in containers was not replaced throughout the experiment, but some water was added to supplement the loss by evaporation though all containers were covered with clear plastic boards.

Table 1. Rearing conditions

Code	Density [L]	Amount of food (mg) [F]	Volume of water (cc) [W]	Food per larvae (mg) [F/L]	Space per larvae (cc) [W/L]	Food per lcc (mg) [F/W]	Replication	Excess amount of food (mg)
L1F1W15	1	1	15	1.00	15.0	0.067	10	1
L1F1W90	1	1	90	1.00	90.0	0.011	10	1
L2F1W15	2	1	15	0.50	7.5	0.067	10	0
L2F2W90	2	2	90	1.00	45.0	0.022	10	2
L5F2W90	5	2	90	0.40	18.0	0.022	5	2
L20F1W90	20	1	90	0.05	4.5	0.011	3	1
L20F3W90	20	3	90	0.15	4.5	0.033	3	3
L20F6W90	20	6	90	0.30	4.5	0.067	3	6
L20F20W135	20	20	135	1.00	6.8	0.148	2	0
L30F6W90	30	6	90	0.20	3.0	0.067	2	6
L30F30W135	30	30	135	1.00	4.5	0.222	2	0
L30F30W1350	30	30	1,350	1.00	45.0	0.022	1	0
L50F1W90	50	1	90	0.02	1.8	0.011	2	7
L50F3W90	50	3	90	0.06	1.8	0.033	2	3
L50F3W135	50	3	135	0.06	2.7	0.022	2	3
L50F5W90	50	5	90	0.10	1.8	0.056	2	5
L50F50W1350	50	50	1,350	1.00	27.0	0.037	2	0
L50F50W2250	50	50	2,250	1.00	45.0	0.022	1	0

Number and age of survivors were examined every day. Molted skins and dead immatures were also counted and the instar was identified as far as possible. The period of each instar in days was determined by difference in timing when the cumulative number of immatures exceeded 50% of a total in two successive instars.

Results and Discussion

No adult emerged from L20F1W90, and only one adult did from L50F1W90 on the fourteenth day after hatching and on the tenth day from L50F3W90 when the majority were still third instar. Therefore, following the method of determining the period of each instar described above, the realistic periods of fourth instar and pupal stage could not calculate.

The larval period became longer and the difference of it was enlarged with progress of development until the third instar. Results were summarized in Table 2. In the first instar, the average periods were around 2 days ranging between 1.7 and 2.5 days. These were around 2.5−3.0 days with range from 1.6 to 6.4 days in the second instar, and around 3.5−4.0 days with range from 2.7 to 10.5 days in the third instar. In the fourth instar, however, both of the period and the difference among varieties shortened because of extra feeding except for L2F1W15, L20F20W135, L30F30W135, L30F30W1350, L50F50W1350 and L50F50W2250. Prominent acceleration in larval development by this extra feeding strongly suggests that the food condition is a decisive factor of larval development.

The effect of the amount of food per larva (F/L) on the overall developmental period was examined. The shortest period, 10.6 days, was observed in L30F30W1350, of which F/L was 1.00 mg. Nearly the same period (10.9 days) was recorded in L20F20W135, L30F30W135 and L50F50W2250, in all of which F/L was also 1.00 mg. In almost all other experimental populations of short immature periods as L1F1W15, L1F1W90, L2F1W15 and L50F50W1350, of which periods were 12.7, 13.5, 11.8, and 11.2 days respectively, larvae were also provided a good amount of foods (1.00 or 0.50 mg per larva). In the contrast with these, in a population of the most delayed larval development (L50F3W135) F/L was 0.06 mg and larvae took 18.9 days to complete the development. It was evident, therefore, that if food per individual (F/L) was decreased, the immature period rationally delayed even if larvae could successfully survive till adults. The relationship between the amount of food per larva (F/L) and the developmental period of immature stages in days was illustrated in Fig. 1. A good negative linear relationship ($r^2=0.812$) was confirmed.

As shown in Table 2 on the range between the first and the last days of immigrants in each stage counted from hatching, irrespective of rearing conditions the timing of immigration to the next immature stage was not so different in the early immigrants. Greater delay rather appeared among the late immigrants. This fact suggests that in poor food condition, this limited food was perhaps consumed solely by stronger or well developed larvae, and these larvae could immigrate into the next stage as quick as those under rich food condition such as L30F30W1350. While many losers in the competition for food, in turn of the early group, suffered the delay of development. The less food resulted in the more losers and the

Table 2. Period of each instar in days, the first and the last day from the hatching of larvae and pupae presence in each instar, and the mortality of immature stage

Code	Period of each instar in days							The first & the last days from hatching					Mortality (%)
	1st.	2nd.	3rd.	4th.	Pupae	Total	(Confidence limit in 90%)	2nd.	3rd.	4th.	Pupae	Adults	
L1F1W15	2.0	2.5	3.5	2.7	2.0	12.7	(0.427)	2−3	4−6	7−12	9−12	11−13	20.0
L1F1W90	2.5	2.9	3.4	2.7	2.0	13.5	(2.463)	2−4	4−13	7−17	9−18	11−21	40.0
L2F1W15	2.0	2.5	3.6	2.6	1.1	11.8	(0.924)	2−3	4−6	4−22	7−13	9−14	60.0
L2F2W90	2.0	2.5	4.3	4.5	1.3	14.6	(1.821)	2−3	4−6	6−17	7−20	9−21	30.0
L5F2W90	2.0	2.7	5.8	4.6	2.8	17.9	(3.107)	2−3	3−15	4−26	7−23	8−24	45.0
L20F1W90	2.5	5.1	10.5	—	—	18.1	—	2−4	4−17	11−27	—	—	100.0
L20F3W90	2.0	3.9	6.1	3.4	2.4	17.8	(1.464)	2−3	3−12	4−25	7−17	16−18	75.0
L20F6W90	2.0	2.4	3.8	3.8	2.1	14.1	(1.025)	2−3	3−6	4−23	8−18	10−19	30.0
L20F20W135	1.9	1.9	2.6	2.6	1.9	10.9	(0.693)	1−3	3−6	4−15	6−15	8−16	5.0
L30F6W90	2.0	4.2	3.3	4.0	2.1	15.6	(1.084)	2−3	3−17	4−23	6−19	8−22	60.0
L30F30W135	1.9	2.0	2.5	2.6	1.9	10.9	(0.713)	1−3	3−6	4−15	6−15	7−16	11.7
L30F30W1350	1.9	1.6	2.7	2.4	2.0	10.6	(0.792)	1−3	3−6	4−20	6−22	8−24	0.0
L50F1W90	2.5	6.4	7.3	—	—	16.2	—	1−7	3−22	5−27	12—	14—	99.0
L50F3W90	1.9	5.9	7.1	—	—	14.9	—	1−3	2−22	4−25	8—	10—	99.0
L50F3W135	2.4	4.8	6.0	4.2	1.5	18.9	(0.740)	2−7	3−18	5−24	12−18	15−19	92.0
L50F5W90	1.8	3.4	5.8	2.0	1.8	14.8	(0.704)	1−7	3−11	4−22	10−17	12−18	84.0
L50F50W1350	1.9	1.9	2.7	2.7	2.0	11.2	(0.877)	1−3	3−6	4−12	6−12	8−14	9.0
L50F50W2250	1.7	1.9	3.0	2.4	1.9	10.9	(1.584)	1−4	3−6	4−17	6−19	8−23	4.0

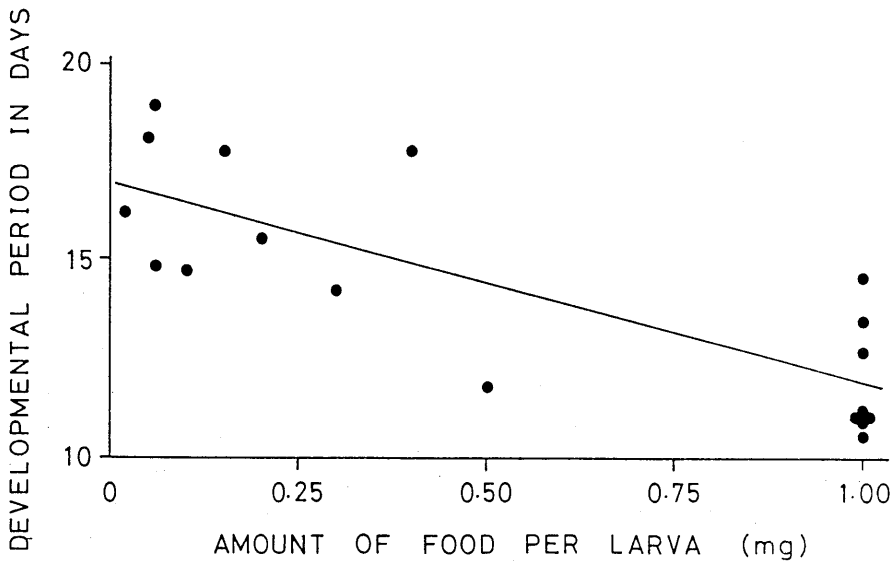

Fig. 1. Relationship between the amount of food per larva (F/L mg) and the developmental period of immature stage in days.

longer delay, so that the average periods in those populations should proportionally prolong.

Mortality of immatures was also heavily affected by the available amount of food per larva. As shown in Fig. 2, when all rearing varieties were grouped into three according to the amount of food (F/L), overall mortality in larvae of 1.0 mg group did not reach to 20% in the average. However, in larvae under poor food condition, of which F/L was between 0.10 and 0.02 mg, continuous death was observed throughout the immature stages, and overall mortality was over 90% in spite of extra feeding on the thirteenth or the fourteenth day. Mortality in larvae fed between 0.5 and 0.15mg was proportionally moderate. Fig. 3 shows the relationship between F/L and the overall mortality. A good linear relationship ($r^2=0.846$) was confirmed between them.

On other two variants, food concentration (F/W), which was reported to be a limiting factor of development in *Aedes aegypti* by Peters *et al.* (1969), and space (L/W) we could not detect clear effects to the larval development in respect to period and mortality through the regression analysis. Therefore we considered that the available food amount per individual might be the most actual and effective factor to developmental success in immature population of *Aedes polynesiensis*.

Wada (1965) observed high mortality, long immature period, and small resulting adults in *Aedes aegypti*, even if the amount of food of immature stage was kept constant when rearing density was increased, and confirmed so called negative density effect in *Aedes aegypti* induced by mutual stimulation. In *Aedes polynesiensis* Lowrie Jr. (1973a, b) reported developmental delay of immature stage under overcrowding conditions, and he also suggested that the delay might be due to mutual interaction. As shown in Table 2, we could

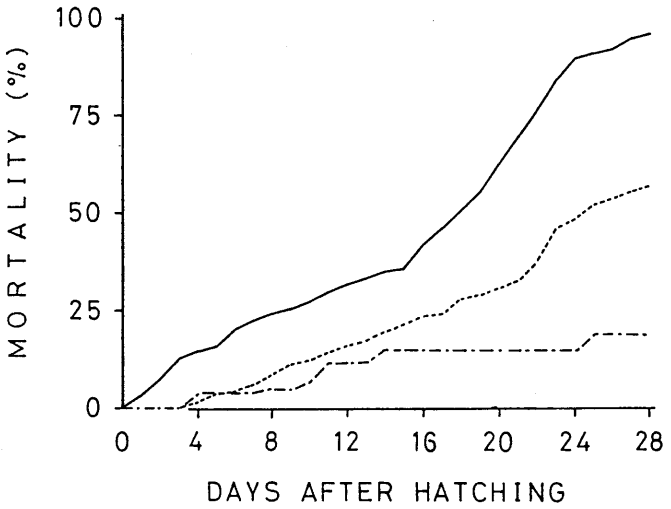

Fig. 2. Cummulative mortality (%) of immatures reared under different F/L. —— 0.02−0.10 mg, ·····, 0.15−0.50 mg, and −•−•−, 1.0 mg.

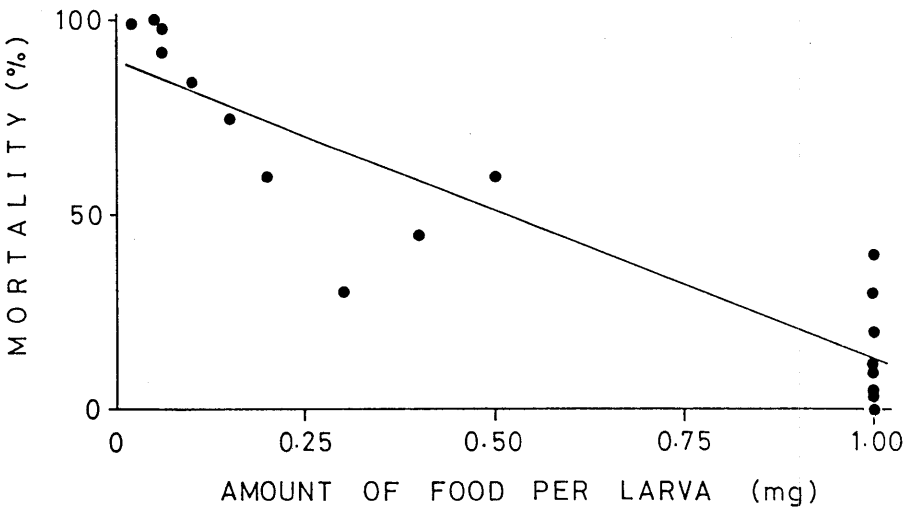

Fig. 3. Relationship between the amount of food per larva (F/L mg) and mortality in immatures.

ascertain again developmental delay and high mortality in immature stage of *Aedes polynesiensis* when no additional food was provided as rearing density was increased. However, once the amount of food was proportionally increased as rearing density increased, neither the developmental delay nor the high mortality came out in our density range (1−50/container). On the contrary, the fast development and the low mortality were observed in crowded population. The overall immature stages in D20F20W135, D30F30W135, D30F30W1350 and D 50F50W2250 took less than 11 days except L50F50W1350 (11.2 days), while those of D1F1W15 and D1F1W90 did 12.7 and 13.5 days, respectively. The overall immature mortalities were from 0. 0% to 11.7% in these crowded populations, while 20.0% and 40.0% in the solitary ones. These facts suggests the possibility of the positive crowding effect in immature stage of the species as it was reported in *Aedes taeniorhynchus* by Nayer (1969) and Nayer and Sauerman Jr. (1969) though further detailed examinations are still necessary.

Different results between ours and Lowrie Jr. (1973a, b), mentioned above, may be due te extraordinary high density in his experiment in which 2,000 larvae were introduced into just small dishes (20×11.5×6 cm). Moreover the difference rather suggests the presence of the optimum density in which the fastest and the most synchronous development and the lowest mortality come out. Either direct mutual contact of biological contamination including some chemical substances released by larvae may control this mode of development in the species.

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