A Laboratory Experiment on the Larval Development of *Culex tritaeniorhynchus* (Diptera: Culicidae) Giles under Different Temperatures and Densities

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Abstract: Rearing experiments of *Cx. tritaeniorhynchus* Giles immatures were conducted under different temperature and densities to ascertain the developmental period and the mortality, which are parameters required to estimate the survivorship of natural populations. Linear relationship was confirmed between the rearing temperature and the developmental velocity, and the developmental zero was estimated between 11 and 15C. No instar specific or temperature dependent mortality was observed. The density effect was represented by the prolongation of immature period and by the increase of mortality. Higher mortality was probably due to longer immature period. The observed density effects might be caused by competition for food in old instar larvae.

Key words: laboratory experiment, larval development, *Culex tritaeniorhynchus*

INTRODUCTION

Culex tritaeniorhynchus Giles is a main rice field breeder and is well known as a principal vector of Japanese encephalitis almost throughout Asia, where the rice cultivation very widely prevails. Because irrigation for rice cultivation to provide sufficient amount of food has been promoted world-widely, the medical importance of this rice field breeder has much increased recently. Many studies on this species have been reported, but we believe that more knowledge on the ecology is still necessary. Especially, precise observation of the larval developmental process is essential to confirm such population parameters as larval developmental velocity. The developmental period of immature stages, which should be required to calculate the daily survival rate of the natural larval population, was examined by Wada and Omori (1971) and Mogi (1978) in *Cx. tritaeniorhynchus* of Nagasaki population. The effects of larval habitat conditions on the development of the species were also experimentally studied,

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the crowding by Nakamura (1979), the photoperiod by Yoshida et al. (1974), the biological conditioning by larvae by Nakamura (1978), and the larval density, the food shortage and the water quality by Siddiqui (1976). Discussion in detail on the immature population was made by Mogi (1978).

We conducted a rearing experiment of *Cx. tritaeniorhynchus* immature populations under different temperature and densities to ascertain the developmental periods of Mie population of the species to prepare a basic parameter to evaluate the survivorship in the natural populations. Results obtained are reported here.

Materials and Methods

Immature stages used were derived from eggs deposited by blood fed females which were caught at cow sheds surrounded by rice fields near Tsu city, Mie Prefecture, central Japan. The area was described in detail by Takagi et al. (1995).

Two series of rearing experiments were performed. In the first experiment (experiment 1), 100 larvae hatched within 12 hours were placed in a plastic jar of $20 \times 30 \times 5$cm in size, under three varieties of water temperature (22, 25 and 28C). Larvae were examined for their instar at 12 hours interval. When the new instar larvae were found, only they were removed to another jar of the same size. On this occasion, density was reset to 80 plus minus 5 individuals per jar. This procedure was continued until they emerged to adults, but the density per jar was reset to 40, 20 and 10 individuals with range of plus minus 5 in the third instar, fourth instar and pupa, respectively. Water depth was 2cm throughout the experiment. An amount of 0.1−0.3 g per jar of finely grained mouse pellets (registered name NMF, manufactured by Oriental Yeast Co. Ltd.) was fed every other day. Water was not renewed during each instar period unless it became turbid or rotten. This rearing method was adopted to confirm the stage specific developmental period and the mortality under little stress from high density and nutritive shortage, and to clarify the temperature dependent velocity of the immature development.

On the other hand, in experiment 2, the first instar larvae were placed in different initial densities of 2^n (n=3...8) per jar, and reared without the reset of density. Feeding schedule was the same as in experiment 1, but the rearing was conducted only under 25C.

Results

Experiment 1

In jars at 28C, 13.8% of the first instar larvae moulted into the second instar between 12 and 24 hours after hatching, while at 25C and 22C, the second instar larvae were firstly observed 36 and 48 hours after hatching. Frequency distribution of the timing that the first instar larvae successfully developed into the second instar under the three rearing temperatures was illustrated in Fig. 1a. The average periods of the first instar were 1.55 days at 28C, 1.86 days at 25C, and 2.73 days at 22C. The mortalities during the period were 30.0%,

16.8% and 19.0%, at 28, 25 and 22C, respectively (Table 1).

Significant differences were found in the developmental period of the instar among the three varieties, but no differences were detected on the mortality ($P<0.05$).

In the successive instars, the average developmental periods in days prolonged as the instar advanced in each rearing temperature, and the frequency distributions of the period flattened (Fig. 1b, c, d). The average periods in days of the second instar were 1.95 (28C), 3.23 (25C) and 3.70 (22C). Those in the third and forth instars were 2.70 (28C), 3.62 (25C), 4.09 (22C); and 4.51 (28C), 5.77 (25C), 6.25 (22C), respectively (Table 1). The differences of periods among three different rearing temperatures were significant ($P<0.05$) in all these instars. No instar specific or temperature specific differences were found in the mortality (Table 1).

The pupal period was the shortest among the immature stages, and less affected by temperature examined. At all varieties of the rearing temperatures, the first group of adults emerged by 24 hours after pupation (Fig. 1d). The average pupal periods in days were 1.29 (28C), 1.49 (25C) and 1.69 (22C) (Table 1). The differences in the developmental period were still significant ($P<0.05$). Mortality during the period ranged from 9.8% to 19.7%.

From these results linear regressions were expected between the rearing temperature (X) and the developmental velocity (the reciprocal of the average instar period in days) in each instar and pupal stage ($Y_{1\cdots p}$). Equations were as follows:

$Y_1=0.0456X-0.6456$ ($r^2=0.9828$, $P<0.01$)

$Y_2=0.0406X-0.6506$ ($r^2=0.8665$, $P<0.05$)

$Y_3=0.0210X-0.2284$ ($r^2=0.9252$, $P<0.05$)

$Y_4=0.0103X-0.0733$ ($r^2=0.9044$, $P<0.05$)

$Y_{1\cdots 4}=0.0056X-0.0669$ ($r^2=0.9392$, $P<0.01$)

$Y_p=0.0293X-0.0473$ ($r^2=0.9880$, $P<0.01$)

Calculated developmental velocities in each instar at 3 temperatures were shown in Table 1. The developmental zero of *Cx. tritaeniorhynchus* immatures estimated from these equations was between 11 and 15C.

Experiment 2

The density effect at 25C was summarized in Fig. 2 and Table 2. Overall developmental period in days was prolonged when the rearing density was increased. The prolongation became more apparent with advance of the instar. It was within 0.11 days difference in the first instar (1.00 day in 16 and 64 individuals per jar and 1.11 days in 128 individuals), but nearly ten days in overall period (8.48 and 17.95 days in 8 and 256 individuals) (Table 2). Emergence of adult was observed synchronously in low densities but it became sluggish as the density increased (Fig. 2). However, the first ecdysis to the next stage including the emergence to adults was occurred nearly on the same day irrespective of rearing density except the highest density group (256 individuals per jar) in which the emergence delayed. Therefore the prolongation in the average period or the median of each instar should be attributed to the sluggish ecdysis to the next instar.

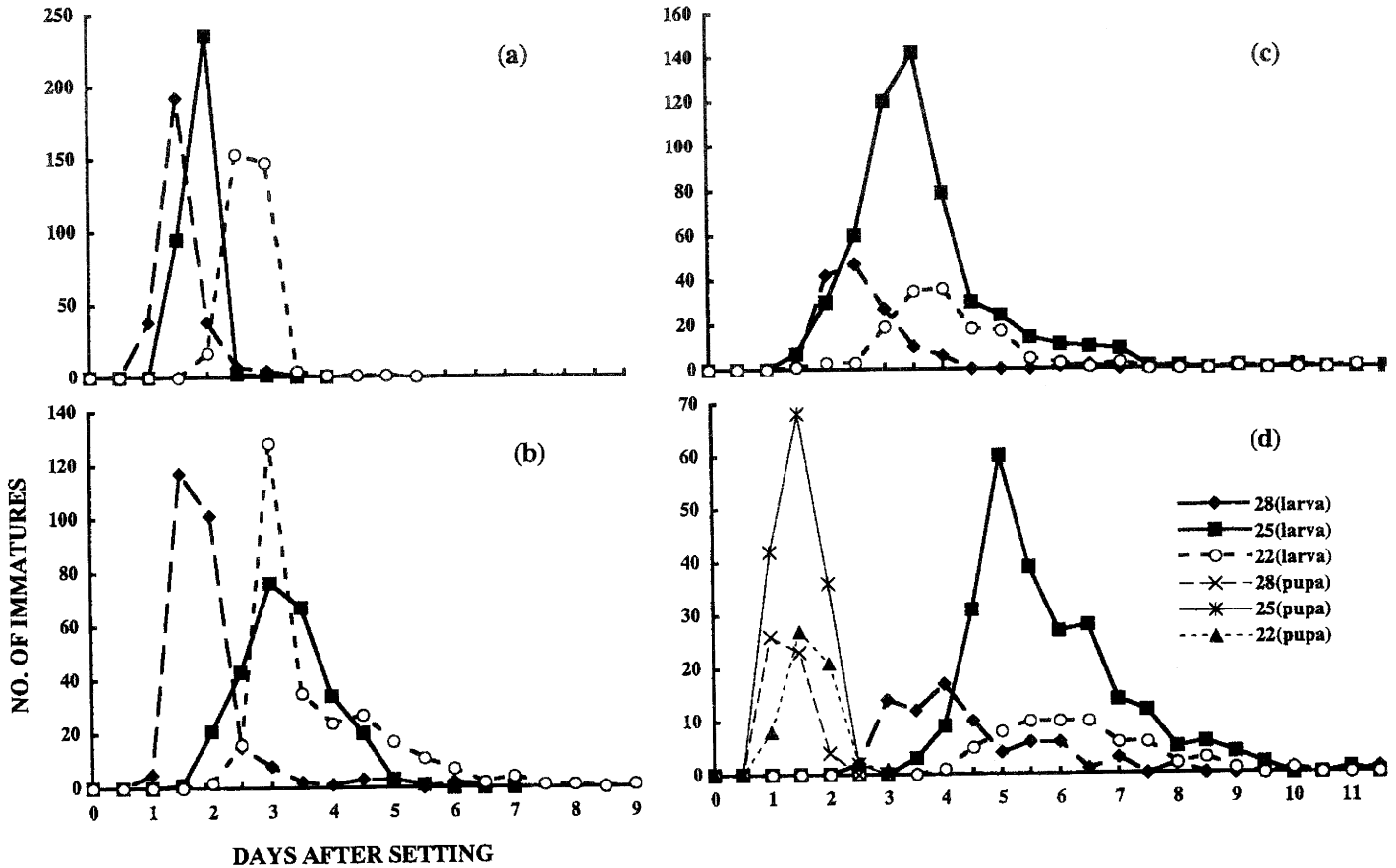

Figure 1. Developmental period in days of immatures at 3 different water temperatures. (a), 1st instar; (b), 2nd instar; (c), 3rd instar; (d), 4th instar and pupa.

Table 1. Average developmental period in days, mortality and developmental velocity of immatures at 3 different water temperatures.

Instar	Water temperature (C)	Density	Replication	No. used	No. survived to the next stage	Mortality (%)	Average developmental period in days [S. D.]	Developmental velocity
1	28	100	4	400	280	30.0	1.55 [0.37]	0.631
1	25	100	4	400	333	16.8	1.86 [0.24]	0.494
1	22	100	4	400	324	19.0	2.73 [0.35]	0.358
2	28	80	4	400	258	19.4	1.95 [0.77]	0.486
2	25	80	4	400	266	16.9	3.23 [0.71]	0.364
2	22	80	4	400	276	13.8	3.70 [1.10]	0.243
3	28	40	4	160	138	31.0	2.70 [0.95]	0.360
3	25	40	15	600	540	10.0	3.62 [1.15]	0.297
3	22	40	4	160	146	27.0	4.09 [1.18]	0.234
4	28	20	5	100	79	21.0	4.51 [1.61]	0.215
4	25	20	15	300	241	19.7	5.77 [1.24]	0.184
4	22	20	4	80	63	21.3	6.25 [1.24]	0.153
P	28	8−17	5	66	53	19.7	1.29 [0.04]	0.773
P	25	20−40	6	164	148	9.8	1.49 [0.03]	0.685
P	22	8−17	6	69	59	14.5	1.67 [0.05]	0.597

The overall mortality was the lowest in 8 individuals group (28.1%). The rate increased as the density increased, and the highest rate was observed in 256 individuals group (84.0%) (Table 2). The age specific increase in the mortality was not distinguished (Fig. 2), and the daily mortality was not different irrespective of density (2.8%, 2.5%, 2.2%, 2.2%, 2.4% and 1.2% in 8, 16, 32, 64, 128 and 256 individuals per jar, respectively). Therefore, it was strongly suggested that the overall mortality was dependent on the overall larval period.

Such prolongation might be caused by the shortage of food in old instars of higher densities through competition for food, because the number of pupae in 256 and 128 individuals groups increased on the next day of the every other daily feeding. The average number of pupae on the day of feeding was 2.86, while that on the next day was 4.43. Difference between two averages was statistically significant by paired t−test ($P<0.005$).

DISCUSSION

This experiment revealed again that the development of immature stages of *Cx. tritaeniorhynchus* follows the law of total effective temperature, and that the development was strongly affected by the larval density possibly through the availability of food amount per larvae. The immature period would actually differ by nearly 20 days according to prevailing temperatures within the mosquito season in Japan, as the period calculated was 31.6 days at 18C or 12.4 days at 28C. Furthermore, the period should change by the larval density, as ascertained by experiment 2. Therefore, if the effect of the larval density is rational in the prevailing temperature, difference of another ten days, totaling 30 days, may be better to take into account of the actual immature period in natural populations.

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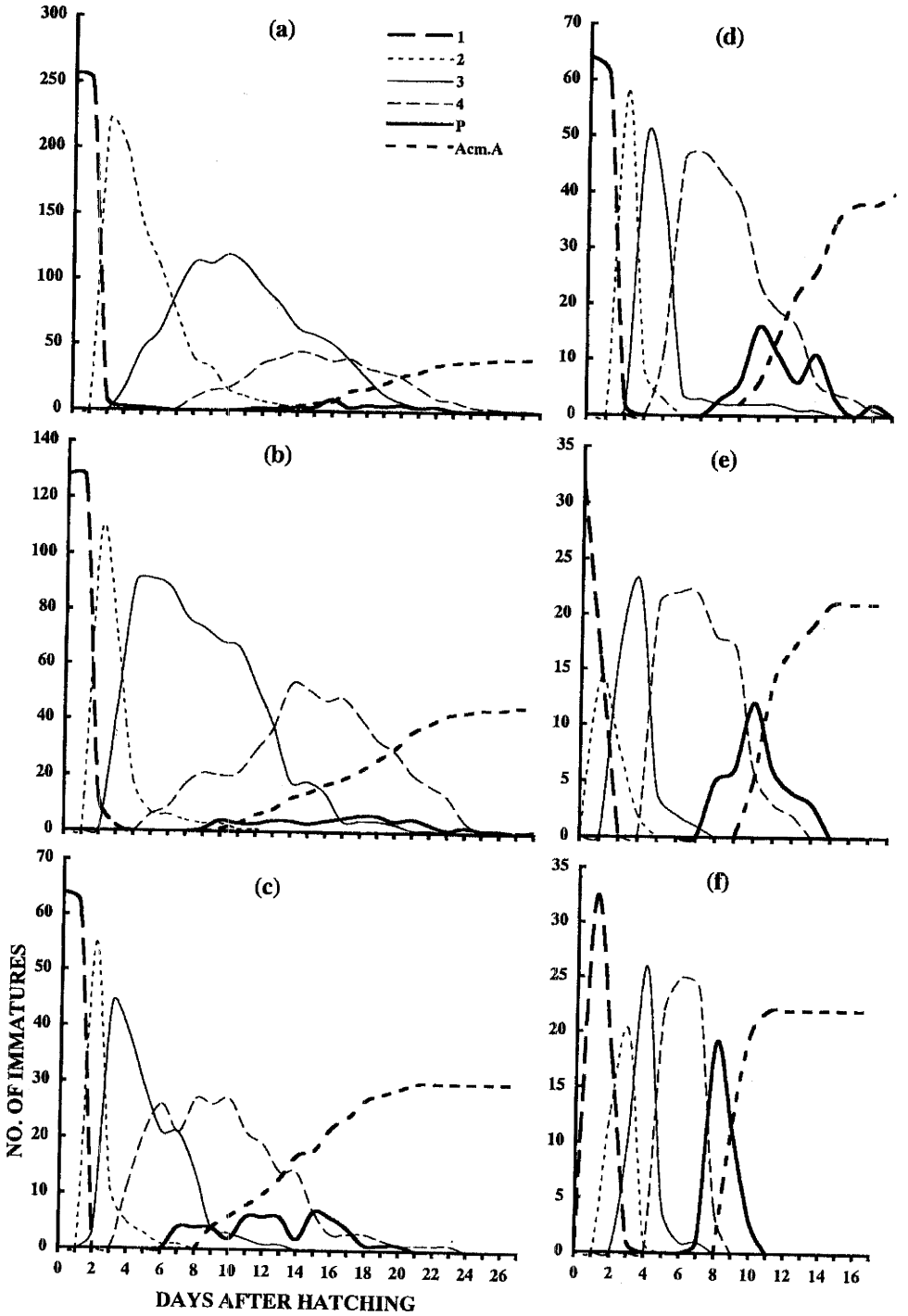

Figure 2. Developmental period in days of immatures reared in different densities ranging from 8 to 256 per jar at 25℃. (a), 256; (b), 128; (c), 64; (d), 32; (e), 16; (f), 8.

Table 2. Accumulated developmental period in days and mortality of immatures reared different density at 25℃

Initial density	Replication	No. of adults emerged	Mortality (%)	Accumulated developmental period in days*					
				1st	2nd	3rd	4th	Pupa	Adult
256	2	82	83.98	1.09	4.13	9.94	14.55	17.06	17.95
128	2	90	64.84	1.11	3.03	7.83	14.37	16.10	16.24
64	3	91	53.13	1.00	2.51	5.15	9.60	12.70	13.40
32	4	80	37.50	1.03	2.20	4.08	7.46	10.17	9.28
16	4	84	34.38	1.00	1.50	2.87	6.25	9.19	8.81
8	4	23	28.13	1.05	1.65	2.98	5.13	7.47	8.48

*Days from the hatch to the time when 50% of total immatures reached to the next stage.

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