

# THE INFLUENCE OF CONSTANT VERSUS FLUCTUATING WATER TEMPERATURES ON THE PREIMAGINAL DEVELOPMENT OF *CULEX TARSALIS*<sup>1</sup>

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**ABSTRACT.** *Culex tarsalis* larvae derived from field-collected egg rafts were reared outdoors in a deep pond with constant water temperature or a shallow pond with fluctuating temperature. No significant differences were found between ponds in the proportion of larvae surviving to adults, female autogeny rates or wing lengths of adults. The average time from 1st instar to adult emergence ranged from 8 days at 31°C to 16 days at 17°C. Males developed faster than females, and both sexes always emerged faster from the shallow pond than from the deep one. However, the temperature-adjusted mean emergence times were equal, and normalized cumulative development curves, fitted to a Weibull function, were identical for the 2 ponds.

## INTRODUCTION

An experiment was carried out in Bakersfield, Kern County, California, in the summer of 1984 to measure the influence of rearing temperature on the vector competence of *Culex tarsalis* Coquillett for western equine encephalomyelitis virus. Newly hatched larvae from field-collected egg rafts were reared in 3 aquatic environments: a shaded deep pond or an uncovered shallow pond outdoors, or in an insectary. Widely fluctuating high and low daily water temperatures were achieved in the shallow pond, whereas relatively constant temperatures were maintained in the deep pond. Although not designed for this purpose, the experiment provided a unique opportunity for retrospective examination of the effects of rearing under constant versus fluctuating temperatures on aquatic development times and adult characteristics of mosquitoes produced in the 3 rearing environments. Results of vector competence evaluations will be presented in a separate publication.

Numerous studies have examined the influence of water temperature on the aquatic stages of *Cx. tarsalis* and other mosquito species. In laboratory observations, thermal stress  $\leq 12^\circ\text{C}$  or  $> 32^\circ\text{C}$  was associated with high mortality of *Cx. tarsalis* larvae (Bailey and Gieke 1968, Reisen et al. 1984). Within the range 15–32°C, preimaginal development was consistently more rapid at higher constant temperatures. We wished to determine if this relationship

would also be true when temperatures fluctuated, as in nature.

## MATERIALS AND METHODS

Mosquitoes were collected as egg rafts at Poso West, 16 km N of Bakersfield, CA. First instar larvae from these rafts were accumulated for 2 days in an insectary at  $27 \pm 1^\circ\text{C}$  and then transferred to 3 environments: a 40 cm deep 1.5m<sup>2</sup> pond shaded with 2 layers of greenhouse screening (90% shade) or a 10 cm deep 1.5m<sup>2</sup> unshaded pond outdoors, or four 5 cm deep 0.8 m<sup>2</sup> rearing trays in the insectary. For each test, outdoor ponds were stocked with about 6,000 larvae each and approximately 1,500 larvae were placed in each insectary tray. The density of larvae per unit of surface area, 0.25/cm<sup>2</sup>, was equal in the 3 environments.

The outdoor ponds were lined with 2 layers of 6 mil clear polyethylene plastic, which was changed for each new cohort. The larvae were fed a diet of finely ground Purina Lab Chow<sup>®</sup>. The larval habitats were filled with tap water 3 days before each cohort of larvae was added. At the time of filling, 25 cc of the diet was added to each insectary tray and 200 cc to each outdoor pond. These same amounts were added again in the insectary trays and the deep pond 4–6 days after larvae were introduced. Food was added to the shallow pond only once as the level of planktonic algal growth in that pond appeared to provide sufficient nutrients and a second feeding was not considered necessary. Additional feeding was needed in the deep shaded pond because the volume of water was greater and the algal bloom developed slowly. The total volume of chow added per liter of water was 0.44 cc in the deep pond, 0.89 cc in the shallow pond and 1.56 cc in the insectary. No attempt was made to quantify the total nutrient content of the 3 rearing environments.

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Six cohorts of mosquitoes were reared in this manner from May through September at intervals of about 4 weeks. Outdoors, pupae were harvested from each pond on alternate days and transferred to 19.5 liter screened and shaded emergence buckets. Emerging adults were removed from the buckets daily by a mechanical aspirator, identified and counted by sex for each pond. In the insectary, pupae were removed on alternate days and transferred to 1 liter plastic containers placed in  $0.5 \times 0.5 \times 0.5$  m screened cages. Emerging adults were removed daily, identified and counted.

Temperatures in each environment were recorded by 7-day continuous recording thermographs. A temperature probe was located 1 cm below the water surface in each pond. Average water temperatures for each cohort were calculated from the recordings in each test site.

Samples of 50 adult females from each environmental cohort were held in the insectary for 7–10 days to determine autogeny rates (Nelson and Milby 1982). Wing lengths of these females and of corresponding samples of 25 males were measured to quantify adult size (Bock and Milby 1981).

Differences in immature survival, autogeny, development time and wing length between the deep pond, shallow pond and insectary groups were compared by analysis of covariance, with mean temperature as the covariable. The cumulative distributions of development time for each cohort were normalized by dividing each percentile value by the midpoint (median development time). The normalized curves for each environment were averaged, then fitted to a Weibull function, using procedures described by Wagner et al. (1984). The cumulative form of the Weibull function is:

$$F(x) = 1 - \exp\{-[x-a]/b\}^c\}$$

where  $F(x)$  is the probability of complete development at normalized time  $x$ . The 3 parameters  $a$ ,  $b$  and  $c$  represent location, spread and skewness (Dell et al. 1983).

## RESULTS

Overall, 43% of 1st instar larvae emerged as adults, and no significant differences in survivorship were found between rearing groups. Approximately 27% of the July, August and September cohorts were *Culex quinquefasciatus* Say. These were not distinguished from *Cx. tarsalis* until pupation. They developed rapidly and most emerged as adults prior to median pupation of the *Cx. tarsalis*. Since the *Cx. quinquefasciatus* were evenly distributed among the *Cx. tarsalis*, their impact on competition for food and space was assumed to be comparable in the 3 environments. Comparable proportions of *Cx. quinquefasciatus* adults were collected during these months in a  $\text{CO}_2$  trap located at the raft collection site.

There was little variation in mean temperatures between tests in the insectary (Table 1). Outdoors, the mean temperatures for the shallow pond were consistently about  $6^\circ\text{C}$  higher than those in the deep pond. Daily water temperatures in the shallow pond fluctuated as much as  $16^\circ\text{C}$ , while those in both the deep pond and the insectary remained essentially constant. The average development time of immature stages ranged from 8 days at  $31^\circ\text{C}$  to 16 days at  $17^\circ\text{C}$ . Development rates correlated significantly with mean, minimum and maximum water temperatures ( $p \leq 0.01$ ).

The time from sunrise to sunset outdoors varied from 12.5 hr in September to 14.5 hr in June, as compared to a constant 14 hr of light each day in the insectary. Day length was not correlated with development time. Autogeny rates averaged 67%, and were lowest for the females reared in the shallow pond, but again, the differences were not statistically significant. No significant differences were observed between adult wing lengths of either sex for the 3 groups. At comparable temperatures, adults reared in the insectary were usually larger than those reared in the outdoor ponds. This may have been due to the higher concentration of diet per liter of water fed to the insectary cohorts.

Table 1. Mean water temperature ( $^\circ\text{C}$ ) and development rate<sup>a</sup> for *Culex tarsalis* reared in 3 environments.

Dates	Indoor insectary		Outdoor			
	Temp.	Rate	Deep pond		Shallow pond	
			Temp.	Rate	Temp.	Rate
May 8–May 23	24.4	0.152	16.8	0.064	22.9	0.096
June 4–June 20	23.3	0.109	18.8	0.070	25.4	0.115
June 28–July 11	22.7	0.107	23.9	0.101	30.5	0.134
July 27–Aug. 9	23.3	0.109	23.1	0.085	29.0	0.099
Aug. 14–Aug. 28	22.1	0.101	22.7	0.086	28.4	0.106
Sep. 8–Sep. 20	21.9	0.109	23.3	0.099	27.7	0.119

<sup>a</sup> The fraction of development completed each day.

The means in Table 2 reflect number of days after the larvae were placed in the 3 rearing sites. Males developed faster than females, and emergence of shallow pond cohorts preceded that of deep pond cohorts by an average of 3 days. The analysis of covariance showed that the difference in development times was entirely attributable to the difference in mean temperatures ( $p$  for temperature  $< 0.001$ , for pond = 0.8). When the mean emergence times were adjusted to account for higher mean temperatures in the shallow ponds, the time differences disappeared. In other words, if the mean temperature had been the same in the 2 ponds, the mean emergence times would also have been equal, despite the daily temperature fluctuation in the shallow pond. A direct comparison with the insectary cohorts could not be made because only pupation times, not emergence times, were recorded for those groups. However, since the mean difference between pupation time in the insectary and emergence time outdoors was about 2 days, we can assume that development rates were not significantly different.

and Geike (1968), Hagstrum and Workman (1971), and Reisen et al. (1984) for *Cx. tarsalis* reared under laboratory conditions. Similar times were recorded by White<sup>3</sup> for cohorts of *Cx. tarsalis* reared in field enclosures at Poso West, where the diurnal temperature range generally exceeded 10°C. The 43% survival rate was low, but within the range of 0–69% observed for field cohorts by White.<sup>3</sup>

Bailey and Geike (1968) found that *Cx. tarsalis* in a field enclosure with a water temperature range of 16–29°C (mean 22°C) required 12–14 days to pupate, while those reared in a laboratory at a constant 21°C pupated in 9–24 days (mean 13.2 days). A similar increased variation in development times at constant temperatures was not observed in the present study.

The presence of *Cx. quinquefasciatus* in the final 3 cohorts of this study may have contributed to a competitive disadvantage for coexisting *Cx. tarsalis*. The normal rate of development of primarily younger instar larvae might have been slowed, but such an impact could not be assessed and must be presumed to have been equal in all 3 environments.

Table 2. Mean development times (days) for 6 cohorts of *Culex tarsalis* reared in 3 environments.

Rearing environment	Life stages	Raw mean	Range of cohort means	Mean adjusted for rearing temperature
Females				
Deep pond	L1 to adult	12.7	9.9–16.7	11.0
Shallow pond	L1 to adult	9.6	7.7–11.0	11.2
Insectary	L1 to pupa	9.2	7.0–10.5	—
Males				
Deep pond	L1 to adult	12.0	9.8–15.0	10.6
Shallow pond	L1 to adult	9.0	7.4–10.1	10.3
Insectary	L1 to pupa	8.7	6.6–10.1	—

The Weibull function described the combined cumulative development curves very well ( $R^2 > 0.99$  for each sex in each environment). The average normalized curves for the 2 outdoor groups coincided almost perfectly, and indicated that the development pattern did not change when temperature fluctuated (Fig. 1). The curves for the insectary group were a bit more spread out. The scale parameter was significantly larger for both sexes and the location parameter was smaller. This difference probably indicated that pupation times and emergence times had slightly different distributions.

#### DISCUSSION AND CONCLUSIONS

Allowing for the 2-day initial holding period, the development times observed in this study were comparable to those reported by Bailey

The algal growth probably combined with overall warmer water temperature to promote the rapid development of *Cx. tarsalis* in the shallow pond. Presence of phytoplankton would effectively reduce density-dependent competition for nutrients by supplying a surplus of food. Studies by Meyer et al. (1982) in productive coastal salt marshes and freshwater foothill sources have shown that abundant phytoplankton can enhance both preimaginal development and survival of immature *Culiseta inornata* (Williston) confined in screened buckets.

The analysis of covariance demonstrated that development time was significantly influenced

<sup>3</sup> White K. D. 1980. Effects of larval density on the growth rate and size of a *Culex tarsalis* mosquito population. Ph.D. Dissertation, University of California, Davis. 156 pp.

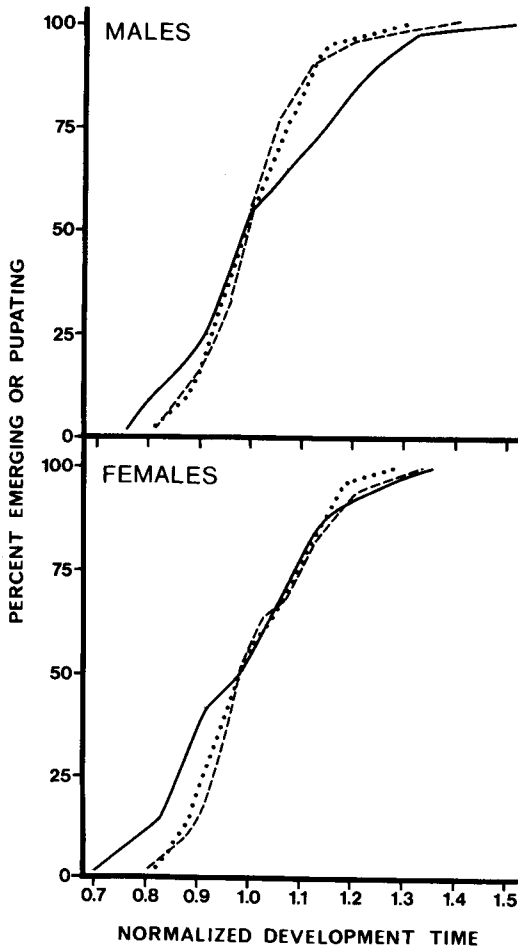


Fig. 1. Cumulative distributions of normalized development time for *Culex tarsalis* males and females in 3 rearing environments: insectary — deep ponds . . . ., or shallow ponds —.

by mean temperature, whereas the independent effect of rearing environment (i.e., deep pond with constant temperature versus shallow pond with fluctuating temperature) was not significant. Although a carefully designed experiment to confirm these observations would be desirable, the outdoor ponds probably provided a reasonable facsimile of predator-free natural breeding sources. Breeding sites in many areas of Kern County are ephemeral (Reisen et al. 1984). Seasonally, water depth, shade, and the amount of daily fluctuation in water temperature vary widely.

The modeling approach used here is based on the assumption that the underlying distributions of normalized development time have the same shape at all temperatures. While this may be true theoretically, a variety of factors can influence the shape of development curves based on observed data (Wagner et al. 1984).

These include measurement errors, differences in sample sizes, differences in sampling interval, and environmental differences such as density and nutrition. A shorter sampling interval in the present study might have revealed irregularities in cumulative development for the shallow pond cohorts that were obscured by counting emerging adults only once each day. However, accuracy of less than 1 day usually is not required for estimates of total aquatic development time.

The conclusion from these analyses is that fluctuating water temperatures have the same impact on the rate of preimaginal development of *Cx. tarsalis* as constant temperatures that are equal to the mean value of the fluctuating ones. This knowledge will facilitate the creation of simulation models to describe the population dynamics of this species.

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