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ABSTRACT   Effects of food, density, and heterospecific interactions on temperature-dependent development of *Aedes albopictus* (Skuse) and *Aedes triseriatus* (Say) larvae and pupae were described using a degree-day model. Under all conditions, the predicted number of degree-days (DD_{T_0}) to complete larval development was less, and the threshold temperature (T_0) for initiation of larval development was higher for *Ae. albopictus* than for *Ae. triseriatus*. The DD_{T_0} for both species was food and density dependent. However, the per capita food ration appeared to exert a greater influence on the developmental times of *Ae. triseriatus* immatures, whereas for *Ae. albopictus* effects of density were not completely eliminated by an increase in the per capita food ration. The presence of heterospecific larvae did not prolong the DD_{T_0} of either species. At the low food ration, DD_{T_0} for both species were significantly greater under conspecific conditions. The DD_{T_0} for *Ae. triseriatus* increased directly as the proportion of conspecific larvae increased. However, when a higher per capita food ration was provided, conspecific effects on DD_{T_0} were mitigated. For *Ae. albopictus*, estimated DD_{T_0} values for larvae reared in pure culture were significantly higher than when heterospecific larvae were present, regardless of the per capita amount of food provided. Survivorship of immatures was density and food-dependent for both species. *Ae. albopictus* exhibited higher immature survivorship under all conditions relative to *Ae. triseriatus*. For both species, survival probabilities were lowest under conditions of high density and low food. Addition of food improved survival for both species. The presence of heterospecific larvae exerted a differential effect on the survivorship response of *Ae. albopictus* immatures to temperature. Lowest probability of survival for *Ae. albopictus* was occurred at low temperature when the proportion of heterospecific larvae in containers was the highest. In contrast, at high temperatures, survivorship of immatures improved, but was lowest for pure species cultures. For *Ae. triseriatus*, survivorship of immatures was consistently lower for pure species cultures regardless of the temperature. Standing crop production of adults of both species was primarily food rather that density-dependent. Under field conditions, *Ae. triseriatus* were predicted to initiate development sooner and exhibit faster population growth early in the season than *Ae. albopictus*. However, because of the shorter DD_{T_0}, *Ae. albopictus* population growth was predicted to surpass that of *Ae. triseriatus* populations later in the season. Based on optimal DD_{T_0} values from laboratory experiments, spring emergence dates of *Ae. triseriatus* females in western North Carolina for 1989 and 1990 were predicted from accumulated degree-days calculated from local air temperature records. Predicted emergence dates were congruent with results of a previous survey for the same locality, indicating that accumulated degree-days can be used to accurately predict the seasonal occurrence of *Ae. triseriatus*. The utility of the degree-day approach in predicting the phenology of *Ae. albopictus* remains to be established.

KEY WORDS   *Aedes albopictus*, *Aedes triseriatus*, immature development, degree-day

CONTAINER-INHABITING MOSQUITOES, such as *Aedes albopictus* (Skuse) and *Aedes triseriatus* (Say), lay desiccation-resistant eggs that overwinter in temperate climates in a diapause condition. *Ae. albopictus* females produce diapausing eggs (Hawley et al. 1987, Focks et al. 1994), whereas *Ae. triseriatus* eggs manifest diapause (Shroyer and Craig 1980) in response to short daylength in late summer. Eggs of both mosquitoes exhibit cold hardiness, which enhances their ability to survive low temperatures during winter (Shroyer and Craig 1980, Hawley et al. 1989, Hanson and Craig 1994). In North America, *Ae. albopictus* is an introduced species that is sympatric over its present geographic distribution with *Ae. triseriatus*, an indigenous species. The geographic extension of *Ae. albopictus* toward its northern limits involved overwintering areas and late summer expansion areas (Nawrocki and Hawley 1987). Hawley et al. (1989) reported greater overwinter survival of eggs of *Ae. albopictus* strains that were collected from the upper Midwest relative to strains collected from southern areas of the

United States. If these North American strains have a common geographic origin, as suggested by Hawley et al. (1989), then *Ae. albopictus* has become increasingly tolerant of cold climates as it has expanded northward. However, at northern latitudes, the overwinter egg survival of *Ae. albopictus* has been reported to vary from year to year and to be consistently lower than for *Ae. triseriatus* (Hawley et al. 1989).

In a given locale, severe overwintering egg mortality could be compensated for partially by rapid larval development, which would facilitate multivoltinism. If differential mortality factors are not exerted, then the relative size of species populations would be dependent, in part, on generation time, which is contingent on their respective rates of larval growth (Southwood 1975). A comparatively high rate of larval growth would increase the number of individuals in a species' population that would achieve the photosensitive stage before the critical daylength would trigger diapause (Tauber et al. 1986). For container-inhabiting mosquitoes that produce desiccation-resistant eggs, the termination of diapause and the timing of rainfall events would be important factors regulating the emergence of adults in spring. Temperature also would be an important determinant of local population dynamics, because the rate of growth and development of mosquito larvae are highly dependent on water temperature (Wagner et al. 1984, Rueda et al. 1990, Focks et al. 1993, Eisenberg et al. 1995). Degree-day models that are based on simple linear regressions are used widely to describe the relationship between temperature and the rate of insect development. These heat summation models can provide adequate predictions of insect population dynamics within the range of temperatures encountered in the field (Campbell et al. 1974; Laing and Heraty 1984; Whitfield 1984; Mahmood and Crans 1997, 1998). In addition, to temperature, rate of growth of mosquito larvae is affected by density-dependent factors, such as quantity of food and larval density (Clements 1992) as well as from heterospecific competition for food resources.

Competition between *Ae. albopictus* and other container-inhabiting mosquitoes, including *Ae. triseriatus*, has been investigated previously under laboratory conditions (Black et al. 1989, Ho et al. 1989, Livdahl and Willey 1991, Novak et al. 1993). These previous studies have measured the effects of competition through changes in life history traits, such as development time or survivorship of immatures; however, no studies of interspecific competition have been conducted using >1 temperature.

The objectives of our current study were to estimate the effects of food ration and larval density on the developmental response of *Ae. albopictus* immatures to temperature with a degree-day model using a factorial experimental approach. In addition, effects of density and food on the survivorship response of immatures of *Ae. albopictus* to temperature were measured. In North Carolina, *Ae. albopictus* is expanding into the mountains where populations of *Ae. triseriatus* occur (Szumlas et al. 1996a, b). Therefore, we also estimated effects of the aforementioned abiotic and biotic variables on the development time and survival of *Ae. triseriatus* immatures in pure as well as mixed species cultures with *Ae. albopictus.*

Materials and Methods

Origin and Maintenance of Laboratory Populations. Eggs from the 1987 colony of *Ae. albopictus* (ALBO-ROC) from Rockingham, NC, were obtained from Notre Dame University. A strain of *Ae. triseriatus* (TRIS-CHER) was established from larvae collected from discarded tires near the town of Cherokee, Jackson County, NC, in 1990. Mosquito colonies were maintained and eggs were collected and stored as described by Teng and Apperson (1996). The F_{3-5} generations of the TRIS-CHER strain were used in experiments. After the ALBO-ROC strain was received in 1989, separate generations were maintained. Experimentation was conducted using the F_{4-6} generations of this strain.

Design of Experiments. Batches of eggs (TRIS-CHER and ALBO-ROC) were hatched in nutrient broth (Novak and Shroyer 1978). Larvae were <6 h old at the start of each experiment. First instars of each species were combined so that larvae were drawn from pooled conspecifics.

Experimental microcosms were created using polyethylene tubs ($\approx$20 by 14 by 7 cm, length, width, height). Food (brewer's yeast lactalbumin:ground Purina rabbit chow, 1:1:1 by volume) (Chambers 1985) was provided at rations of 0.5 and 2.0 mg per larva. The low food ration was provided at the beginning of each experiment. The high food ration was added twice, with 1 mg per larva added on day 0 and on day 4. Water depth was $\approx$0.7 cm at the start of each experiment. Containers were covered to retard evaporation. The photoperiod during experiments was set at a long daylength conditions (16:8 [L:D] h) to prevent *Ae. triseriatus* larvae from entering diapause (Sims 1982).

Because leaf litter is a major source of organic matter in treeholes (Walker and Merritt 1988), 0.5 g of leaves were placed in each container on day 0. Oak tree leaves were used because *Quercus* sp. is an abundant tree in the forests of western North Carolina. Leaves were collected from the ground and oven dried at 30°C overnight. Petioles were removed and the leaf blades were coarsely chopped into $\approx$4 cm^2 pieces before they were used in experiments (Fish and Carpenter 1982).

Experiments were conducted using a factorial design to determine effects of food ration, larval density, and the proportion of conspecifics on the developmental and survivorship responses of immatures of the 2 species to temperature. A split-plot design was employed with temperature as the main plot factor and combinations of 3 larval densities (20, 200, and 800 larvae per 200 ml of water), 2 food rations (0.5 and 2.0 mg per larva) and 4 proportions of conspecific larvae (0.25, 0.5, 0.75, 1.0) as subplot factors that were randomly assigned to rearing containers within each of 3 environmental chambers, each set at a different temperature (15, 23, and 31°C). For each species, 4 rep-

licates of each combination of larval population level (larval density and conspecific larval proportion) and food ration were completed for each temperature.

Populations were censused daily. At each census, dead larvae and pupae were removed from the containers. Live pupae were removed from the experimental containers and placed into vials partially filled with water. Vials, plugged with cotton, were held in the same environmental chambers and checked daily for adults. Adults were removed and kept in a freezer until the entire experimental replicate for each temperature was completed, after which adults were sexed and counted.

Model Development. In a degree-day (DD_{T_0}) model, the rate of development in physiological time (reciprocal of the number of days required to complete development per individual, $1/D_T$) is a linear function of temperature (T). Larval development occurs when a threshold temperature (T_0) is exceeded. An estimate of DD_{T_0} represents the degree-days above T_0 required for immatures to complete development to the adult stage. Both thermal coefficients (DD_{T_0} and T_0) can be used to describe the growth response of an insect species to temperature (Southwood 1975).

An estimate of T_0 can be obtained through regression of development rate ($1/D_T$) against temperature (T), using the following equation:

$$1/D_T = b_0 + b_1 * T, \qquad [1]$$

where b_0, the intercept point on the temperature axis of the regression, provides the estimate of T_0. In a degree-day model, T_0 is equal to $-b_0/b_1$ (Southwood 1975), and when $-b_0/b_1$ is substituted into the degree-day equation (Southwood 1975):

$$1/DD_{T_0} = 1/D_T (T - T_0), \qquad [2]$$

then,

$$DD_{T_0} = 1/b_1, \qquad [3]$$

where b_1 is the slope coefficient of the 2nd regression.

In estimating DD_{T_0} in our experiments, development rates of males and females of each species were averaged separately for each replicate container. The mean values for each replicate within each treatment (i.e., each combination of larval density, food, and proportion of conspecifics) were regressed against temperature (PROC REG, SAS Institute 1989) using equation 1 to estimate a T_0 for each species. A single value of T_0 was calculated for each sex of *Ae. albopictus* and *Ae. triseriatus* by averaging estimates of T_0 across all treatments. The assumption that T_0 is constant has been made in other models (Gilpin and McClelland 1979, McCallister 1992). We validated the assumption that T_0 was constant under our experimental conditions by analysis of variance (ANOVA) (Neter et al. 1996). Significant main effects of sex, larval density, food ration, and proportion of conspecific larvae were not found for *Ae. albopictus* and only density significantly affected T_0 for *Ae. triseriatus.*

For each species, DD_{T_0} is the cumulative product of physiological development time and temperature (Southwood 1975); consequently, 1 value of DD_{T_0} was

estimated for each treatment using all 3 temperatures simultaneously. In our analyses, using the mean T_0 for each species, linear regression models (PROC REG; SAS Institute 1989) without intercept were fit for $1/D_T$ against $T - T_0$ (equation 2). For each experimental treatment, DD_{T_0} was estimated as $1/b_1$, where b_1 was the slope coefficient of the regressions (equation 3).

Prediction of Field Populations. To evaluate our degree-day model, the spring emergence dates for both species were predicted for the 1989 and 1990 mosquito seasons. Accumulated degree-days exceeding the T_0 for larvae of both species were calculated (McCallister 1992) using air temperature data for 1989 and 1990 that were provided by the State Climate Office of North Carolina at N.C. State University for the Oconaluftee weather station on the Cherokee Indian Reservation. Thermal sums for these years were calculated because some phenology data for *Ae. triseriatus* from the Indian Reservation in the mountains of North Carolina have been reported by Szumlas et al. (1996a) for these mosquito seasons. Accumulated degree-days for dates corresponding to the lowest estimates of DD_{T_0} when larvae were reared in pure species cultures were used to predict when the spring emergence of females of each mosquito would occur.

Survivorship of Immatures. Survivorship data were analyzed, because mortality of young instars would reduce competition for food resources between surviving larvae, which would potentially alter effects of food and density on DD_{T_0}. Survival probabilities were calculated as the ratio of the number immatures of each species surviving from 1st instar to the adult stage and the number of 1st instars of each species added to each container.

Statistical Analyses. Planned comparisons were made between species to evaluate effects of sex, density, food and proportion of conspecific larvae on DD_{T_0}. An ANOVA was carried out on the DD_{T_0} values for each species separately. Because a single value of DD_{T_0} was derived for each experimental treatment, there were not enough degrees of freedom for all interactions in the ANOVA. Therefore, only main effects and one-way interactions of sex, and other main effect variables were included in the ANOVA.

Survival probabilities were analyzed by a factorial ANOVA to determine effects of density, food and proportion of conspecific larvae on the survivorship responses of immatures of each species to temperature. A visual examination of a scatter plot of predicted values ($\hat{y}_i$) against residuals (Draper and Smith 1981) revealed that the residuals were abnormally distributed about a mean of zero, indicating that the error variance was not constant. Error variance was stabilized through an arcsine transformation of survival probability values (arcsine $\sqrt{y_i}$) for each species in each container and, the ANOVA for each species was repeated.

To further elucidate differences between *Ae. albopictus* and *Ae. triseriatus,* when significant interaction effects of treatment variables were found by ANOVA for one species but not the other, these variables were examined for simple effects involving one variable at

Table 1. Results of ANOVA for effects of sex, larval density, per capita food ration, and proportion of conspecific larvae on larval development times in degree-days for *Aedes albopictus* and *Ae. triseriatus*

Source	df	*Ae. albopictus*		*Ae. triseriatus*	
		F	$P > F$	F	$P > F$
Sex	1	82.30	0.0001	49.88	0.0001
Density	2	19.16	0.0001	84.40	0.0001
Food	1	74.98	0.0001	296.53	0.0001
Proportion[a]	3	10.13	0.0002	1.66	0.20
Sex × Density	2	6.98	0.0043	1.00	0.38
Sex × Food	1	37.85	0.0001	3.22	0.086
Sex × Proportion	3	3.27	0.0395	0.9	0.046
Density × Food	2	6.34	0.0064	54.26	0.0001
Density × Proportion	6	1.18	0.3490	3.35	0.016
Food × Proportion	3	5.25	0.0066	6.87	0.0018
Error	23				

[a] Proportion for *Ae. albopictus* or *Ae. triseriatus* was calculated as the initial number of *Ae. albopictus* or *Ae. triseriatus* larvae/initial total number of larvae per container.

Table 2. Effects of larval density, per capita food ration, and proportion of conspecific larval density on development times in degree-days ($DD_{8.8}$) for male and female *Ae. albopictus*

Variable	Male			Female		
	n	Mean $DD_{8.8}$	SE	n	Mean $DD_{8.8}$	SE
Larval density (no. larvae/200 ml)						
20	8	145.1a	2.9	8	170.9a	10.3
200	8	154.6ab	5.6	8	233.3b	28.7
800	8	171.3b	7.6	8	223.7b	21.9
Food ration (mg/larva)						
0.5	12	164.2a	6.7	12	252.0a	19.7
2.0	12	149.8a	2.9	12	166.6b	4.0
Proportion of conspecific larvae[a]						
0.25	6	155.0a	7.0	6	188.0a	19.3
0.5	6	149.6a	3.2	6	193.7a	20.7
0.75	6	154.0a	3.9	6	204.6a	21.0
1.0	6	169.3a	12.5	6	250.9b	38.7

Means within each level of each variable for each sex followed by the same letter are not significantly different by an LSD test ($P > 0.05$). n, Represents the number of treatments.

[a] Larval proportion for *Ae. albopictus* was calculated as the initial number of *Ae. albopictus* larvae/initial total number of larvae per container.

fixed levels of the other. Fisher protected least significant difference (LSD) tests (Steel et al. 1997) were used for planned comparisons to determine the statistical significance of differences between mean DD_{T_0} and survival probability values for the treatment variables examined. Single degree of freedom tests (Neter et al. 1996) were conducted across the levels of some variables at fixed levels of an opposing variable to determine if linear, quadratic or lack-of-fit effects were achieved in DD_{T_0} and survival probabilities.

Results

Thermal Coefficients. Temperature thresholds (T_0) for initiation of larval development were estimated to be 8.81 ($\pm$SE = 0.46) and 5.30 ($\pm$SE = 0.79)°C for *Ae. albopictus* and *Ae. triseriatus*, respectively. In estimating DD_{T_0} required to complete development of male and female *Ae. albopictus* and *Ae. triseriatus* for each combination of food ration, larval density and, proportion of conspecifics, 96 separate regressions were carried out. All regression coefficients were significantly different from zero ($P < 0.01$). The regression models explained a biologically significant amount of variation in DD_{T_0}, because coefficients of determination (R^2) ranged from 0.59 to 0.99. Degree-days required to complete development of *Ae. albopictus* varied from 132.3 to 220.9 $DD_{8.8}$ and 143.8 to 387.2 $DD_{8.8}$ for males and females, respectively. For *Ae. triseriatus*, degree-days varied from 229.4 to 853.9 $DD_{5.3}$ and 304.9 to 990.1 $DD_{5.3}$ for males and females, respectively.

Effects of Food, Density, and Proportion of Conspecific Larvae on DD_{T_0}. In addition to sex, larval density and food ration had highly significant ($P < 0.005$) main effects on DD_{T_0} for both species (Table 1). The proportion of conspecifics significantly affected ($P < 0.05$) only the DD_{T_0} of *Ae. albopictus* immatures. However, the proportion of conspecific larvae and food had significant nonadditive effects on the degree-days required to complete development of immatures of both species.

Density-dependent factors affected the response of immatures to temperature differently for each sex of *Ae. albopictus*, but not of *Ae. triseriatus* (Table 1). Generally, *Ae. albopictus* males were less responsive to changes in conditions than were females. Estimates of $DD_{8.8}$ for males increased in a linear fashion ($F = 6.89$; df = 1, 23; $P = 0.015$) over the 3 larval densities; however, for females, effects of larval density on $DD_{8.8}$ were curvilinear (linear effect: $F = 14.56$; df = 1, 23; $P = 0.0009$; quadratic effect: $F = 30.69$, df = 1, 23; $P = 0.0001$) (Table 2). Increasing food did not affect $DD_{8.8}$ for males, but for females, $DD_{8.8}$ declined significantly at the high food ration. Heterospecific interactions did not appear to affect the development time of males, but females exhibited a curvilinear response (linear effect: $F = 30.02$; df = 1, 23; $P = 0.0001$; quadratic effect: $F = 6.19$; df = 1, 23; $P = 0.021$) as the proportion of conspecifics increased with the highest mean $DD_{8.8}$ value estimated for pure species cultures.

In contrast, *Ae. albopictus*, effects of larval density, food, and the proportion of conspecific larvae on DD_{T_0} for *Ae. triseriatus* were independent of sex (Table 1). A strong curvilinear response in $DD_{5.3}$ over the 3 levels of density was exhibited by *Ae. triseriatus* males (quadratic effect: $F = 69.09$; df = 1, 23; $P = 0.0001$) and females (quadratic effect: $F = 100.50$; df = 1, 23; $P = 0.0001$) (Table 3). Highest values for both males and females were estimated at the intermediate density of 200 larvae per container. Increasing the food ration significantly lowered the estimated development time in degree-days for both males and females. The response curve for mean $DD_{5.3}$ values of males did not assume any specific functional form as the proportion of conspecific larvae increased. However, $DD_{5.3}$ values of females increased in a linear fashion ($F = 6.15$; df = 1, 23; $P = 0.021$) as the proportion of conspecific larvae increased.

Table 3. Effects of larval density, per capita food ration, and proportion of conspecific larval density on development times in degree-days ($DD_{5.3}$) for male and female *Ae. triseriatus*

Variable	Male			Female		
	n	Mean $DD_{5.3}$	SE	n	Mean $DD_{5.3}$	SE
Larval density (no. larvae/200 ml)						
20	8	286.2a	11.7	8	387.8a	26.7
200	8	511.9b	89.0	8	652.5b	97.4
800	8	381.2c	49.7	8	469.8c	65.4
Food ration (mg/larva)						
0.5	12	513.5a	57.8	12	651.8a	65.2
2.0	12	272.7b	7.6	12	355.0b	11.8
Proportion of conspecific larvae[a]						
0.25	6	375.0a	52.9	6	471.6a	66.9
0.5	6	408.0a	85.7	6	488.6ab	89.8
0.75	6	391.8a	94.2	6	505.9ab	100.4
1.0	6	397.7a	90.0	6	547.4b	115.9

Means within each level of each variable for each sex followed by the same letter are not significantly different by an LSD test ($P > 0.05$). n, Represents the number of treatments.

[a] Larval proportion for *Ae. triseriatus* was calculated as the initial number of *Ae. triseriatus* larvae/initial total number of larvae per container.

For both species, density significantly affected development times of immatures. Over the 3 larval densities, significantly lower values of DD_{T_0} were estimated when larvae were least crowded. Generally, the highest values of DD_{T_0} were estimated for larvae at the intermediate density. Increasing per capita food ration ameliorated the effects of density on development time. At each density of *Ae. albopictus* and *Ae. triseriatus*, estimates of development times decreased when larvae were provided the high food ration (Fig. 1 and 2), although decreases were most substantial at higher densities. Estimates of $DD_{5.3}$ over the 3 densities of *Ae. triseriatus* larvae at the high food ration were not significantly different. However, for *Ae. albopictus* at the high food ration, differences in larval development times between the highest and lowest densities declined, but remained significant.

Mean development times of immatures of both species were lower in the presence of heterospecific larvae than when larvae were reared in pure cultures of conspecifics and provided the low food ration (Figs. 1 and 2). Degree-days required to complete development for *Ae. albopictus* increased in a curvilinear fashion over the 4 proportions of conspecific larvae evaluated. Both linear ($F = 31.69$; df = 1, 23; $P = 0.0001$) and quadratic ($F = 12.27$; df = 1, 23; $P = 0.0019$) effects were significant. In comparison, estimates of DD_{T_0} for *Ae. triseriatus* increased in a linear fashion ($F = 22.65$; df = 1, 23; $P = 0.0001$).

Survival Probabilities. ANOVA indicated that among the temperatures evaluated, density and food exerted significant differential effects ($F = 8.58$; df = 4, 207; $P = 0.0001$) on survival probabilities of *Ae. albopictus* immatures. In comparison, nonadditive temperature-dependent effects of density and food on survivorship responses of immature *Ae. triseriatus*

were not found ($F = 1.42$; df = 4, 207; $P = 0.23$) (Fig. 3) in our experiments. Generally, regardless of the level of density or food ration, *Ae. albopictus* immatures exhibited higher survivorship in comparison to *Ae. triseriatus.* The probability of survival of *Ae. albopictus* immatures at 15°C was significantly lower than at the 2 higher temperatures under conditions of low density and low food. Conversely, survival of *Ae. triseriatus* was lowest at 31°C under the same conditions of density and food. At the lowest density, increasing food substantially improved survivorship of *Ae. albopictus* larvae at 15°C, but not at the higher temperatures. In fact, survivorship of immatures at the 2 highest temperatures declined slightly under high food conditions. In contrast, for *Ae. triseriatus* at the lowest density, increasing the food ration improved survivorship of immatures equivalently for all 3 temperatures.

Higher survivorship of *Ae. albopictus* under all conditions of food and density was reflected in a higher standing crop production of adults compared with *Ae. triseriatus* (Fig. 4). Production of adults of both species increased substantially under high density conditions when the food ration was increased. Comparable mean numbers of adults were produced per container under high food conditions for both species at all 3 densities (Fig. 4).

Generally, survival probabilities for both species over all experimental conditions were lowest when the proportion of conspecifics was highest. The 4 proportions of conspecific larvae evaluated in our experiments did not have significant differential impacts on survival probabilities for *Ae. albopictus* ($F = 1.61$; df = 3, 207; $P = 0.188$); however, for *Ae. triseriatus* ($F = 4.77$; df = 3, 207; $P = 0.0031$) survivorship of immatures at the lowest proportion of conspecifics was significantly higher than at other proportions. For both species, heterospecific interactions did not have significant nonadditive effects in combination with food or density on the survival probabilities of *Ae. albopictus* (proportion × food, $F = 2.01$; df = 3, 207; $P = 0.11$; proportion × density, $F = 0.33$; df = 6, 207; $P = 0.24$; proportion × food × density, $F = 0.24$; df = 6, 207; $P = 0.65$) or *Ae. triseriatus* (proportion × food, $F = 0.49$; df = 3, 207; $P = 0.69$; proportion × density, $F = 1.64$; df = 6, 207; $P = 0.14$; proportion × food × density, $F = 0.68$; df = 6, 207; $P = 0.66$). Nonetheless, temperature-dependent effects of the proportion of conspecifics on survival probabilities were found for *Ae. albopictus* ($F = 3.22$; df = 6, 207; $P = 0.0048$), but not found for *Ae. triseriatus* ($F = 0.76$; df = 6, 207; $P = 0.60$) (Fig. 5). At 15°C survival probabilities increased as the proportion of *Ae. albopictus* conspecifics increased. At the 2 higher temperatures, survival probabilities either did not vary or declined as the proportion of conspecifics increased. In general, *Ae. triseriatus* exhibited the same response form for survival probabilities over the proportion of conspecifics at each temperature (Fig. 5). Survival probabilities declined as the proportion of conspecifics increased. Lowest survival probabilities were exhibited at 31°C.
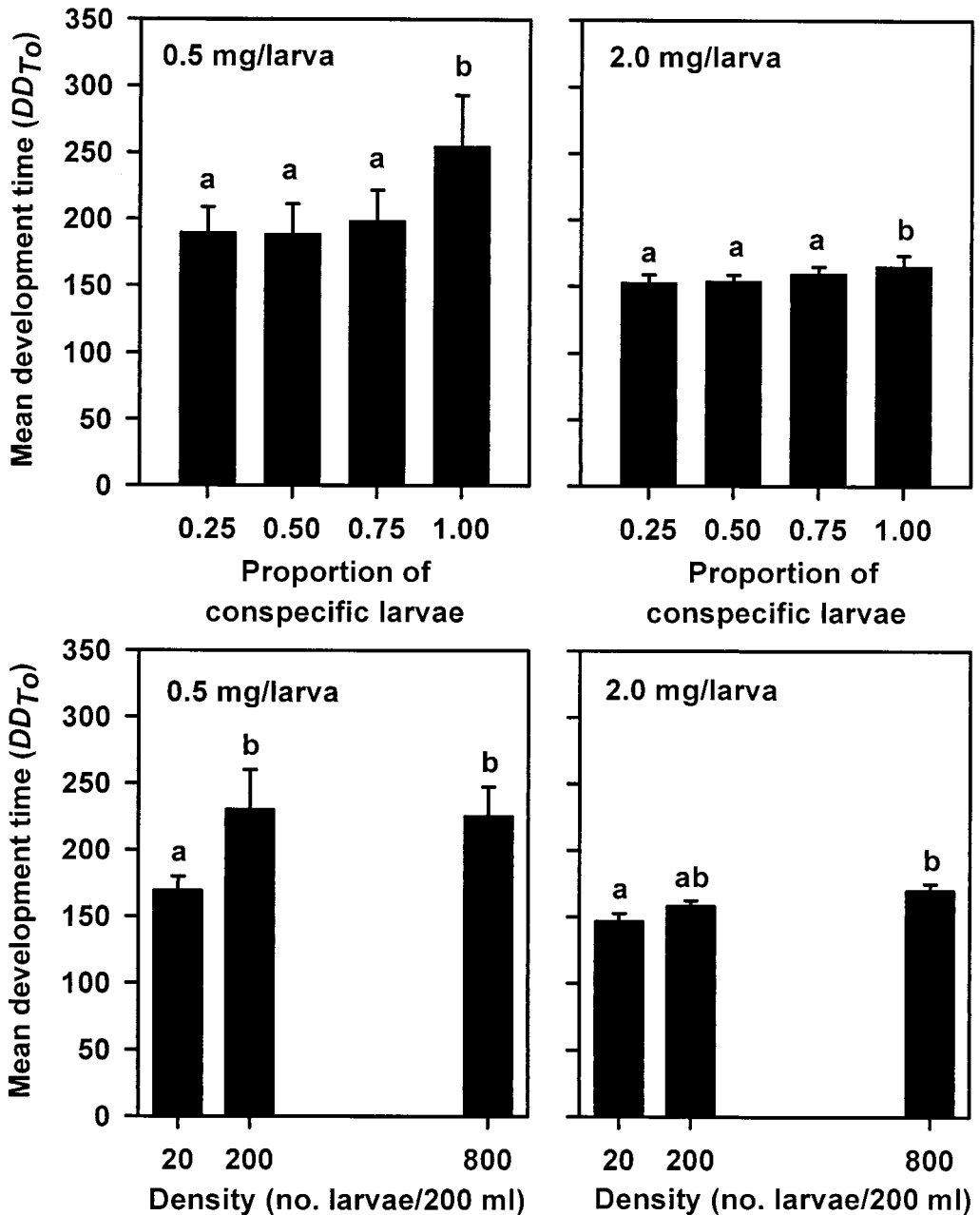
Fig. 1. Effects of conspecific larval density ($n = 8$) and the proportion of conspecific density and heterospecific larval density ($n = 6$) on larval development time in degree-days ($DD_{8.8}$) for *Ae. albopictus* at a low and high per capita food ration. Means for each food ration within each level of each variable followed by the same letter are not significantly different at $P = 0.05$ by an LSD test. Bars are 1 SE of mean $DD_{8.8}$.

Prediction of Field Populations. From our degree-day model, *Ae. triseriatus* females were predicted to have emerged in late April of both years after an accumulation of 304 DD (Fig. 6). Our model predicted that *Ae. albopictus* females would have emerged ≈1 mo later in late May or early June after 160 DD had accumulated (Fig. 7).

Discussion

Effects of Temperature on Development Time and Survival Probabilities of Immatures. Few previous studies of effects of temperature on degree-days required to complete development of *Ae. triseriatus* and *Ae. albopictus* immatures have been conducted. Jalil
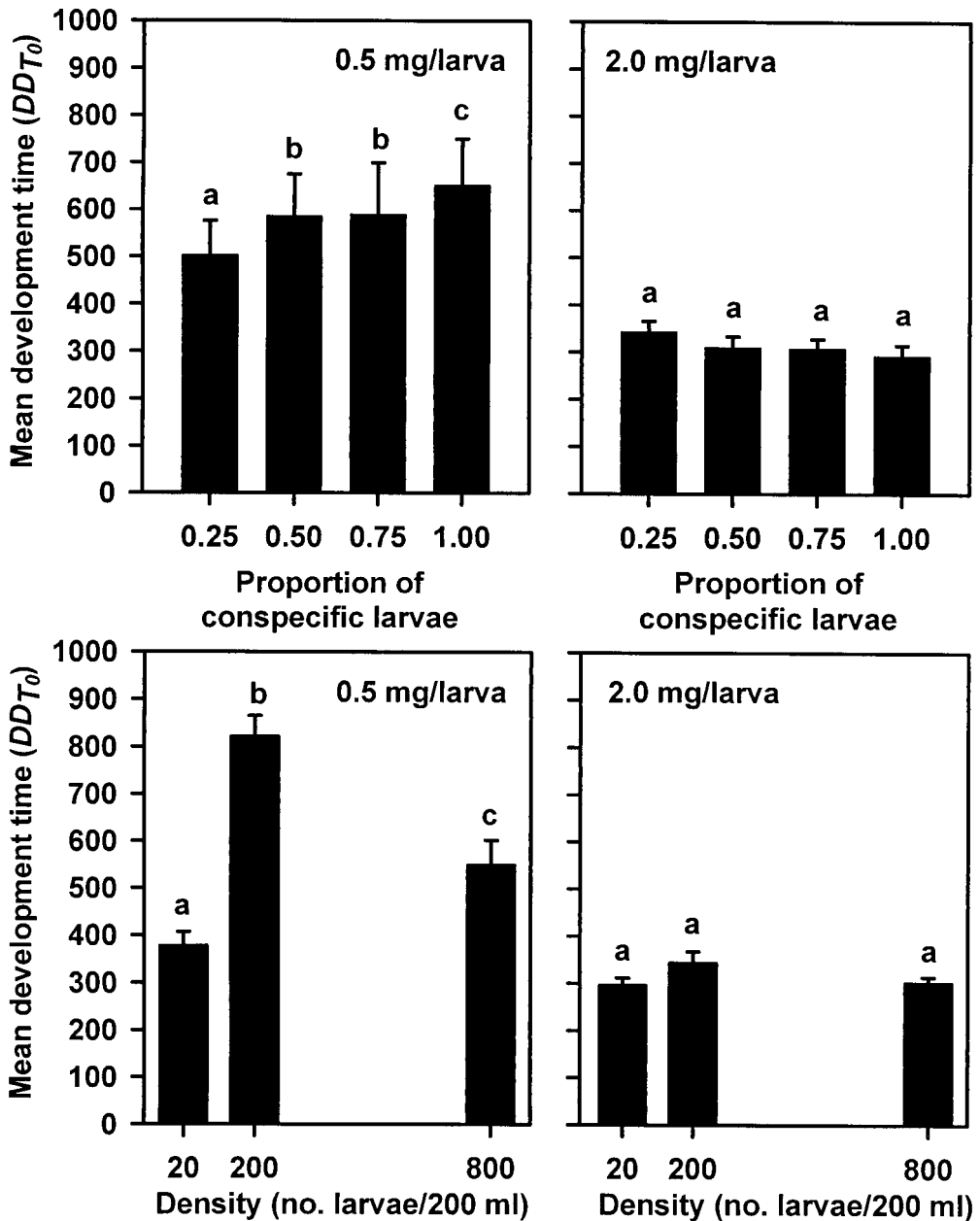
Fig. 2. Effects of conspecific larval density ($n = 8$) and the proportion of conspecific density and heterospecific larval density ($n = 6$) on larval development time in degree-days ($DD_{5.3}$) for *Ae. triseriatus* at a low and high per capita food ration. Means for each food ration within each level of each variable followed by the same letter are not significantly different at $P = 0.05$ by an LSD test. Bars are 1 SE of mean $DD_{5.3}$.

(1972) reported that some larvae initiated development at 9°C. A T_0 of 12°C was extrapolated for *Ae. triseriatus* that were reared individually in test tubes at a density of 1 larva per 2.3 ml of water, and provided fresh water and food daily. Calculated values of DD_{T_0} for *Ae. triseriatus* in Jalil's study ranged from 140 to 160 $DD_{12.0}$ compared with 229–944 $DD_{5.3}$ in our study.

Estimates of T_0 (5.3°C) and $DD_{5.3}$ in our study probably differed because Jalil's estimates were derived over a much narrower range of conditions of food and larval density, and also because Jalil used development times for the first 50% of larvae completing development instead of all larvae in each experimental replicate. Differences in the strain of *Ae. triseriatus* also
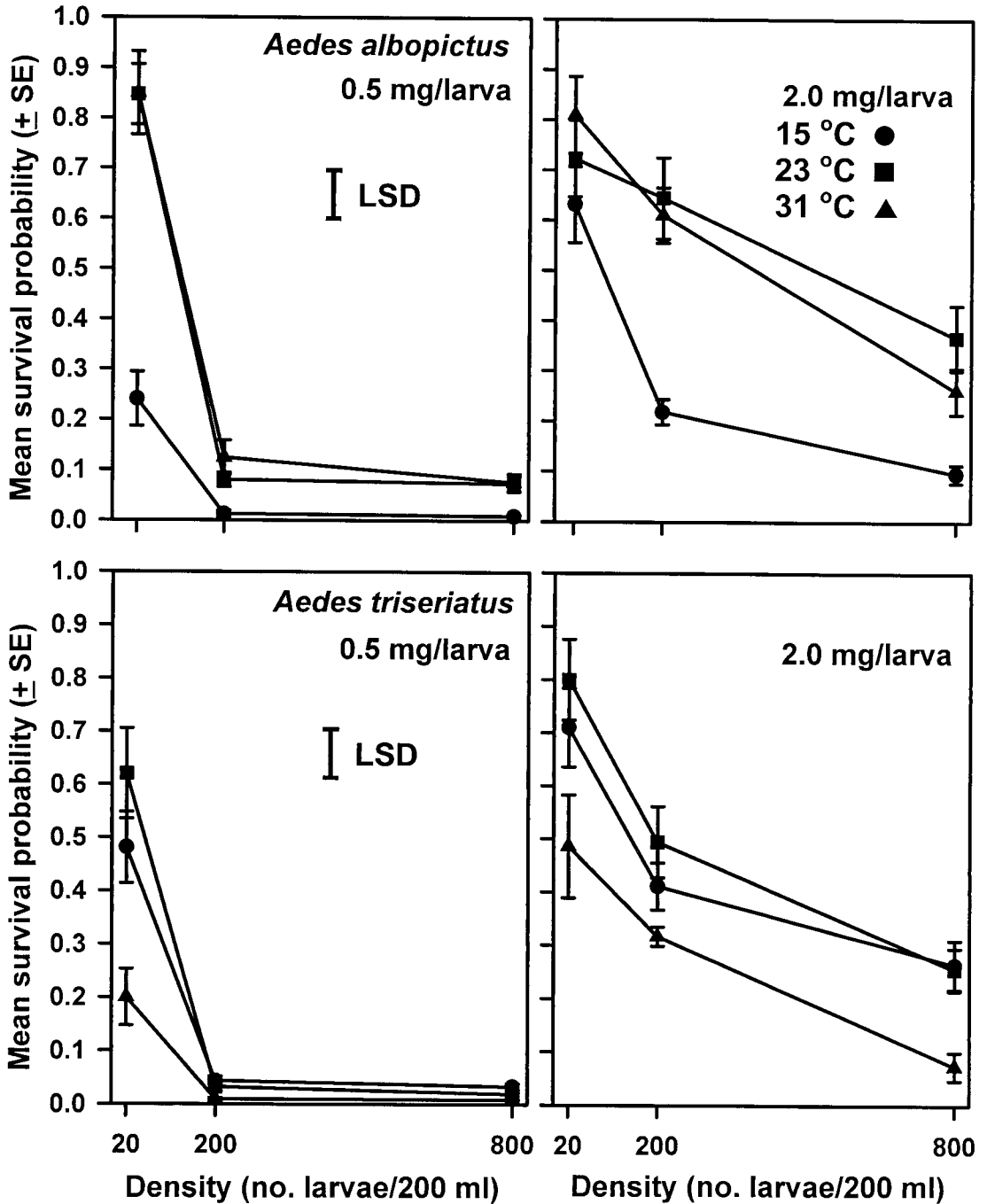
Fig. 3. Effects of larval density and food ration on the temperature-dependent survivorship of *Ae. albopictus* and *Ae. triseriatus* immatures. Survival probabilities were subjected to an arcsine $\sqrt{y}$ transformation before data were analyzed. Means are back-transformed.

may have accounted for differences in estimates of T_0. Jalil used a strain of *Ae. triseriatus* that was originally collected in Alabama. Livdahl (1984) found that 2 different stains of *Ae. triseriatus*, one from Connecticut and the other from North Carolina, exhibited markedly different larval development times under the same conditions.

Udaka (1959) reported that for a Japanese strain of *Ae. albopictus* larval development ceased at 11°C which is comparable to the T_0 (8.8°C) of our study. For
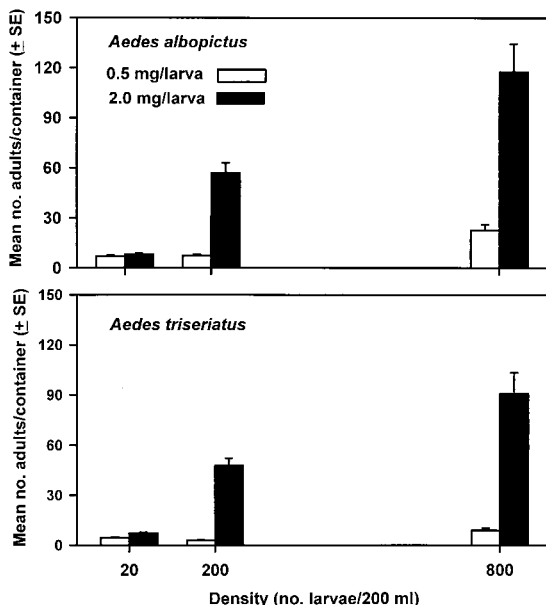
Fig. 4. Effects of density and food ration on the standing crop production of adult *Ae. albopictus* and *Ae. triseriatus.*

Ae. albopictus, Hien (1975) reported development of larvae required 4–5 d at 30°C, 5–8 d at 25°C, and 7–12 d at 20°C.

Aedes albopictus and *Ae. triseriatus* exhibited contrasting survivorship responses to temperature. *Aedes*
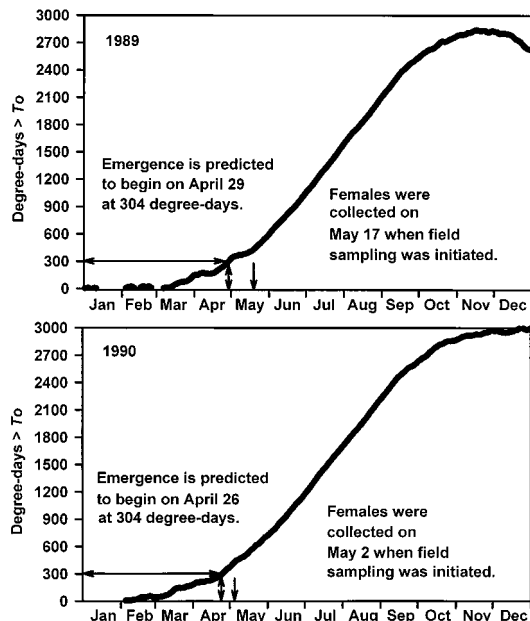

Fig. 5. Effects of the proportion of conspecific larvae on the temperature-dependent survivorship of *Ae. albopictus* and *Ae. triseriatus* immatures. Survival probabilities were subjected to an arcsine $\sqrt{y}$ transformation before data were analyzed. Means are back-transformed.

Fig. 6. Accumulated degree-days that exceed the threshold temperature for initiation of development ($T_0 = 5.3$°C) of *Ae. triseriatus* larvae. *Ae. triseriatus* females are predicted to first emerge on 29 April 1989 and 26 April 1990, based on a development time of 304 $DD_{5.3}$. Females were reported by Szumlas et al. (1996a) to have been collected on 17 May 1989 and 2 May 1990.
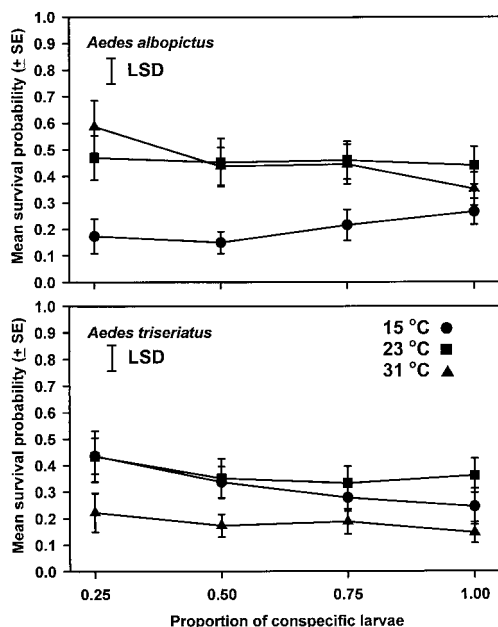
albopictus manifested highest survivorship at the intermediate and higher temperatures, whereas *Ae. triseriatus* exhibited higher survivorship at lower and intermediate temperatures. Temperature sensitivities exhibited by both species are congruent with estimates of their T_0.

Effects of Food Ration and Larval Density on DD_{T_0} and Survival Probabilities of Immatures. Larvae of *Ae. albopictus* and *Ae. triseriatus* responded to changes in food and density in a comparable fashion. For both species, DD_{T_0} significantly declined when the per capita amount of food was increased fourfold from 0.5 to 2.0 mg per larva. A comparable effect of increasing food availability on larval development time has been reported previously for other container-inhabiting *Aedes* mosquitoes (Wada 1965, Keirans and Fay 1968, Hard et al. 1989). Larval density affects population growth by lowering survivorship and slowing development times (Hawley 1985). Density can exert an indirect effect on development times by mediating the availability of food resources (Hard et al. 1989, Focks et al. 1993) or act directly through growth retardants (Dye 1982), by physical contact (Broadie and Bradshaw 1991) or through predation (Livdahl 1982). In fully factorial studies, food and density have exerted both additive and multiplicative effects on development times and other fitness traits of larvae (Livdahl 1982, Carpenter 1983, Hard et al. 1989, Focks et al. 1993, Léonard and Juliano 1995, Walker et al. 1997, Lord 1998). In our study, the interaction of food and
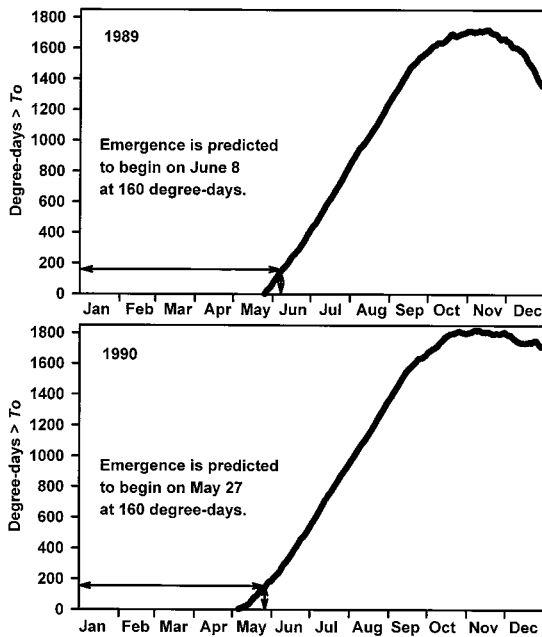
Fig. 7. Accumulated degree-days that exceed the threshold temperature for initiation of development ($T_0 = 8.8°C$) of *Ae. albopictus* larvae. *Ae. albopictus* females are predicted to 1st emerge on 8 June 1989 and 27 May 1990, based on a development time of 160 $DD_{8.8}$.

density were highly significant for both species. Estimates of DD_{T_0} for both species increased in a curvilinear fashion as density increased with highest values of DD_{T_0} exhibited at the intermediate density under low food conditions. Mean survival probabilities for both species at the intermediate density were ≈1.4 times lower than for immatures surviving high density conditions; however, mean standing crop production of adults was threefold lower for both species than at the highest density. Lower larval densities should have reduced competition for food, resulting in smaller mean values of DD_{T_0} for immatures surviving at the intermediate density. Increased development times at the intermediate density could not have resulted from chemical interference, because microcosms stocked at higher densities of larvae exhibited lower mean values of DD_{T_0}. The reasons for the slower development times exhibited by both species under conditions of intermediate density and low food are not presently understood.

In our experiments, *Ae. triseriatus* larvae appeared to be affected more by changes in food availability than density. Increasing the food ration negated the effects of density on $DD_{5.3}$. In contrast, values of $DD_{8.8}$ of *Ae. albopictus* immatures in our experiments seemed to be more sensitive to changes in density than food. Even at the high food ration, mean $DD_{8.8}$ values for the lowest and highest densities were significantly different. The sensitivity of *Ae. triseriatus* to food availability may relate to its weaker ability to withstand starvation relative to *Ae. albopictus* (Barrera 1996).

Effects of Heterospecific Interactions on DD_{T_0} and Survival Probabilities. Generally, under our experimental conditions, conspecific interactions exerted greater effects on DD_{T_0} than did heterospecific interactions. For *Ae. triseriatus*, increasing the per capita amount of food rendered differences among $DD_{5.3}$ values insignificant across all proportions of conspecific larvae evaluated. Likewise, increasing food availability diminished deleterious effects of conspecific interactions on $DD_{8.8}$ for *Ae. albopictus*, but the mean $DD_{8.8}$ was still significantly higher with conspecifics than estimates for experiments involving heterospecific larvae. It is noteworthy that some temperature-dependent heterospecific effects on survival of *Ae. albopictus* immatures were found at the lowest temperature, indicating that at low temperatures, the presence of *Ae. triseriatus* would have deleterious effects on survival of *Ae. albopictus* immatures.

Comparable studies of heterospecific interactions over multiple temperatures are lacking. In single temperature studies, involving both species, *Ae. albopictus* has been found to be the superior competitor. Ho et al. (1989) reported *Ae. triseriatus* to exhibit a longer developmental period and lower larval survivorship in mixed cultures with *Ae. albopictus* and *Ae. aegypti*. The lower competitiveness of *Ae. triseriatus* compared with the other species was attributed, in part, by Ho et al. (1989) to "the high temperature during the experimental period . . ." In their low-diet treatment, Novak et al. (1993) found *Ae. albopictus* was a stronger competitor than *Ae. triseriatus*. However, experiments were conducted at 27°C that would likely have favored *Ae. albopictus*. Differences in resource utilization have been postulated by Livdahl and Willey (1991) to explain the outcome of laboratory competition between these 2 *Aedes* species. At 22°C, using tire and treehole fluids in experimental microcosms, *Ae. albopictus* was predicted to coexist with *Ae. triseriatus* in treehole habitats, but competitively exclude *Ae. triseriatus* from tires.

Equivocal conclusions can be drawn concerning the outcome of heterospecific mosquito interactions from previous investigations that involved at least one of the species in our experiments. In some studies, conspecific competition was reported to be more important in mediating population growth or larval performance than were heterospecific interactions (Black et al. 1989, Copeland and Craig 1992, Washburn and Hartmann 1992). Results of other investigations indicate that heterospecific interactions from resource competition or chemical interference can have differential deleterious effects on life history and fitness traits of container-inhabiting mosquitoes (Wilton 1968, Moore and Fisher 1969, Lowrie 1973, Juliano 1996).

Prediction of Field Populations. Unquestionably, the population dynamics of natural populations of both mosquitoes are governed by a variety of interacting environmental factors. The growth response of *Ae. albopictus* and *Ae. triseriatus* immatures to temperature was density dependent but significantly influenced by the availability of food resources. Interactions with heterospecific larvae did not prolong the

DD_{T_0} of either species; however, survival of *Ae. albopictus* immatures was lowest under low temperature conditions when the proportion of heterospecific larvae was highest. Our results indicate that the presence of *Ae. triseriatus* would not likely impede the establishment, but may affect the seasonal rate of population growth of *Ae. albopictus* in the mountains of North Carolina. Admittedly, we only examined 2 fitness traits, development time and survivorship of immatures, in single cohort experiments. Albeit, under field conditions, when combined with the deleterious effects of overwinter egg mortality on the size of the base population of *Ae. albopictus* larvae and a higher threshold temperature of larval development, competition from *Ae. triseriatus* may suppress the rate of growth of *Ae. albopictus* populations early in the mosquito season until populations with cold tolerance characteristics comparable to *Ae. triseriatus* are selected. Whether lower overwintering egg mortality augments the competitive ability of *Ae. triseriatus*, as has been previously suggested (Hanson and Craig 1995), in locales where *Ae. albopictus* experiences high egg mortality certainly merits further research.

In the field, egg diapause for both species would terminate in spring (eggs containing pharate larvae would hatch) after prolonged exposure to low winter temperatures (Shroyer and Craig 1983). Larval growth would start when temperature thresholds for initiation of development are exceeded. From our degree-day models, *Ae. triseriatus* larvae are predicted to have a larger DD_{T_0} but a lower T_0 than *Ae. albopictus* larvae. These results indicate that the overwintering generation of *Ae. triseriatus* would initiate development earlier in the season and that *Ae. triseriatus* adults would be more abundant than *Ae. albopictus* adults in the spring. Because of the smaller mean DD_{T_0}, *Ae. albopictus* population growth is predicted to exceed that of *Ae. triseriatus* later in the season. Unfortunately, there is scant direct evidence to support our prediction. Results of a field survey reported by Moore et al. (1990) indirectly confirm our prediction. *Ae. albopictus* was geographically most abundant in the southern United States from July to September, whereas *Ae. triseriatus* larvae were collected most frequently from more northern locales during the same time period. In other investigations, *Ae. triseriatus* occurred early in the spring when temperatures were ≈17°C (Bradshaw and Holzapfel 1984, Haramis 1984).

The predicted emergence dates for *Ae. triseriatus* appear to be accurate, because Szumlas et al. (1996a) reported collecting small numbers of females in vacuum aspiration samples from vegetation when field sampling was initiated on 17 May 1989 and 2 May 1990. It should be noted that our estimates of emergence dates are based on air temperatures, which would differ from the water temperature in containers (Cheng et al. 1998). Changes in the water temperature in artificial containers and treeholes would likely lag behind changes in air temperature during spring. Consequently, we may be underestimating the spring emergence dates of *Ae. triseriatus*.

According to Hawley et al. (1989), larval mortality, fecundity, and voltinism influence the level of overwintering egg survival required for populations to rebound to prewinter levels. Our experimental findings indicated that larval development times, resulting from the independent and combined effects of temperature, per capita food ration and larval density, also would affect the population dynamics of mosquito species. Despite their ability to produce diapausing, cold hardy eggs in fall, both *Ae. albopictus* and *Ae. triseriatus* would be expected to experience some mortality of overwintering eggs in the mountains of North Carolina. Based on comparative experiments conducted in Indiana at a latitude of 39° N (Hawley et al. 1989; Hanson and Craig 1994, 1995), *Ae. albopictus* would probably exhibit lower levels of survival of overwintering eggs than would *Ae. triseriatus*, resulting in a smaller base population of larvae in spring. Consequently, in western North Carolina, to achieve fall population levels comparable to or exceeding those of *Ae. triseriatus*, *Ae. albopictus* larvae would have to exhibit higher rates of larval development and survival to compensate for lower overwinter survival of eggs. In our study, optimal development times of *Ae. albopictus* were ≈2 times that of *Ae. triseriatus* larvae. However, based on a higher T_0, *Ae. albopictus* was predicted to initiate development ≈1 mo later than *Ae. triseriatus*. In fall, population growth of both species would decline as the critical photoperiod that induces egg diapause is reached. Based on critical photoperiods (Shroyer and Craig 1983, Focks et al. 1994) and day lengths (Meeus 1982), in western North Carolina at a latitude of 35° N, *Ae. albopictus* females are predicted to produce diapausing eggs in late July, whereas eggs of *Ae. triseriatus* are predicted to enter diapause in mid-August. For *Ae. albopictus*, the critical photoperiod would be expected to vary between populations at the same latitude based on elevation (Focks et al. 1994) and within populations based on larval nutrition (Pumpuni et al. 1992). Effects of elevation and larval nutrition on the critical photoperiod for *Ae. triseriatus* egg diapause are presently unknown. Our estimated dates of spring emergence and diapause would allow ≈2 and 3.5 mo for *Ae. albopictus* and *Ae. triseriatus* population growth, respectively. It is unknown whether the faster development of *Ae. albopictus* immatures could compensate for lower survival of overwintering eggs and a shorter growth season.

Results of our laboratory study provide insight into the population dynamics of *Ae. albopictus* and *Ae. triseriatus* that potentially would occur under field conditions. Our estimate of degree-days required to complete larval development and accumulated degree-days on dates of collection of females reported by Szumlas et al. (1996a) are sufficiently close to indicate that accumulated degree-days can be used to predict the date of spring emergence of *Ae. triseriatus* females. The utility of the thermal summation approach to predicting the seasonal occurrence of *Ae. albopictus* remains to be established. In this regard, results of field studies that are currently being conducted with sym-

patric populations of these mosquitoes should be informative.

Acknowledgments

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