

Effects of Developmental Asynchrony Between *Aedes triseriatus* (Diptera: Culicidae) and its Predator *Toxorhynchites rutilus* (Diptera: Culicidae)

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ABSTRACT Newly hatched *Toxorhynchites rutilus* (Coquillett) were added to experimental populations of *Aedes triseriatus* (Say) at varying days after prey hatch to test the hypothesis that a developmental asynchrony of *Ae. triseriatus* and *Tx. rutilus* leads to escape from predation by *Ae. triseriatus* in small water bodies. Presence of *Tx. rutilus* significantly affected prey survivorship. Regression of survivorship [$\log_{10}(x + 1)$ transformed] versus days head start for prey yielded a small, but significant positive slope, indicating that survivorship increased slightly with an increasing number of days head start. For females, mean weight at emergence was not significantly affected by treatments; however, median days to emergence differed significantly between the treatments, with females taking significantly longer to emerge with *Tx. rutilus* absent than when the predator was present. For males, neither mean mass nor median days to emergence was significantly affected by treatments. Treatments had no significant effect on the frequency of deaths or on mean weight of *Tx. rutilus*. Thus, a developmental asynchrony between *Tx. rutilus* and *Ae. triseriatus* appears to have no effects on the predator, but does have a weak effect on prey performance at high hatch densities.

KEY WORDS *Toxorhynchites rutilus*, *Aedes triseriatus*, developmental escape, predation, ontogenetic stage, phenology

Aedes triseriatus (Say) is a tree hole mosquito distributed throughout the eastern United States (Darsie and Ward 1981). In the southern part of its range, *Ae. triseriatus* cooccurs with a predatory mosquito, *Toxorhynchites rutilus* (Coquillett) (Bradshaw and Holzapfel 1988). *Tx. rutilus* can cause considerable mortality of *Ae. triseriatus* populations (Bradshaw and Holzapfel 1988); however, most studies on this predator-prey system have used *Tx. rutilus* and *Ae. triseriatus* in developmental synchrony (e.g., Russo 1986, Juliano 1989, Juliano and Reminger 1992). Such synchrony in the development of this predator and prey may not always occur in nature. In fact, overwintering and 1st summer generations are somewhat asynchronous (Bradshaw and Holzapfel 1984). The containers in which these 2 species naturally occur are subject to frequent drying and reflooding (Bradshaw and Holzapfel 1983). Upon reflooding, *Ae. triseriatus* larvae hatch from dormant eggs, whereas *Tx. rutilus* must colonize the containers later, hatching from eggs oviposited only after the container has flooded (Bradshaw and Holzapfel 1983, Lounibos 1985). This natural pattern results in a developmental head start for *Ae. triseriatus*, so that *Tx. rutilus* hatch into an environment with many potential prey that are older and larger than themselves. Because *Toxorhynchites* primarily take prey smaller

than themselves (Steffan and Evenhuis 1981), it is important to understand the impact of this developmental asynchrony on this predator-prey interaction.

Although the effects of differences in ontogenetic stage and size on predator-prey interactions are probably widespread, particularly in aquatic systems (Wilbur 1988), such effects have been studied systematically in only a few insect and amphibian systems (e.g., Thompson 1975; Lounibos 1979, 1985; Alford 1989; Sredl and Collins 1991; Blaustein and Margalit 1996). Even these limited data show that asynchrony can radically alter prey death rate, predator feeding rate, or effects of predation (Thompson 1975, Lounibos 1985, Alford 1989, Sredl and Collins 1991), and in some cases alter which member of a species pair is predator and which is prey (Blaustein and Margalit 1996).

In this article we test the hypothesis that a developmental asynchrony of *Ae. triseriatus* and *Tx. rutilus* leads to an escape from predation and increased survivorship relative to synchronous development.

Materials and Methods

Aedes triseriatus used in this experiment were the progeny of mosquitoes collected near Vero Beach, FL, reared to adulthood, and allowed to mate freely in a 0.6-m³ colony cage (Juliano 1989, Juliano and Reminger 1992). *Tx. rutilus* were collected in tires

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at the Florida Medical Entomology Laboratory, Vero Beach, FL, as eggs 1–2 d before the experiment. The experiment was carried out in a walk-in incubator, at 27°C and a photoperiod of 14:10 (L:D) h. The experiment was conducted in 2 runs, started at different times. Three replicates were part of run 1 (started 11 June 1993), whereas 4 replicates were part of run 2 (started 23 June 1993). Twenty-four hours before day 0, *Ae. triseriatus* eggs were hatched synchronously (Novak and Shroyer 1978). On day 0, 100 newly hatched *Ae. triseriatus* larvae were placed into 400-ml beakers containing 340 ml of tap water and 2 g (dry mass) of live oak, *Quercus virginiana*, leaf litter that had soaked for 4 d. The density used in this experiment simulated a large hatch after a reflooding event. This hatch density (≈ 29 larvae per 100 ml) corresponds roughly to the 75th percentile of hatching densities observed after major reflooding of natural oak tree holes near Vero Beach (L. P. Lounibos, personal communication) and is close to observed mean crowding of 27/100 ml in tree holes in northern Florida (Bradshaw and Holzapfel 1983). One newly hatched *Tx. rutilus* larva was added at 1 of 5 times: day 0, day 3, day 6, day 9, day 12, or never. Numbers of replicates in each treatment were determined by the numbers of *Tx. rutilus* larvae that hatched on a particular addition day. After *Tx. rutilus* were added, they were monitored daily for survival and instar. If mortality of *Tx. rutilus* occurred in a particular replicate, a *Tx. rutilus* larva of equivalent instar and size was added to that replicate. On days 5, 10, and 15 containers were emptied and the *Ae. triseriatus* larvae counted by instar. Treatments were monitored daily for the emergence of adults, which were collected and dried in vials labeled for treatment, sex, and days to emergence. This continued until either all larvae had been consumed or had emerged as adults. Remaining *Tx. rutilus* larvae were dried after all prey larvae had been consumed. Dry masses of both species were determined to the nearest 0.1 μ g using a Cahn C31 ultra microbalance (ATI; Boston, MA).

The primary statistical analysis consisted of a mixed model analysis of variance (ANOVA) on the number of individuals surviving to the adult stage transformed as $\log_{10}(x + 1)$. Transformation was necessary because raw data did not meet ANOVA assumptions of normality and homogeneous variance. Treatment was analyzed as a fixed effect and run as a random effect. The Ryan test (SAS Institute 1987) was used for multiple comparisons to determine which treatments differed. Data on the number of individuals surviving to adulthood also were analyzed by regression versus days head start for the prey. For this analysis, survival data for prey in treatments lacking a predator were omitted, and the number surviving to adulthood also were transformed to $\log_{10}(x + 1)$ to meet the regression assumptions of normality, homogeneous variance, and linearity. Mass at and days to adulthood for *Ae. triseriatus* were analyzed using ANOVA, with the sexes analyzed separately. In preliminary analyses of mass at and days to emer-

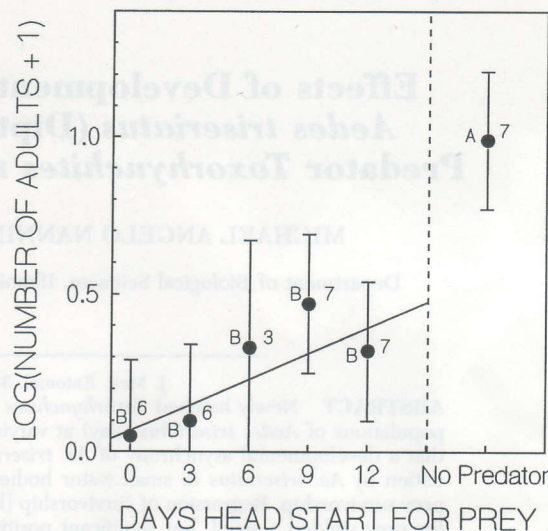


Fig. 1. \log_{10} number of surviving adults (mean \pm 2 SE) for each of the asynchrony treatments. Means preceded by same letter do not differ significantly ($P < 0.05$, Ryan test). The regression of \log_{10} (number surviving to adulthood + 1) versus days head start is also shown (solid line) for treatments containing a predator. The regression is $\log_{10}(\text{survivors} + 1) = 0.030(\text{days}) + 0.68$, or, on the original scale, survivors = $(4.79 \cdot 10^{0.030(\text{days})}) - 1$. Numbers adjacent to data points indicate the number of replicates in a particular treatment.

gence, there were no significant differences between the runs, and the 2 runs were combined.

The number of *Tx. rutilus* that died during the experiment was analyzed using a Kruskal–Wallis test because no *Tx. rutilus* died in some replicates, rendering the data irretrievably nonnormal. The mass of the 4th-instar *Tx. rutilus* in each of the treatments also was analyzed using ANOVA. Only *Tx. rutilus* surviving throughout the entire experiment were used in this analysis. Treatments were compared using the Ryan test.

Results

There was a significant effect of run on survivorship ($F = 5.38$; $df = 1, 25$; $P = 0.03$) that was a result of an overall greater survivorship in run 2. However, the interaction between treatment and run was not significant ($F = 0.41$; $df = 4, 25$; $P = 0.80$) indicating that the significant effect of treatment ($F = 21.12$; $df = 5, 4.32$; $P < 0.01$) did not differ between runs. Therefore, pairwise comparisons of survivorship were done on treatment means averaged across the 2 runs. The Ryan test indicated that the predator-free control differed significantly from all of the predator treatments, but that there were no significant pairwise differences among the predator treatments (Fig. 1). Regression indicated that there was a small but significantly positive slope of 0.030 ($SE = 0.012$) ($F = 5.88$; $df = 1, 27$; $P = 0.02$) for the \log_{10} (number surviving to adulthood + 1) versus

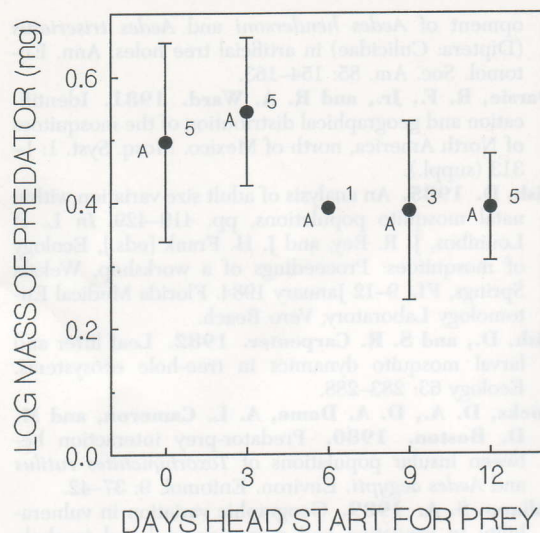


Fig. 2. Mean dry weights ± 2 SE of 4th-instar *Tx. rutilus* that survived through the entire experiment. Means do not differ significantly ($P < 0.05$, Ryan test). Numbers adjacent to data points indicate the number of replicates for a particular treatment.

days head start (Fig. 1). Though significant, this relationship was weak ($r^2 = 0.148$).

Analyses of the mean mass and median days to emergence of *Ae. triseriatus* indicated that only female median days to emergence was significantly affected by treatments ($F = 11.43$; $df = 2, 7$; $P < 0.01$). Median days to emergence for males ($F = 2.56$; $df = 5, 14$; $P = 0.08$), and mean masses of both females ($F = 0.22$; $df = 2, 7$; $P = 0.80$) and males ($F = 1.82$; $df = 5, 14$; $P = 0.17$) did not differ significantly among treatments. For females, days to emergence was significantly greater for the treatment in which no predator was added (mean \pm SE, 40.0 ± 3.0 ; $n = 7$) compared with those treatments in which the prey were given a 9-d (16.5 ± 1.5 , $n = 2$) or a 12-d (mean 15, $n = 1$) head start. These were the only treatments that yielded surviving females.

There was no significant difference in the number of *Tx. rutilus* dying among the different treatment groups (Kruskal-Wallis $\chi^2 = 5.72$; $df = 4$; $P = 0.22$) or the mass of the 4th-instar *Tx. rutilus* that survived throughout the entire experiment ($F = 3.67$; $df = 4, 22$; $P = 0.3751$) (Fig. 2).

Discussion

These results indicate that, for ≤ 12 d, and at relatively high hatch densities that are characteristic of reflooded containers, a developmental head start for *Ae. triseriatus* had a limited benefit for the survivorship of *Ae. triseriatus* exposed to *Tx. rutilus* predation. One way to interpret this is that *Ae. triseriatus* larvae never attain a size large enough to escape predation. But, considering the relative sizes of 1st-instar *Tx. rutilus* (≈ 4 mm) and 4th-instar *Ae. triseriatus* (≈ 7 mm), perhaps a better interpretation would be

that variability in growth and development among *Ae. triseriatus* larvae within a cohort enable *Tx. rutilus* to overcome the head start. When growth and development of *Ae. triseriatus* larvae vary, stragglers provide food for the small 1st-instar *Tx. rutilus* and enable the predator to grow large enough to prey upon larger *Ae. triseriatus*. Evidence for this interpretation comes from the treatment in which *Ae. triseriatus* larvae received a 12-d developmental head start. Second-instar *Ae. triseriatus* represented $\approx 21\%$ of the surviving population 2-d before the addition of *Tx. rutilus*. This represents an ample supply of small larvae for the 1st-instar *Tx. rutilus*. It also seems that, given food, *Tx. rutilus* are able to grow and develop sufficiently rapidly to close the gap between *Ae. triseriatus* and themselves, and eventually to decimate the *Ae. triseriatus* population before many adults can emerge.

These findings are important because some investigators deemphasize the importance of early instar *Toxorhynchites* in limiting populations of their prey (e.g., Focks et al. 1980). Although early instar predators might not consume as many larvae as large, late instar predators, they seem to grow and to develop faster than do *Ae. triseriatus* under high density situations, and may limit the population once they achieve their larger size. Whatever the mechanism, the limited effect of developmental asynchrony is clear. There is only a minimal advantage to a developmental head start (up to 12 d) for *Ae. triseriatus* at the high hatch densities used in our experiment. *Ae. triseriatus* survivorship seems to depend primarily on the presence or absence of this predator, and not on the number of predators.

Our study was designed specifically to simulate high density conditions that occur when there is a major hatch after a container refills. For a number of reasons, the effects of a developmental head start for *Ae. triseriatus* may be greater when hatching densities are low. At low hatching densities, growth and development rates are likely to be faster (e.g., Léonard and Juliano 1995), enhancing the chance that prey can outgrow vulnerability to the predator. Also, at low hatching densities, it is more likely that all larvae will grow and develop at near-maximal rates, resulting in lower variation in size at any one time (e.g., Fish 1985). This may limit the availability of small larvae that seem to be important as prey for young *Tx. rutilus*, and therefore limit the ability of young predators to catch up to a cohort of prey with a developmental head start. A final effect of a developmental head start relative to *Tx. rutilus* is that *Ae. triseriatus* females reach adulthood significantly faster when given a large head start compared with females developing in the absence of the predator. This may be caused by the predator consuming most of the slow-developing females before they have time to emerge, selecting for females with high growth and development rates when *Ae. triseriatus* populations are developing asynchronously with *Tx. rutilus*. This significant effect on mean development time also may indicate that although predation is very in-

tense, it is able to substantially alleviate intraspecific competition for *Ae. triseriatus* survivors. The easing of intraspecific competition has been shown to shorten the time to emergence in *Ae. triseriatus* in other studies (Fish and Carpenter 1982, Carpenter 1983, Copeland and Craig 1992, Léonard and Juliano 1995).

Although *Tx. rutilus* seems to be able to develop at a faster rate than *Ae. triseriatus*, at 27°C, a large developmental head start for prey does not seem to negatively affect *Tx. rutilus* populations by reducing the size of the *Tx. rutilus* larvae. *Tx. rutilus* also does not appear to have a greater probability of dying as the head start for the *Ae. triseriatus* increases. However, it is important to note that the effect of developmental asynchrony on *Tx. rutilus* was a secondary aspect of this study, which was directed at measuring the effect of the developmental asynchrony on *Ae. triseriatus*. An experiment specifically aimed at determining the effect of developmental asynchrony on *Tx. rutilus* would seem to be important if we are to determine what actually happens in this predator-prey interaction when the prey receive a developmental head start.

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