Simulated Seasonal Photoperiods and Fluctuating Temperatures Have Limited Effects on Blood Feeding and Life History in *Aedes triseriatus* (Diptera: Culicidae)

K. M. WESTBY^{1,2,3} AND S. A. JULIANO¹

J. Med. Entomol. 52(5): 896–906 (2015); DOI: 10.1093/jme/tjv116

ABSTRACT Biotic and abiotic factors change seasonally and impact life history in temperate-zone ectotherms. Temperature and photoperiod are factors that change in predictable ways. Most studies testing for effects of temperature on vectors use constant temperatures and ignore potential correlated effects of photoperiod. In two experiments, we tested for effects of larval rearing environments creating ecologically relevant temperatures and photoperiods simulating early and late season conditions (June and August), or constant temperatures (cool and warm) with the June or August photoperiods, respectively. We determined effects on survivorship, development, size, and a composite performance index in a temperate-zone population of *Aedes triseriatus* (Say). We followed cohorts of resulting females, all held under the same environmental conditions, to assess carry-over effects of rearing conditions for larvae on longevity, blood feeding, and egg production. Larval survivorship was affected by treatment in one experiment. Development time was greater in the June and cool treatments, but the constant and fluctuating temperatures did not differ. Significantly larger mosquitoes were produced in fluctuating versus constant temperature treatments. There were no significant treatment effects on the composite performance index. Adult female longevity was lower after rearing at constant versus fluctuating temperature, but there was no difference between June and August, nor did size affect longevity. There was no effect of treatments on blood feeding and a limited effect on egg production. We conclude that seasonal temperatures and photoperiods during development have limited effects on this population of *A. triseriatus* and find little evidence of strong effects of fluctuating versus constant temperatures.

KEY WORDS *Aedes triseriatus*, season, thermoperiod, blood feeding, longevity

Like many other infectious diseases, vector-borne diseases often show marked seasonal patterns in human incidence (Altizer et al. 2006). These seasonal patterns may be due to temperature and rainfall events that change the quantity of larval habitats or the rates of development of vectors and pathogens. Other seasonal changes may influence mosquito life history, such as pulses of terrestrial detritus inputs into larval habitats, degradation of larval resources, and photoperiodic-induced changes in adult feeding behavior (Bowen 1992, Macia and Bradshaw 2000, Bevins 2007, O'Neal and Juliano 2013). Biotic and abiotic environments of mosquitoes, in all life stages, can alter adult female production, female longevity, fecundity, blood-feeding behavior, vector competence, and vector immunity (Straif and Beier 1996, Suwanchaichinda and Paskewitz 1998, Delatte et al. 2009, Murdock et al. 2012a, Alto and Bettinardi 2013, Costanzo et al. 2013). All of these traits are thought to alter vectorial capacity, which is a

mathematical expression of the rate of new infections arising from a single infected mosquito in a given place and time (Dye 1986).

Changes in photoperiod, and to a lesser extent temperature, are two of the most reliable cues to season in temperate climates. Temperature is especially important for determining life-history characteristics for ectotherms like mosquitoes, though photoperiod can alter larval development rate, female fat body size at eclosion, and ovary size (Harwood and Halfhill 1964, Depner and Harwood 1966, Bradshaw and Lounibos 1972). Generally, hotter temperatures decrease development time and produce smaller adults (Kingsolver and Huey 2008). Most of the published studies of the effects of rearing temperature on adult characteristics have focused on changes in vector competence and a few studies have tested for combined effects of temperatures experienced during both larval and adult life stages (Kay et al. 1989, Brubaker and Turell 1998, Alto and Bettinardi 2013). Laboratory studies that test for “carry-over effects” of conditions in the larval habitat on adult longevity or fecundity have largely focused on effects of resource availability or sublethal effects of insecticide exposure (Hawley 1985; Reiskind and Lounibos 2009; Muturi et al. 2010, 2012). To our knowledge, there is only one other published study that tested for

¹ School of Biological Sciences, Illinois State University, Normal, IL 61790-4120.

² Corresponding author, e-mail: katiwestby206@gmail.com.

³ Present address: Washington University in St Louis, Tyson Research Center, 6750 Tyson Valley Road, Eureka, MO 63025.

effects of larval rearing temperature on adult longevity in mosquitoes (Alto and Bettinardi 2013). This is surprising, as both resource availability and temperature affect the size of resulting females and there is some evidence that size is related to longevity in mosquitoes (Walker et al. 1987, Landry et al. 1988, Maciel-de-Freitas et al. 2007). Further, size changes seasonally in field-collected mosquitoes (Day et al. 1990), suggesting the hypothesis that temperature and photoperiod in the larval environment drive variation in the size distribution of adults.

Many insects use cues from both temperature and photoperiod to make decisions about entering or terminating diapause, which is a mechanism that insects use to cope with harsh conditions (Tauber and Tauber 1976). They may also react to changing seasonal photoperiod as an indication of impending unfavorable conditions by altering their immature development rate (Bradshaw and Lounibos 1972, Bradshaw and Holzapfel 1975), though these effects may only be observed in conjunction with other unfavorable conditions (e.g., food limitation; Luker et al. 2002, Yee et al. 2012). Life history theory predicts that organisms can respond to environmental cues in ways that maximize an individual's fitness by allocating resources to maintenance or reproduction accordingly (Williams 1966). For example, *Culex pipiens* (L.) mosquitoes respond to end-of-season cues (temperature and photoperiod) by terminating egg production and switching from blood feeding to sugar feeding. *C. pipiens* diapause as adults during the winter (Eldridge 1968, Bowen 1992, Robich and Denlinger 2005), and this strategy of reducing current reproduction and increasing energy acquisition and storage likely enhances survival and fitness in this species by increasing winter survival and future reproduction. More generally, we predict that organisms will respond to seasonal cues (e.g., short photoperiod) in ways that maximize fitness for their specific life history. Thus, we expect that although mosquitoes that overwinter in the egg stage (e.g., *Aedes*) should show changes in reproductive tactics in response to seasonal temperature and photoperiod, those changes would not be the same as those documented for *C. pipiens*. For *Aedes*, end-of-season cues should induce a shift toward terminal investment (Kight et al. 2000), changing from investing in longevity and future reproduction, to investing maximally in current reproduction, increasing the quantity or quality of the offspring they currently produce and increasing their offspring's overwintering ability, even at the cost of reduced adult longevity.

Under natural conditions, temperatures fluctuate daily (thermoperiodism). Although most studies that examine temperature and photoperiod effects on mosquitoes use constant temperatures, experiments with fluctuating temperatures are not new (reviewed by Beck 1983). There has, however, been increasing interest in the effects of temperature fluctuations on mosquito and parasite dynamics, particularly for vectors of malaria and dengue (Lambrechts et al. 2011; Carrington et al. 2013a, b). If we assume that populations are locally adapted to seasonal climatic conditions, it may then be important to test for

environmental effects on fitness using ecologically appropriate diel temperature fluctuations.

In this article, we test the effects of simulated seasonal conditions of temperature and photoperiod experienced in the larval habitat only, on a suite of characteristics that are likely to influence population dynamics and vectorial capacity in a population of *Aedes triseriatus* (Say), vector of La Crosse virus (LACV). *A. triseriatus* has a native range that extends over most of the eastern United States and southern Canada. Northern populations overwinter mainly as diapause eggs and more southerly populations also diapause as late-stage larvae (Simms 1985). Diapause is induced in the mature embryo or late-stage larva, and it is believed that there are no maternal effects on diapause in this species (Shroyer and Craig 1980). It remains to be tested if seasonal cues in the larval habitat "carry-over" to influence reproductive decisions by adult females involving blood-feeding behavior or reproductive investment.

We quantified measures of population performance such as larval survival, female development time, female size, and r' (Livdahl and Sugihara 1984) and followed cohorts of adult females to test for "carry-over" effects of larval rearing conditions on female longevity, blood feeding, and egg production. These last three potential responses were included because they are important for both population growth and for vectorial capacity for LACV, which is transovarially transmitted (i.e., mother-offspring) by *A. triseriatus* at high rates (Woodring et al. 1998, Hughes et al. 2006).

We tested the following three general hypotheses in a temperate mosquito population: 1) simulated early and late season temperatures and photoperiods affect life-history characteristics that are related to LACV transmission, particularly larval survivorship to adulthood, willingness to blood feed, and adult longevity (i.e., characteristics that are related to vectorial capacity); 2) end-of-season cues encountered by developing larvae will induce resulting adult females to increase investment in current reproduction; and 3) fluctuating, as opposed to constant temperatures of the same mean, will alter larval and adult life-history characteristics. We predicted that mosquitoes experiencing early-season cues would have lower survivorship to adulthood, be less willing to blood-feed on a suboptimal blood source (artificial membrane feeder), have greater adult longevity, and invest less in current reproduction (number of eggs produced) relative to mosquitoes that experienced late-season cues.

Materials and Methods

Experiment 1

Aedes triseriatus used in this experiment were F_1 generation of a population originating from Tyson Research Center, ~32 km west of St. Louis, MO (38° 31' N, 90° 33' W). One hundred first-instar larvae were placed in experimental microcosms with 450 ml of deionized water, 1.6 g of senescent oak leaves (*Quercus virginiana* Miller), and 0.06 g of dried crushed crickets

from a colony maintained at Illinois State University (*Gryllobates* spp.) after 4 d of infusing at a constant 25°C and a photoperiod of 14:10 (L:D) h. The population of *A. triseriatus* used in this experiment would not encounter leaves from *Q. virginiana* in the field. We used this oak species as the detritus base because it is an established detritus source used in experiments in our laboratory, was available in large quantities, and was deemed preferable to using an artificial diet. There were 12 replicates per treatment, though female size, r' , and longevity were followed for only six replicates per treatment, which were randomly chosen from the 12 total replicates. Additional food spikes of 0.6 g of oak leaves were added on day 5 and 0.6 g of leaves and 0.06 g of cricket were added on day 12. Microcosms were randomly assigned to one of three temperature treatments and will be referred to as the June, cool, and August treatments, respectively (Table 1). Microcosms were kept in environmental chambers set to the higher temperature during the light phase and the lower temperature during the dark phase. The June and August temperature and photoperiod treatments were chosen to mimic the average daily high and low temperature and photoperiods for June and August in St. Louis, MO (http://www.crh.noaa.gov/lstx/?n=cli_archive, accessed 28 July 2015). These temperature treatments mimicked the extremes of daily water temperature fluctuations recorded at Tyson in 2013 (K. M. W. unpublished data). June and August also represent the peak and decline, respectively, of *A. triseriatus* oviposition at Tyson (K. M. W. unpublished data). The constant temperature and a photoperiod of 14:10 (L:D) h was chosen, as it is a commonly used laboratory condition and it yielded the same mean temperature as the June treatment. Actual water temperatures in the environmental chambers were recorded hourly using an iButton (Maxim Integrated, San Jose, CA) sealed inside a water-proof battery container (www.lighthouse.com, accessed 28 July 2015) product #LHSTTB). Water temperatures never deviated from the set temperatures in all chambers by more than $\pm 1^\circ\text{C}$.

Pupae were removed daily and allowed to eclose in 20-ml vials. Adult eclosion was recorded daily. Males

were discarded and females were transferred to 1-liter plastic containers covered with mosquito netting in groups by treatment, replicate, and day of eclosion. Our experience maintaining colonies of this *A. triseriatus* population in the laboratory has been that they require large wooden cages (60 cm³) and a dawn and dusk period as part of the daily light cycle to mate readily. We did not have sufficient space in our environmental chambers to house experimental mosquitoes in large cages, thus we expected limited to no mating to occur in the 1-liter containers. To avoid heterogeneity created if some individuals mate and some do not, we ensured that all females were virgins by not exposing them to males. Females were held in a walk-in environmental chamber set at 25°C with a photoperiod of 14:10 (L:D) h with *ad libitum* access to 5% sugar. Mortality was recorded daily. Temperature and photoperiod treatments were only applied to larvae and pupae, not adults. Females from all treatments were held under the same conditions to isolate the effects of larval rearing temperature and photoperiod on adult longevity. Upon death, females were dried at 50°C and one wing was removed and measured to quantify female size (Blackmore and Lord 2000).

We calculated r' , which is a composite performance index designed to estimate instantaneous rate of change, using the method of Livdahl and Sugihara (1984). The index combines time to adult eclosion, cohort survivorship to adulthood, and fecundity estimated from the relationship between female size (wing length) and egg production:

$$r' = \frac{\ln \left[\left(\frac{\sum_x A_x f(w_x)}{N_0} \right) \right]}{D + \left[\frac{\sum_x x A_x f(w_x)}{\sum_x A_x f(w_x)} \right]},$$

where $f(w_x)$ is a function predicting the number of female eggs produced (assuming a 50:50 sex ratio) by a female having the mean wing length for females eclosing on day x , N_0 is the number of females in the experimental cohort (assuming 50:50 sex ratio), x is day of female eclosion, A_x is the number of females eclosing from a cohort on day x , and D is the estimated number of days from eclosion to oviposition in the first gonotrophic cycle. The function $f(w_x)$ was $0.9272(\text{mean wing}^{3.0024})$ and D was estimated to be 12 d (Aspbury and Juliano 1998). For Experiment 2, there were three females for which wing lengths were missing, and there were no other females that eclosed from that container on that day. For these females, we used the overall mean wing length for all females in Experiment 2 as an approximate measure of size.

The effects of treatment on larval survivorship to adulthood, female median days to eclosion, female mean wing length, and r' were analyzed with individual microcosms as the experimental unit with one-way analyses of variance (ANOVAs; PROC GLM). Differences among least squares means were determined using Tukey's correction for multiple comparisons. The

Table 1. Temperature and photoperiod regimens simulated in the laboratory that mimic seasonal conditions experienced in the St. Louis, MO, area and corresponding constant temperature treatments

	Temp. (°C)	Photoperiod (L:D)	Daily mean (°C)	Daily fluctuation (°C)
Experiment 1				
June	28:20	15:9	25	8
Cool	25:25	14:10	25	0
August	31:23	13:11	27:3	8
Experiment 2				
June	28:20	15:9	25	8
Cool	25:25	15:9	25	0
August	32:24	13:11	28	8
Warm	28:28	13:11	28	0

The temperature listed before the colon was the temperature experienced during light hours (L) and the temperature after the colon was the temperature experienced during dark hours (D). Actual temperatures recorded in the larval microcosms were $\pm 1^\circ\text{C}$.

effects of treatment and size (wing length) on adult female longevity were analyzed with two separate models using Cox proportional hazards survival analysis with replicate nested in treatment as a random effect (PROC PHREG). The first model included all females and only the main effect of treatment because wings were too damaged to measure for 35% of females. Damaged wings were most frequent among the longest-lived females. The second model included both treatment and wing length for those females with measurable wings. Differences among treatment groups were further analyzed using the Sidak correction for multiple comparisons. All analyses were performed in SAS 9.3 (SAS 9.3 User's Guide, SAS Institute, Cary, NC).

Experiment 2. Microcosms for larvae were set up with same food regimen as Experiment 1 with six replicates per treatment. Mosquitoes used in this experiment were a mix of F₂ and F₃ from the same population as Experiment 1. Temperature and photoperiod treatments were slightly modified so that June and cool treatments now had the same photoperiod (different from Experiment 1) and same mean temperature, and the August treatment had the same photoperiod and same mean temperature as a fourth treatment referred to as "warm" (Table 1). By conducting two separate experiments, we were able to test for "carry-over" effects on a larger number of females, allowing more statistical power. As with Experiment 1, pupae were moved to 20-ml vials, daily eclosion was recorded, males were discarded, and females were housed in group cages by treatment, replicate, and day of eclosion. Temperature and photoperiod treatments were again only applied to larvae and pupae, not adults.

Adult females were kept at a constant 25°C and a photoperiod of 14:10 (L:D) h in a walk-in environmental chamber and given ad libitum access to 10% sugar for 4 d. On the fifth day, sugar was removed and they were given only water for 48 h to encourage blood feeding. We increased the sugar concentration to 10% in Experiment 2 (from 5% in Experiment 1), which may increase adult survivorship that we wanted to maximize in Experiment 2 (Lambrechts et al. 2006). Six days posteclosion, mosquitoes were offered a bloodmeal in the morning (between 8 and 10 am) for 15 min. Bloodmeals were prepared by heating defibrinated bovine blood (Hemostat Laboratories, Dixon, CA) to 37°C in a water bath, transferring 3 ml of blood to 5-ml petri dishes, which were then covered with a porcine intestine membrane secured with a rubber band. Petri dishes were placed, membrane side down, on the screen on the top of the group cages and a bag of hot water was placed on the top of the dish to maintain blood temperature. At the end of the 15-min feeding period, the blood source was removed and feeding status was determined visually. We only included females with fully distended abdomens, which we determined with the naked eye. We did not quantify the amount of blood imbibed. Blood-fed mosquitoes were transferred to new group cages by treatment, replicate, and date of feeding. No oviposition substrate was given to force the females to retain their eggs. This procedure allowed us

to house multiple females in a single cage and still get individual egg production data as well as eliminated the need to both count laid eggs and dissect ovaries to count retained eggs. To maximize the sample size of blood-fed mosquitoes, group cages were offered blood up to four times over 2 d. Only mosquitoes that fed on the first offered bloodmeal were included in the analysis of willingness to blood feed. Blood-fed females were held for 7 d under the same constant laboratory conditions and then frozen at -20°C. We determined, based on the literature, that 7 d was sufficient time for female *A. triseriatus* to mature eggs to Christophers Stage IV (Christophers 1960, Walker et al 1987). One wing was removed from each female (those that blood fed and those that did not) and measured. Ovaries of blood-fed females were removed and placed in a small amount of Hank's buffered salt solution and mature eggs (Christophers Stage IV or V) counted (Christophers 1960).

Effects of treatment on larval survivorship to adulthood, female median day to eclosion, female mean wing length, and r' were analyzed as in Experiment 1. Willingness to blood feed was analyzed as the proportion that fed the first time females were offered blood, with individual microcosms as the experimental unit. We only analyzed the proportion that fed the first time they were offered, as not all mosquitoes were offered blood the same number of times. Additionally, except for the first feeding, time of day at which blood was offered varied. We also assumed that the number of times blood was offered was not independent from the likelihood of taking a bloodmeal. The effect of treatment on number of mature eggs produced was analyzed as a mixed-model analysis of covariance with wing length as the covariate and replicate nested in treatment as the random effect (PROC MIXED). Estimates of the intercept and slope for the relationship between size and egg production for each treatment group were obtained by simple linear regression (PROC REG). All analyses were performed in SAS 9.3 (SAS 9.3 User's Guide, SAS Institute).

Results

Experiment 1. There were significant effects of treatment on survivorship to adulthood, female median day to eclosion, and female mean wing length, but not on r' (Table 2). Survivorship to adulthood was lower in the August treatment than in the June and cool treatments, which did not differ (Fig. 1A). Median days to eclosion was significantly lower for females in the August treatment than in the June and cool treatments, which did not differ (Fig. 1B). Females in the June treatment were significantly larger than those in the other treatments, and those from the cool treatment were significantly larger than those from the August treatment (Fig. 1C). There was no significant effect of treatment on r' (Fig. 1D). There was a significant effect of treatment on female longevity in the first model that included only the effect of treatment and used the entire data set ($n = 474$; $\chi^2 = 10.84$; $df = 2$; $P = 0.0009$). After adjusting for multiple comparisons, the cool treatment differed from both June and August treatments,

which did not differ. The cool treatment had the greatest hazard of death and showed earlier senescence than the two fluctuating treatments (Fig. 2). The model with both female size and treatment yielded no significant effects of treatment ($n=308$; $\chi^2=4.19$; $df=2$; $P=0.095$) and size ($\chi^2=0.1$; $df=1$; $P=0.743$).

Experiment 2. There was a significant effect of treatment on female median days to eclosion, and mean female wing length but not on survivorship to adulthood and r' (Table 3). Larval survivorship was high in Experiment 2, approaching 90% for all treatment groups (Fig. 3A). Females in the June treatment took significantly longer to eclose than did females in the August and warm treatments, but not in the cool treatment. The cool and August treatment did not differ, but females from the cool treatment took longer to

eclose than those from the warm treatment. The August and warm treatments did not differ (Fig. 3B). Females from the June treatment were significantly larger than those in the August and warm treatments, but not those in the cool treatment. Females from the August treatment were significantly larger than the warm treatment but were not significantly different from those in the cool treatment (Fig. 3C). As in Experiment 1, means for r' were all rather similar among treatment groups (Fig. 3D). There was no effect of treatment on the proportion of females that blood fed ($F_{3,20}=1.78$; $P=0.184$) (Fig. 4). There was no significant main effect of treatment on the number of eggs produced ($F_{3,20}=1.72$; $P=0.1953$), but there was a significant interaction between wing length and treatment ($F_{4,220}=3.29$; $P=0.0121$). The relationship between wing length and egg production for the June and cool treatments had slopes that were not significantly different from zero, indicating no size–fecundity relationship for these treatments (Fig. 5A and B). The slopes were positive and significantly greater than zero for the August and warm treatments (Fig. 5C and D).

Table 2. ANOVA table of the effects of rearing temperature and photoperiod on larval survivorship, median day to eclosion for females only, female wing length, and a composite cohort performance index r' from Experiment 1

Effect	df	F	P
Survivorship			
Treatment	2	11.88	0.0001
Error	33		
Median day of selection			
Treatment	2	52.37	<0.0001
Error	33		
Wing length			
Treatment	2	42.89	<0.0001
Error	15		
r'			
Treatment	2	0.54	0.5961
Error	15		

Discussion

In this article, we tested several hypotheses about the effects of seasonal cues experienced in the larval habitat, and of constant and fluctuating temperatures, on a population of *A. triseriatus*, a primary vector of LACV. In Experiment 1, all larvae had lower survivorship compared with Experiment 2, with the August treatment having significantly lower survivorship compared with the June and cool treatments. In

Fig. 1. Least squares means and SEs for the effect of treatment in Experiment 1 on (A) larval survivorship, (B) median day of eclosion for females only (C) female wing size, and (D) r' , which is a composite performance index. Means associated with the same letter are not significantly different from one another.

Fig. 2. Cumulative survival probabilities of female *A. triseriatus* reared as larvae under three different temperature and photoperiod regimens. Letters denote groups that were significantly different.

Table 3. ANOVA table of the effects of rearing temperature and photoperiod on larval survivorship, median day to eclosion for females only, female wing length, and a composite cohort performance index r' from Experiment 2

Effect	df	F	P
Survivorship			
Treatment	3	1.81	0.1784
Error	20		
Median day of selection			
Treatment	3	13.02	<0.0001
Error	20		
Wing length			
Treatment	3	12.29	<0.0001
Error	20		
r'			
Treatment	3	1.34	0.2890
Error	20		

Experiment 2, larvae from all treatment groups had high survivorship and the overall effect of treatment on survivorship was not significant. Although the microcosms were set up with exactly the same detritus amounts and densities, the leaves used in the two experiments were collected at different times, but at the same location, and may have been of different quality. Other laboratory experiments have shown that leaf quality, including leaf age, can affect larval survivorship (Walker et al. 1997, Macia and Bradshaw 2000). In another study, *A. triseriatus* larvae reared at 31°C exhibited reduced survivorship to adulthood compared with larvae reared at 15° or 23°C (Teng and Apperson 2000). The mean temperature of our August treatment was 27.3°C, though during the 13-h diurnal period, the temperature was 31°C, which is consistent with Teng and Apperson (2002). These results suggest that if resources are of poor quality, *A. triseriatus* may have reduced survivorship at higher temperatures even if the daily mean temperature is lower. Temperature has been shown to alter the effects of poor resource quality, or starvation, on insect survival (Adamo et al. 2012). There is a lack of published data on the effects of photoperiod on mosquito larval survival. Megaloptera (Diptera: Phoridae) larvae exhibited increased larval survivorship in one study when reared under short

versus long photoperiods, though only at the lowest temperature measured (21°C), which is consistent with our data from Experiment 1 (Trumble and Pienkowski 1979).

Median days to eclosion for females was longer in the June and cool treatments compared with the August treatment in Experiment 1. In Experiment 2, this same pattern of longer development at lower mean temperatures was evident (Fig. 3B), although in contrast to Experiment 1, the cool and August treatments were not different. Though differences between the two experiments are relatively minor, differences in leaf quality between the experiments may again play a role, although the lack of significant differences in Experiment 2 compared with Experiment 1 may also be explained by the lower number of replicates (6) in Experiment 2 compared with Experiment 1 (12). In both experiments, the constant and corresponding fluctuating temperature treatments with the same mean temperatures did not differ, suggesting that under these conditions, temperature fluctuations of up to 8°C do not alter the development rate compared with constant mean temperatures. Photoperiod, independent of temperature, can affect development time in mosquitoes with the direction of the effect dependent on the specific biology of the species. For example, predatory *Toxorhynchites* spp., which overwinter in the larval stage, increase development rate in response to long photoperiods (Bradshaw and Holzapfel 1975), whereas *Aedes albopictus* (Skuse) reduces its development rate under long photoperiods, presumably allowing for increased size and fecundity (Yee et al. 2012).

All pair-wise comparisons of wing size were significant in Experiment 1 but not Experiment 2, though the trend was the same (compare Figs. 1C and 3C). The June treatment produced the largest females and the warm treatment produced the smallest females. Wing length is often negatively correlated with temperature and positively correlated with development time (Briegleb and Timmermann 2001, Dodson et al. 2012). Our data support this, but also show that the metabolic relationship between temperature and size can be independent of the relationship between temperature and development time when temperatures fluctuate. In other words, fluctuating temperatures compared with constant temperatures alter female size without significantly affecting development time. The mechanism behind this is not clear, but it could be because of differences in foraging and assimilation rates at different temperatures. In a similar study that compared fluctuating versus constant rearing temperatures for *Aedes aegypti* L., development time also was not affected, but they did not report female size. Other investigators reported development times and wing size for adults reared as larvae under constant and fluctuating regimens but did not test for differences between the regimens, so we cannot be sure if our result is specific to *A. triseriatus* or a more general property of *Aedes* or insects in general (Mohammed and Chadee 2011; Carrington et al. 2013a, c).

In both experiments, r' , our cohort performance estimate, was unaffected by treatments. This was true

Fig. 3. Least squares means and SEs for the effect of treatment in Experiment 2 on (A) larval survivorship, (B) median day of eclosion for females only (C) female wing size, and (D) r' , which is a composite performance index. Means associated with the same letter are not significantly different.

Fig. 4. Proportion of females that took a bloodmeal the first time they were offered. There was no significant effect of temperature and photoperiod treatment on blood feeding.

despite significant effects of treatments on adult size and development time (both experiments) and lower survivorship to adulthood in the warmer treatment (August) in Experiment 1. It appears that the effects of warmer temperature decreasing development time and decreasing adult size (and estimated fecundity) approximately cancel one another, resulting in similar estimated rates of increase for cohorts under the conditions used in these experiments. For Experiment 1, although overall survivorship differed among treatments (Fig. 1A), numbers of female survivors, which determines r' , were similar (means $\pm$ SE: 26.5 ± 2.3 ; 29.2 ± 2.4 ; 23.2 ± 2.9 for June, cool, and August, respectively) and these small differences were evidently not sufficient to cause differences in r' .

In Experiment 1, we tested for the effect of rearing temperature and photoperiod on adult female longevity. Treatment had a significant effect, with constant cool temperature females exhibiting reduced longevity compared with the June and August females. The main factor producing this difference in longevity between treatment groups was the earlier senescence of the cool treatment females (Fig. 2). The two seasonal temperature treatments were not different, which suggests that, at least under benign laboratory conditions, seasonally fluctuating temperatures and photoperiods in the larval habitat do not produce adults that differ significantly in survival probabilities. Thus, effects of rearing temperatures and photoperiods on female longevity, which is one of the most important parameters of vectorial capacity, (Dye 1986) cannot explain the observed greater LACV incidence among humans associated with late summer and fall (Haddow and Odoi 2009).

Why the cool temperature females had the lowest cumulative survivorship estimates is not entirely clear. One possibility is that teneral reserves are greater in *A. triseriatus* when the rearing temperature fluctuates, though there is no experimental evidence for this hypothesis. Teneral reserves are highly correlated with size (Van Handel and Day 1988), and we found no effect of size on longevity. The only other variable measured in this experiment that indicated that the constant temperature groups fared worse than the fluctuating temperature groups was female size (Fig. 1C). When the model was expanded to include wing size, the effect of treatment was marginally not significant, which is likely a result of sample size being reduced by

Fig. 5. Plot of observed egg production (dots) and predicted size–fecundity relationship (solid line) for (A) June, (B) cool, (C), August, and (D) hot treatments. Asterisks denote slopes that are significantly different than zero.

35% owing to damaged wings. Size was clearly unrelated to longevity. Most of the wings that were too damaged to use came from females >50 d old when they died and thus are not randomly distributed among the population. Thus, we think that the analysis of the full data set without size is the most accurate indicator of the effect of treatment on longevity.

The utility of size as a measure of vector “quality” is not resolved. Size can affect vector competence for arboviruses, though the mechanism is not clear (Paulson and Hawley 1991, Alto et al. 2008). Some studies have shown that size may be a predictor of success in acquiring a bloodmeal, adult longevity, and fecundity (Hawley 1985, Takken et al. 1998, Stone et al. 2012, Xue et al. 2012). Not all experiments reach the same conclusions about size, however. A survey of field-collected *A. triseriatus* found no evidence for a benefit of being large (Landry et al. 1988). Temperature may affect size directly via metabolic effects and indirectly via effects on the bacterial resource that is the food supply for developing larvae, and these effects may lead to different physiological or immunological phenotypes, and ultimately to different outcomes in traits related to vector competence and other life-history traits. In the current experiment, we found no evidence for an effect of size on longevity and limited evidence for an effect of size on fecundity.

Most researchers find a significant size fecundity relationship in mosquitoes (Blackmore and Lord 2000, Armbruster and Hutchinson 2002). Only a handful of studies have investigated how different environmental conditions may alter the size–fecundity relationship (Clauss and Aarsen 1994, Arendt 2010) or investigated

how the relationship may change as females age (Mccann et al. 2009). In this study, we found no significant main effect of treatment on egg production, despite significant differences among treatments for mean female size. There was a significant interaction between treatment and size; the slopes of the size–fecundity relationships were significant and positive for females coming from the August and warm treatments, whereas the slopes for the June and cool treatments were not significantly different from zero. The significant size–fecundity relationship in the warmer treatments appears to arise from the smallest females in each group producing a small number of eggs. The predicted number of eggs for larger females is similar for all treatments (compare Fig. 5A–D), suggesting that the apparent difference owing to rearing conditions may indeed be a product of the presence or absence of the smallest females. As with many of the other variables measured in this study, there is weak support for the hypothesis that fluctuating versus constant temperatures have large effects on individual performance or of population growth in this system.

In Experiment 2, we tested the effects of larval rearing treatment on willingness to take a bloodmeal and egg production for one gonotrophic cycle. As the population of *A. triseriatus* used in this experiment primarily overwinters as diapausing eggs, we predicted that if *A. triseriatus* females make reproductive decisions based on seasonal cues they experience as larvae, late-season females (i.e., August) would be more willing to feed and would produce more eggs than early-season females (i.e., June). We specifically chose our temperatures and photoperiods to mimic late-season cues that

were just above the threshold when this population is predicted to be going to produce diapause eggs (Sims 1985). We found no evidence of a treatment effect on willingness to take a bloodmeal. It may be that as the decision to enter diapause is made by eggs and that larvae of this midwestern population generally do not respond to seasonal cues, though it is known that larvae from populations of *A. triseriatus* from the same latitude as the population used in this study will enter diapause at a photoperiod of 11:13 (L:D) h (Sims 1985). It may also be that seasonal cues in the larval habitat alone are not enough to elicit a change in reproductive tactics in the resulting adults. We used an artificial membrane feeding system and a short feeding period (15 min), which could have contributed to the lack of treatment effect and overall low blood feeding rates, although if *Aedes* do change their feeding behavior based on seasonal cues, it may only be detectable with relatively unattractive blood sources, like an artificial membrane feeder.

The number of eggs produced was our only measure of reproductive effort, and there may have been a change in egg quality that was not accounted for. All of the females in this experiment were held under the standard laboratory conditions that were the same as rearing conditions for larvae in the cool treatment. It seems likely that the conditions experienced during adulthood may have over-ridden any effect of larval rearing conditions on life history. In this species, seasonal cues experienced by adults are not transmitted to offspring; in other words, eggs and larvae make the physiological decision to enter or to terminate diapause, and adult females do not make that physiological decision for them, as is the case in *Aedes albopictus* (Skuse) (Kappus and Venard 1967, Focks et al. 1994). This biological property of *A. triseriatus* was what led to the prediction that if females make reproductive decisions based on seasonal cues, the cues would have to be experienced in immature stages. To our knowledge, there are no published tests for a shift in feeding behavior in *Aedes* mosquitoes owing to seasonal cues, in contrast to studies that have shown seasonal shifts in *Culex* feeding behavior (Eldridge 1968, Bowen 1992, Robich and Denlinger 2005).

Other studies have demonstrated that fluctuating temperatures give a more accurate measure of performance than constant temperatures (Bradshaw 1980, Murdock et al. 2012a, b, 2013). Many of these studies compare large and small daily fluctuations, and use mainly species from warmer climates and used temperature regimens that were relevant to those systems. In this experiment, we were interested in testing not only the difference between daily thermoperiods but also seasonally realistic temperatures and photoperiods to determine if seasonal climate impacts life history and, ultimately, vectorial capacity. Though a greater magnitude of fluctuation, or greater mean temperature difference between groups, may have produced more dramatic effects, we chose to design our experiment with treatments within the range of observed temperatures and photoperiods for this population. Our results suggest that at least in this temperate-zone *A.*

triseriatus–LACV system, the use of constant temperatures in laboratory studies may be justified. Although the potential effects of thermoperiodism are intriguing, and likely important in some systems, they do not appear to be so here. One of the striking results of this study is that female size is altered by temperature fluctuations. In this study, size did not affect longevity, and had little impact on fecundity. Thus, we find limited evidence that rearing temperature fluctuations and their effect on female size are strongly related to vectorial capacity in this mosquito–virus system.

One of our hypotheses was that seasonal conditions experienced by larvae would impact variables related to LACV transmission (survivorship to adulthood, willingness to blood feed, and adult longevity). We find little or no evidence for this postulated effect. June versus August conditions did affect survivorship in one of two experiments, but even in that case, production of females was similar. Blood feeding was unaffected by any treatment, and adult female longevity was similar for June and August rearing conditions. Though other effects of seasonal rearing conditions are possible (e.g., effects on vector competence), our data suggest that seasonal conditions experienced by larvae are unlikely to influence seasonal differences in these traits related to LACV transmission.

Acknowledgments

We thank P.J. McCormick, A.B. Wiggins, M.K. Schumacher, and G.D. Ower for laboratory assistance, and E.J. Muturi, V.A. Borowicz, W.A. Nichols, and W.L. Perry for useful discussion during the planning stages of this research and on earlier versions of the manuscript. This work was funded by National Institute of Allergy and Infectious Disease (NIAID) grant 1R15AI094322-01A1 and by a grant from the Illinois State University Office of Research to S.A.J., a grant from the Beta Lambda chapter of the Phi Sigma Biological Honors Society, and a Dissertation Completion Grant from the Graduate School at Illinois State University awarded to K.M.W.

References Cited

- Adamo, S. A., J. L. Baker, M.M.E. Lovett, and G. Wilson. 2012. Climate change and temperate zone insects: The tyranny of thermodynamics meets the world of limited resources. *Environ. Entomol.* 41: 1644–1652.
- Altizer, S., A. Dobson, P. Hosseini, P. Hudson, M. Pascual, and P. Rohani. 2006. Seasonality and the dynamics of infectious diseases. *Ecol. Lett.* 9: 467–484.
- Alto, B. W., and D. Bettinardi. 2013. Temperature and Dengue virus infection in mosquitoes independent effects on the immature and adult stages. *Am. J. Trop. Med. Hyg.* 88: 497–505.
- Alto, B. W., M. H. Reiskind, and L. P. Lounibos. 2008. Size alters susceptibility of vectors to dengue infection and dissemination. *Am. J. Trop. Med. Hyg.* 79: 688–695.
- Arendt, J. D. 2010. Size-fecundity relationships, growth trajectories, and the temperature-size rule for ectotherms. *Evolution* 65: 43–51.
- Armbruster, P., and R. A. Hutchinson. 2002. Pupal mass and wing length as indicators of fecundity in *Aedes albopictus* and *Aedes geniculatus* (Diptera: Culicidae). *J. Med. Entomol.* 39: 699–704.

- Aspbury, A. S., and S. A. Juliano. 1998. Negative effects of habitat drying and prior exploitation on the detritus resource in an ephemeral aquatic habitat. *Oecologia* 115: 137–148.
- Beck, S. D. 1983. Insect thermoperiodism. *Annu. Rev. Entomol.* 28: 91–108.
- Bevins, S. N. 2007. Timing of resource input and larval competition between invasive and native container-inhabiting mosquitoes (Diptera: Culicidae). *J. Vector Ecol.* 32: 252–262.
- Blackmore, M. S., and C. C. Lord. 2000. The relationship between size and fecundity in *Aedes albopictus*. *J. Vector Ecol.* 25: 212–217.
- Bowen, M. F. 1992. Patterns of sugar feeding in diapausing and nondiapausing *Culex pipiens* (Diptera: Culicidae) females. *J. Med. Entomol.* 29: 843–849.
- Bradshaw, W. E. 1980. Thermoperiodism and the thermal environment of the pitcher-plant mosquito, *Wyeomyia smithii*. *Oecologia* 46: 13–17.
- Bradshaw, W. E., and L. P. Lounibos. 1972. Photoperiodic control of development in the pitcher plant mosquito *Wyeomyia smithii*. *Can. J. Zool.* 50: 713–719.
- Bradshaw, W. E., and C. M. Holzapfel. 1975. Biology of tree-hole mosquitoes: Photoperiodic control of development in northern *Toxorhynchites rutilus* (Coq.). *Can. J. Zool.* 53: 889–893.
- Briegleb, H., and S. E. Timmermann. 2001. *Aedes albopictus* (Diptera: Culicidae): Physiological aspects of development and reproduction. *J. Med. Entomol.* 38: 566–571.
- Brubaker, J. F., and M. J. Turell. 1998. Effect of environmental temperature on the susceptibility of *Culex pipiens* (Diptera: Culicidae) to Rift Valley Fever virus. *J. Med. Entomol.* 35: 918–921.
- Carrington, L. B., M. V. Armijos, L. Lambrechts, C. M. Barker, and T. W. Scott. 2013a. Effects of fluctuating daily temperatures at critical thermal extremes on *Aedes aegypti* life-history traits. *PLoS ONE* 8: e58824.
- Carrington, L. B., M. V. Armijos, L. Lambrechts, and T. W. Scott. 2013b. Fluctuations at a low mean temperature accelerate dengue virus transmission by *Aedes aegypti*. *PLoS Negl. Trop. Dis.* 7: e2190.
- Carrington, L. B., S. N. Seifert, N. H. Willits, L. Lambrechts, and T. W. Scott. 2013c. Large diurnal temperature fluctuations negatively influence *Aedes aegypti* (Diptera: Culicidae) life-history traits. *J. Med. Entomol.* 50: 43–51.
- Clauss, M. J., and L. W. Aarsen. 1994. Phenotypic plasticity of size-fecundity relationships in *Arabidopsis thaliana*. *J. Ecol.* 82: 447–455.
- Costanzo, K. S., E. J. Muturi, A. V. Montgomery, and B. W. Alto. 2013. Effect of oral infection of La Crosse virus on survival and fecundity of native *Ochlerotatus triseriatus* and invasive *Stegomyia albopicta*. *Med. Vet. Entomol.* 28: 77–84.
- Day, J. F., A. M. Ramsey, and J. T. Zhang. 1990. Environmentally mediated seasonal variation in mosquito body size. *Environ. Entomol.* 19: 469–473.
- Delatte, H., G. Gimonneau, A. Triboire, and D. Fontenille. 2009. Influence of temperature on immature development, survival, longevity, fecundity, and gonotrophic cycles of *Aedes albopictus*, vector of Chikungunya and Dengue in the Indian Ocean. *J. Med. Entomol.* 46: 33–41.
- Depner, K. R., and R. F. Hardwood. 1966. Photoperiodic responses of two latitudinally diverse groups of *Anopheles freeborni* (Diptera: Culicidae). *Ann. Entomol. Soc. Am.* 59: 7–11.
- Dodson, B. L., L. D. Kramer, and J. L. Rasgon. 2012. Effects of larval rearing temperature on immature development and West Nile virus vector competence of *Culex tarsalis*. *Parasit. Vectors* 5: 199.
- Dye, C. 1986. Vectorial capacity: Must we measure all its components? *Parasitol. Today* 2: 203–209.
- Eldridge, B. F. 1968. The effect of temperature and photoperiod on blood-feeding and ovarian development in mosquitoes of the *Culex pipiens* complex. *Am. J. Trop. Med. Hyg.* 17: 133–140.
- Focks, D. A., S. B. Linda, G. B. Craig, W. A. Hawley, and C. B. Pumpuni. 1994. *Aedes albopictus* (Diptera: Culicidae): A statistical model of the role of temperature, photoperiod, and geography in the induction of egg diapause. *J. Med. Entomol.* 31: 278–286.
- Haddow, A. D., and A. Odoi. 2009. The incidence risk, clustering, and clinical presentation of La Crosse virus infections in the eastern United States, 2003–2007. *PLoS ONE* 4: e6145.
- Harwood, R. F., and E. Halfhill. 1964. The effect of photoperiod on fat body and ovarian development of *Culex tarsalis* (Diptera: Culicidae). *Ann. Entomol. Soc. Am.* 57: 596–600.
- Hawley, W. A. 1985. The effect of larval density on longevity of a mosquito, *Aedes sierrensis*: Epidemiological consequences. *J. Anim. Ecol.* 54: 955–964.
- Hughes, M. T., J. A. Gonzalez, K. L. Reagan, C. D. Blair, and B. J. Beaty. 2006. Comparative potential of *Aedes triseriatus*, *Aedes albopictus*, and *Aedes aegypti* (Diptera: Culicidae) to transovarially transmit La Crosse virus. *J. Med. Entomol.* 43: 757–761.
- Kay, B. H., I. D. Fanning, and P. Mottram. 1989. Rearing temperature influences flavivirus vector competence of mosquitoes. *Med. Vet. Entomol.* 3: 415–422.
- Kight, S. L., M. Batino, and Z. Zhang. 2000. Temperature-dependent parental investment in the giant waterbug *Belostomatidae flumineum* (Heteroptera: Belostomatidae). *Ann. Entomol. Soc. Am.* 93: 340–342.
- Kingsolver, J. G., and R. B. Huey. 2008. Size, temperature, and fitness: Three rules. *Evol. Ecol. Res.* 10: 251–268.
- Lambrechts, L., J. M. Chavatte, G. Snounou, and J. C. Koella. 2006. Environmental influence on the genetic basis of mosquito resistance to malaria parasites. *Proc. Biol. Sci.* 273: 1501–1506.
- Lambrechts, L., K. P. Paaijmans, T. Fabsiri, L. B. Carrington, L. D. Kramer, M. B. Thomas, and T. W. Scott. 2011. Impact of daily temperature fluctuations on dengue virus transmission by *Aedes aegypti*. *Proc. Natl. Acad. Sci. USA* 108: 7460–7465.
- Landry, S. V., G. R. Defoliart, and D. B. Hogg. 1988. Adult body size and survivorship in a field population of *Aedes triseriatus*. *J. Am. Mosq. Control Assoc.* 4: 121–128.
- Livdahl, T. P., and G. Sugihara. 1984. Non-linear interactions of populations and the importance of estimating per capita rates of change. *J. Anim. Ecol.* 53: 573–580.
- Luker, L. A., J. D. Hatle, and S. A. Juliano. 2002. Reproductive responses to photoperiod by a south Florida population of the grasshopper *Romalea microptera* (Orthoptera: Romaleidae). *Environ. Entomol.* 31: 702–707.
- Macia, A., and W. E. Bradshaw. 2000. Seasonal availability of resources and habitat degradation for the western tree-hole mosquito, *Aedes sierrensis*. *Oecologia* 125: 55–65.
- Maciel-de-Freitas, R., C. D. Codeco, and R. Lourenco-De-Oliveira. 2007. Body size-associated survival and dispersal rates of *Aedes aegypti* in Rio de Janeiro. *Med. Vet. Entomol.* 21: 284–292.
- Mccann, S., J. F. Day, S. Allan, and C. C. Lord. 2009. Age modifies the effect of body size on fecundity in *Culex quinquefasciatus* Say (Diptera: Culicidae). *J. Vector Ecol.* 34: 174–181.
- Mohammed, A., and D. D. Chadee. 2011. Effects of different temperature regimens on the development of *Aedes aegypti* (L.) (Diptera: Culicidae) mosquitoes. *Acta Trop.* 119: 38–43.
- Murdock, C. C., L. L. Moller-Jacobs, and M. B. Thomas. 2013. Complex environmental drivers of immunity and resistance in malaria mosquitoes. *Proc. Biol. Sci.* 280: 20132030.

- Murdock, C. C., K. P. Paaijmans, A. S. Bell, J. G. King, J. F. Hillyer, A. F. Read, and M. B. Thomas. 2012a. Complex effects of temperature on mosquito immune function. *Proc. Biol. Sci.* 279: 3357–3366.
- Murdock, C. C., K. P. Paaijmans, D. Cox-Foster, A. F. Read, and M. B. Thomas. 2012b. Rethinking vector immunology: The role of environmental temperature in shaping resistance. *Nat. Rev. Microbiol.* 10: 869–876.
- Muturi, E. J., B. F. Allan, and J. Ricci. 2012. Influence of leaf detritus type on production and longevity of container-breeding mosquitoes. *Environ. Entomol.* 41: 1062–1068.
- Muturi, E. J., K. Costanzo, B. Kesavaraju, R. Lampman, and B. W. Alto. 2010. Interaction of a pesticide and larval competition on life history traits of *Culex pipiens*. *Acta Trop.* 116: 141–146.
- O'Neal, P. A., and S. A. Juliano. 2013. Seasonal variation in competition and coexistence of *Aedes* mosquitoes: Stabilizing effects of egg mortality or equalizing effects of resources? *J. Anim. Ecol.* 82: 256–265.
- Paulson, S. L., and W. A. Hawley. 1991. Effect of body size on the vector competence of field and laboratory populations of *Aedes triseriatus* for La Crosse virus. *J. Am. Mosq. Control Assoc.* 7: 170–175.
- Reiskind, M. H., and L. P. Lounibos. 2009. Effects of intra-specific larval competition on adult longevity in the mosquitoes *Aedes aegypti* and *Aedes albopictus*. *Med. Vet. Entomol.* 23: 62–68.
- Robich, R. M., and D. L. Denlinger. 2005. Diapause in the mosquito *Culex pipiens* evokes a metabolic switch from blood feeding to sugar gluttony. *Proc. Natl. Acad. Sci. USA* 102: 15912–15917.
- Shroyer, D. A., and G. B. Craig. 1980. Egg hatchability and diapause in *Aedes triseriatus* (Diptera: Culicidae): Temperature- and photoperiod- induced latencies. *Ann. Entomol. Soc. Am.* 73: 39–43.
- Stone, C. M., B. T. Jackson, and W. A. Foster. 2012. Effects of bed net use, female size, and plant abundance on the first meal choice (blood vs sugar) of the malaria mosquito *Anopheles gambiae*. *Malar. J.* 11: 3.
- Straif, S. C., and J. C. Beier. 1996. Effects of sugar availability on the blood-feeding behavior of *Anopheles gambiae* (Diptera: Culicidae). *J. Med. Entomol.* 33: 608–612.
- Suvanchaichinda, C., and S. M. Paskewitz. 1998. Effects of larval nutrition, adult body size, and adult temperature on the ability of *Anopheles gambiae* (Diptera: Culicidae) to melanize Sephadex beads. *J. Med. Entomol.* 35: 157–161.
- Takken, W., M. J. Klowden, and G. M. Chambers. 1998. Effect of body size on host seeking and blood meal utilization in *Anopheles gambiae* sensu stricto (Diptera: Culicidae): The disadvantage of being small. *J. Med. Entomol.* 35: 639–645.
- Tauber, M. J., and C. A. Tauber. 1976. Insect seasonality: Diapause maintenance, termination, and postdiapause development. *Annu. Rev. Entomol.* 21: 81–107.
- Teng, H., and C. S. Apperson. 2000. Development and survival of immature *Aedes albopictus* and *Aedes triseriatus* (Diptera: Culicidae) in the laboratory: Effects of density, food, and competition on response to temperature. *J. Med. Entomol.* 37: 40–52.
- Trumble, J. T., and R. L. Pienkowski. 1979. Development and survival of *Megaselia scalaris* (Diptera: Phoridae) at selected temperatures and photoperiods. *Proc. Entomol. Soc. Wash.* 81: 207–210.
- Van Handel, E., and J. F. Day. 1988. Assay of lipids, glycogen and sugars in individual mosquitoes: correlations with wing length in field-collected *Aedes vexans*. *J. Am. Mosq. Control Assoc.* 4: 549–550.
- Walker, E. D., R. S. Copeland, S. L. Paulson, and L. E. Munstermann. 1987. Adult survivorship, population density, and body size in sympatric populations of *Aedes triseriatus* and *Aedes hendersoni* (Diptera: Culicidae). *J. Med. Entomol.* 24: 485–493.
- Walker, E. D., R. W. Merritt, M. G. Kaufman, M. P. Ayres, and M. H. Riedel. 1997. Effects of variation in quality of leaf detritus on growth of the eastern tree-hole mosquito, *Aedes triseriatus* (Diptera: Culicidae). *Can. J. Zool.* 75: 706–718.
- Williams, G. C. 1966. Natural selection, the costs of reproduction, and a refinement of Lack's principle. *Am. Nat.* 100: 687–690.
- Woodring, J., L. J. Chandler, C. T. Oray, M. M. McGaw, C. D. Blair, and B. J. Beaty. 1998. Short report: Diapause, transovarial transmission, and filial infection rates in geographic strains of La Crosse virus-infected *Aedes triseriatus*. *Am. J. Trop. Med. Hyg.* 58: 587–588.
- Xue, A. R., A. Ali, and D. R. Barnard. 2012. Mating status and body size in *Aedes albopictus* (Diptera: Culicidae) affect host finding and host repellency. *Florida Entomol.* 95: 268–272.
- Yee, D. A., S. A. Juliano, and S. M. Vamosi. 2012. Seasonal photoperiods alter developmental time and mass of an invasive mosquito, *Aedes albopictus* (Diptera: Culicidae), across its north-south range in the United States. *J. Med. Entomol.* 49: 825–832.

Received 10 March 2015; accepted 16 July 2015.