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Biological Sciences

7-2012

Seasonal Photoperiods Alter Developmental Time and Mass of an Invasive Mosquito, Aedes albopictus (Diptera: Culicidae), Across Its North-South Range in the United States

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Seasonal Photoperiods Alter Developmental Time and Mass of an Invasive Mosquito, *Aedes albopictus* (Diptera: Culicidae), Across Its North-South Range in the United States

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ABSTRACT The Asian tiger mosquito, *Aedes albopictus* (Skuse), is perhaps the most successful invasive mosquito species in contemporary history. In the United States, *Ae. albopictus* has spread from its introduction point in southern Texas to as far north as New Jersey (i.e., a span of ≈14° latitude). This species experiences seasonal constraints in activity because of cold temperatures in winter in the northern United States, but is active year-round in the south. We performed a laboratory experiment to examine how life-history traits of *Ae. albopictus* from four populations (New Jersey [39.4° N], Virginia [38.6° N], North Carolina [35.8° N], Florida [27.6° N]) responded to photoperiod conditions that mimic approaching winter in the north (short static daylength, short diminishing daylength) or relatively benign summer conditions in the south (long daylength), at low and high larval densities. Individuals from northern locations were predicted to exhibit reduced development times and to emerge smaller as adults under short daylength, but be larger and take longer to develop under long daylength. Life-history traits of southern populations were predicted to show less plasticity in response to daylength because of low probability of seasonal mortality in those areas. Males and females responded strongly to photoperiod regardless of geographic location, being generally larger but taking longer to develop under the long daylength compared with short day lengths; adults of both sexes were smaller when reared at low larval densities. Adults also differed in mass and development time among locations, although this effect was independent of density and photoperiod in females but interacted with density in males. Differences between male and female mass and development times was greater in the long photoperiod suggesting differences between the sexes in their reaction to different photoperiods. This work suggests that *Ae. albopictus* exhibits sex-specific phenotypic plasticity in life-history traits matching variation in important environmental variables.

KEY WORDS daylength, life history, phenotypic plasticity, survival

The Asian tiger mosquito, *Aedes albopictus* (Skuse), is an important invasive container-dwelling species (Juliano and Lounibos 2005) having colonized all continents except mainland Australia (Ritchie et al. 2006) and Antarctica (Lounibos 2002). In the United States, *Ae. albopictus* is now one of the most common mosquitoes occupying human-associated containers east of the Mississippi River (Yee 2008). This species is also of medical importance as a vector of several arboviruses (dengue, LA Crosse encephalitis, eastern equine encephalitis, West Nile encephalitis; Mitchell et al. 1992; Gerhardt et al. 2001; Turell et al. 2001, 2005), and has negative ecological effects on resident mosquito species (Juliano 1998, Lounibos 2002). Understanding the factors that explain the successful invasion and

spread of this species across the United States and elsewhere is of fundamental importance for predicting its future expansion, disease transmission, and interactions with native vectors (Lounibos 2002, Juliano and Lounibos 2005).

One area of investigation that may be useful for understanding invasion and spread of exotic mosquitoes is their pattern of allocation of limited resources to life-history parameters (Juliano and Lounibos 2005). Phenotypic plasticity, the expression of multiple environmentally cued phenotypes by a single genotype (West–Eberhart 1989), has been identified for life-history traits in response to different environments in a number of insects, including butterflies (Leimar 1996), damselflies (Johansson and Rowe 1999), psyllids (Miles et al. 1998), grasshoppers (Luker et al. 2002, Homeny and Juliano 2007), and crickets (Olvido and Mousseau 1998). Environmental cues are often accurate indicators of season and the potential for catastrophic environmental impacts (e.g., temperature, drought). Thus, such cues may induce plasticity in life-history traits, including traits

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such as development time (egg to adult) and adult body size or mass (Nylin and Gotthard 1998). For many insects, although several environmental factors or cues signal the end of the active season (e.g., temperature, diminishing food quality), daylength is often a strong cue for alterations in life-history traits (Beck 1980, Nylin and Gotthard 1998) because it is a reliable indicator of seasonal time, independent of weather variation (Tauber et al. 1986). Photoperiod and temperature covary in nature, and thus isolating the effect of photoperiod on life-history traits may not reflect natural conditions for mosquitoes. However, because photoperiod is not subject to interannual and seasonal variation it has been described as the main seasonal cue for insects (Nylin and Gotthard 1998). A number of studies have quantified the effects of photoperiod alone on mosquito life-history traits (e.g., Jordon and Bradshaw 1978, Lounibos et al. 2003), which allows for the isolation of the developmental response to photoperiod. Many mosquitoes in temperate environments use daylength as an important cue for the initiation of egg or larval diapause (Anderson 1968, Jordan and Bradshaw 1978, Holzapfel and Bradshaw 1981). One general prediction for the developmental response to photoperiod is that an individual that experiences short or diminishing day lengths may accelerate development at a cost to final body size (Leimar 1996).

The source of *Ae. albopictus* in the United States is likely temperate Asia (Hawley et al. 1987), and genetic analyses suggest that the current populations in the United States derive from a single invasion (Birungi and Munstermann 2002). In northern latitudes, female *Ae. albopictus* lay diapausing eggs (Lounibos et al. 2003) that can tolerate winter conditions, but larvae or adults that experience freezing will die. Thus, larvae developing in the north late in the season experience pronounced time constraints (Johansson and Rowe 1999, Stoks et al. 2006b). Adaptive responses to such constraints in environments with short growing seasons may include compensation by accelerating reproduction or development and ovipositing before weather-induced death (Luker et al. 2002, Leisnham et al. 2008). Therefore, in colder climates that experience annual freezing temperatures, there is likely selection for *Ae. albopictus* to alter life-history patterns when it encounters cues like short or decreasing daylength that signal the arrival of potentially fatal temperatures.

There is evidence of differentiation of life-history traits for *Ae. albopictus* subsequent to the invasion into North and South America. Lounibos et al. (2003) investigated whether the occurrence of egg diapause varied among populations of *Ae. albopictus* from across North and South America, and showed that variation in these rates had occurred since its introduction into both regions, with reduction of likelihood of diapause in the southern United States. Rapid adaptive evolution of *Ae. albopictus* in response to photoperiod recently has been show for U.S. populations compared with native populations (Urbanski et al. 2012). Armbruster and Conn (2006) examined growth rates of larvae from three sites (Florida, Texas, and New Jersey) and found weak but consistent trends toward increased rates of larval growth in northern relative to southern populations, although these differences were small relative to the effects of other factors (e.g., temperature). Leisnham et al. (2008) examined variation in adult survival and reproductive traits (e.g., total reproductive output, reproductive investment per offspring) between populations in the northern (Indiana and Virginia) and southern (Florida) range extremes in the United States, but found no consistent differences in these traits between northern and southern populations. They did, however, find a single northern population to be dissimilar in some traits compared with all other populations (Leisnham et al. 2008). These studies collectively suggest fairly rapid local differentiation of populations of *Ae. albopictus* after invasion, an idea corroborated by isozyme analyses (Black et al. 1988, Kambhampati et al. 1991). At least some of this differentiation may be adaptive and associated with latitudinal variation in climate (Lounibos et al. 2003).

There is an increasing awareness of sex-based differences in certain life-history patterns among insects (Vincent 2006). For instance, male and female mosquitoes frequently exhibit dissimilar trade-offs because they are under different reproductive constraints (Kleckner et al. 1995). Specifically, in some species male and female mosquitoes demonstrate trade-offs in development time versus mass at eclosion, with males sacrificing mass for quicker development times and females maximizing mass, which is positively related to life-time fecundity, but taking longer to develop. This trade-off leads to protandry, which was first conceived by Darwin (1871) as a form of sexual selection for increased male mating opportunities via access to virgin females and for greater size and correspondingly longer prereproductive period for females. More recent work with the western tree hole mosquito (*Aedes sierrensis* Ludlow) suggests that for mosquitoes protandry has instead evolved via differential selection on sex-specific, size-based reproduction (Kleckner et al. 1995). Because of protandry, and the underlying intersexual difference in life history trade-offs, we might expect that males and females would differ in their plastic responses to changing photoperiods. Males, having been selected for minimum development time, are expected to show little or no plastic response to a short photoperiod. In contrast, females, selected for maximum size at the cost of prolonged development, are expected to show a pronounced reduction in development time in response to the threat of seasonal death indicated by short photoperiod. Several studies have documented sex-specific trade-offs in development time and mass in *Ae. albopictus* under a variety of biotic (e.g., competition; Juliano 1998) and abiotic conditions (e.g., drying, Costanzo et al. 2005, food resources, Yee et al. 2007), although it is unknown if sex-specific mass-development trade-offs are differentially affected by changing seasonal conditions (e.g., photoperiod), or whether this plastic response varies with latitude.

Table 1.   Collection sites of *Ae. albopictus*, percent freeze, and date of percent freeze under three photoperiods used in this exp

| Location (city) | Latitude (°N) | Percent chance of freeze and date associated with each photoperiod | | |
		Short stable	Short declining	Long
New Jersey (Salem)	39° 36′	70%, 31 Oct	30%, 14 Oct	0%, 14 Aug
Virginia (Manassas)	38° 36′	90%, 3 Nov	70%, 20 Oct	0%, 11 Aug
North Carolina (Raleigh-Durham)	35° 45′	70%, 11 Nov	30%, 25 Oct	0%, 2 Aug
Florida (Vero Beach)	27° 35′	30%, 26 Dec	0%, 6 Nov	0%, 15 Jun

Here we test the hypotheses that: 1) larval *Ae. albopictus* exhibit photoperiodic sensitivity in life history traits; and 2) this photoperiodic sensitivity in life history traits shows evidence for adaptive geographic variation across the current range of this species in North America. Our study, following several others (e.g., Jordon and Bradshaw 1978, Lounibos et al. 2003, Urbanski et al. 2012), focued solely on the effect of photoperiod while holding temperature constant. We tested the response of four populations of *Ae. albopictus* spanning 12° latitude across the United States to different photoperiods assumed to indicate approaching harsh (high freeze probability) or benign (low freeze probability) environments. Specifically, we predicted that if the mass-development trade-off for males and females was flexible, then individuals from latitudes with greater seasonal likelihood of lethal temperatures (i.e., the north) would exhibit stronger plastic response to photoperiod. Specifically, individuals in the north would have shorter development times and be smaller under the photoperiods indicative of harsh future temperatures, but would be larger with longer development times when reared under a photoperiod indicative of benign future conditions, compared with southern populations. We predicted that this mass-development time trade-off for southern populations would be invariant under different photoperiods that normally do not indicate temperatures fatal to mosquitoes. We also explicitly tested for differences in responses of males and females (i.e., sexual dimorphism) to our treatments, and predicted that when there is a plastic response of development time or mass to photoperiod cues, females would show a greater response than males. This latter prediction is based on patterns of protandry in a related species (Kleckner et al. 1995).

Materials and Methods

Mosquitoes. We obtained *Ae. albopictus* eggs from four locations in the eastern United States: New Jersey (NJ), Virginia (VA), North Carolina (NC), and Florida (FL). Three populations (NJ, VA, NC) came from laboratory colonies (F_5–F_7) originally collected from single counties within each state (Table 1). The Florida eggs were F_1 progeny from adults collected from Vero Beach (Table 1). All eggs were hatched in a solution of 0.33 g nutrient broth per 750 ml deionized water (DI). After 24 h, first instars were rinsed with water before being added to experimental containers.

Photoperiods. We used three photoperiods that we predicted would modify mass-development time

trade-offs for these four populations: short-stable (SS), short-declining (SD), and long-stable (LS). Larvae subjected to the SS and LS photoperiods experienced the same daylength throughout the experiment, whereas individuals in the SD photoperiod experienced a decreasing daylength through time. We used the SD in addition to the SS photoperiod to test whether natural changes in photoperiod are a cue to season. The SS photoperiod was defined by the date of maximal mid- to late-year (July through December) freeze probability from the FL location (30%; Table 1). The maximal freeze probability is the greatest probability of a freeze during the period of interest. This information was based on data (1923–2007) from the Southeast Regional Climate Center (http://www.sercc.com/). After determining the date of maximal freeze probability for Florida (i.e., 26 December), we used data from the United States Naval Observatory (http://www.usno.navy.mil/) to determine daylength for that date (i.e., 10 h 28 min). We then found this identical daylength during fall and identified the corresponding freeze probability for the other three sites using data from the Southeast Regional Climate Center (VA, NC) or the Northern Regional Climate Center (NJ; http://www.nrcc.cornell.edu/). This process yielded freeze probabilities for VA, NC, and NJ that ranged from 70 to 90% (Table 1). This approach enables us to expose all populations to the same photoperiod, yet assures that all populations were subjected to a daylength that reflects high freeze probability for the site of origin in nature.

For the SD photoperiod, we targeted the declining photoperiod to be identical to the SS (i.e., 10 h 28 min) on the 18th day after adding larvae to the experiment. Based on a preliminary run of this experiment (data not shown), the highest percentage of all adults from the NJ population eclosed on day 18. To determine the daylength at the start of the SD treatment, we determined the daylength for the NJ location 17 d before the date generated for the SS treatment (31 Oct − 17 d = 14 Oct). We then determined the daylength for this date (i.e., 11 h 8 min) and divided the difference between the 2 daylengths by number of days (40 min/17 d). Because this process did not yield a whole number of minutes per day (i.e., 2.35 min), we alternated between daily 2 and 3 min reductions in photoperiod until the conclusion of the experiment.

For the LS, we chose the longest summer daylength for the FL location (i.e., 13 h 49 min) and determined the date of that daylength using the same procedure

Table 2. Multivariate ANVOA results for male and female *Ae. albopictus* mass (mg) and development time (d) across photoperiods, densities, and locations

	Females					Males				
				SCC					SCC	
	df	Pillai's Trace	*P* value	Mass	Development time	df	Pillai's Trace	*P* value	Mass	Development time
Density (D)	**2, 89**	**0.138**	**0.001**	**0.694**	**1.141**	**2, 85**	**0.094**	**0.015**	**0.177**	**1.512**
Location (L)	**6, 180**	**0.623**	**<0.001**	**0.827**	**1.025**	**6, 172**	**1.110**	**<0.001**	**0.968**	**0.957**
Photoperiod (P)	**4, 180**	**0.409**	**<0.001**	**0.760**	**1.088**	**4, 172**	**0.302**	**<0.001**	**0.409**	**1.414**
D × L	6, 180	0.120	0.080	−0.450	1.300	**6, 172**	**0.159**	**0.025**	**1.407**	**−0.919**
D × P	4, 180	0.038	0.488	0.231	1.360	4, 172	0.047	0.388	1.502	−0.490
L × P	12, 180	0.131	0.401	0.677	1.154	12, 172	0.211	0.073	−0.216	1.616
D × P × L	12, 180	0.076	0.843	0.408	1.304	12, 172	0.150	0.315	−0.417	1.620

Effects significant at $\alpha = 0.05$ are shown in bold. The sizes of the standardized canonical coefficients (SCC) were used to indicate which dependent variable (mass or development time) were important for significant multivariate effects.

as for the SS for all locations. This process yielded a 0% freeze probability for all locations (Table 1, as above).

Experimental Design. After hatching, larvae were added to 100-ml plastic beakers containing 100 ml DI water. Because sex-specific trade-offs often are subject to density-dependent effects (Kleckner et al. 1995), larvae were grown under two initial densities: 20 or 40 larvae/100 ml water. These densities were higher than the density that produces sex-specific trade-offs (1 larva/20 ml; Yee et al. 2007). Larvae were fed 0.001 g/larva powered Lacalbumin and Brewer's yeast (1:1 ratio by weight) initially (i.e., 0.02 g for 20 larvae, 0.04 g for 40 larvae), then given 0.02 and 0.01 g for 40 and 20, respectively, every 5 d after. We replicated all density (2) by population (4) treatments five times within each photoperiod (3) for a total of 120 experimental units. Larvae were raised under constant temperature (20°C) so the effect of temperate on metabolic and development rates would not be confounded with effects of photoperiod on development. This temperature was chosen as a compromise between summer and fall/winter conditions that the photoperiods were meant to mimic, and was similar to the temperature used in another study examining the photoperiodic diapuses response in *Ae albopictus* (Lounibos et al. 2003). Beakers ($n = 6–8$) were randomly assigned to plastic shoe boxes and loosely covered to reduce evaporation. Shoe box positions were rotated every other day to homogenize variation within incubators. Pupae were removed every day, isolated, and adults allowed to eclose in individual glass shell vials. Adults were sexed, dried at 50°C for 48 h, and weighed to the nearest 0.0001 mg using an ultramicrobalance (Mettler-Toledo, Columbus, OH). Mean mass and development time (from egg to adult) for each sex were generated from each replicate and represented the dependent variables for each beaker.

Data Analysis. We analyzed mosquito mass and development time for each sex, separately, using multivariate analysis of variance (MANOVA) with locations (4), photoperiods (3), and density (2), as well as their interactions as independent variables. Significant MANOVA effects were interpreted using standardized canonical coefficients (SCCs; Scheiner 2001), which quantify the magnitude of the contributions of the individual dependent variables in producing significant multivariate differences. When necessary, significant effects were analyzed further using multivariate pair-wise contrasts (Scheiner 2001) with a Bonferroni correction to control experiment-wise error rate. Differences in sexual dimorphism were explicitly tested by subtracting the mean mass and development time of males from those for females in each beaker that produced each sex. These data were then analyzed using three-way MANOVA with photoperiod, density, and location as independent factors, and differences in mass and development time as independent variables. Finally, we analyzed survival among treatments (locations, photoperiod, density) using three-way ANOVA, after applying an arcsine square-root transformation to meet assumptions of normality and homoscedasticity. All analyses were conducted in SAS (2004).

Results

Female mass and development time were significantly affected by larval density, location, and photoperiod, but not by any of their interactions (Table 2). Based on standardized canonical coefficients, development time contributed more to the significant effects than did mass (Table 2). Under low density, adult females were smaller ($425.7 \pm 9.2 \mu g$) but developed faster (19.1 ± 0.19 d) compared with the high density ($453.9 \pm 9.2 \mu g$, 20.0 ± 0.18 d). Females from NC were larger and took significantly longer to develop compared with all other locations (Fig. 1a). In addition, females from NJ and VA took less time to develop compared with females from FL (Fig. 1a). Females in the LS photoperiod took significantly longer to develop and achieved a significantly larger mass compared with either SS or SD, which did not differ (Fig. 1b).

Males displayed significant differences in mass and development time with larval density, location, and photoperiod, but also showed a significant density by location interaction (Table 2). Males usually took longer to develop under high compared with low density, with no density differences in male development time from the NC and FL populations (Fig. 2b). Males from
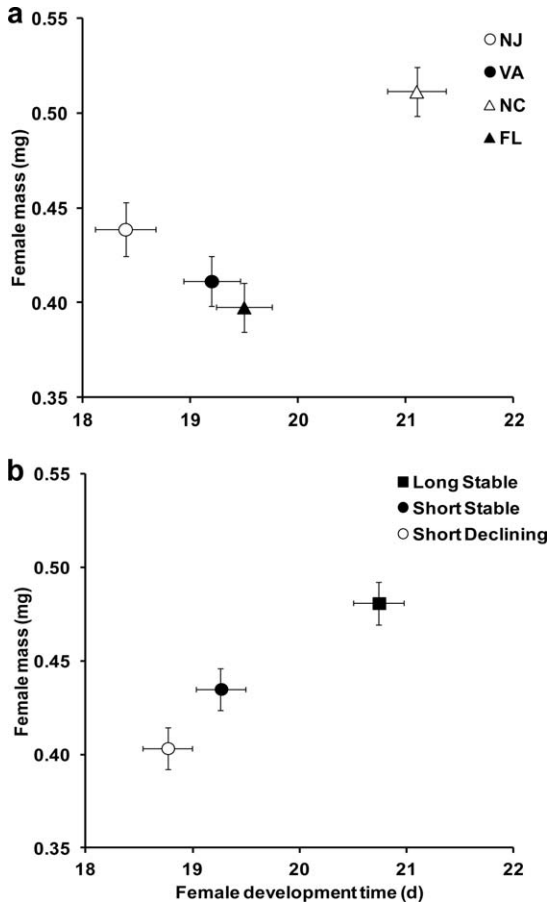
Fig. 1. Means ($\pm$SE) for female *Ae. albopictus* mass and development time for the significant effect of (a) location and (b) photoperiod.

Fig. 2. Means ($\pm$SE) for male *Ae. albopictus* mass and development time for the significant (a) effect of photoperiod and (b) interaction of location (NJ, New Jersey; VA, Virginia; NC, North Carolina; FL, Florida) and density (low = 20, high = 40 larvae per 100 ml). Means for SS and SD in panel a are coincident.

FL were smaller in the low density versus the high density treatment, and smaller overall compared with other locations, with no differences in mass for other locations between density treatment levels (Fig. 2b).

There were strong differences in sexual dimorphism across photoperiods (Pillai's Trace$_{4,166}$ = 0.268; $P <$ 0.001), but not among locations (Pillai's Trace$_{6,166}$ = 0.082; P = 0.311) or between densities (Pillai's Trace$_{2,83}$ = 0.027; P = 0.325); no interactions among factors were significant. Both mass (SCC = 0.583) and development time (SCC = 0.868) were similar in contributing to difference in dimorphism among photoperiods. Specifically, differences in sexual dimorphism were greater for mass and development time in the LS photoperiod compared with either short photoperiod (Fig. 3).

Survival differed significantly for density ($F_{1,96}$ = 28.11; $P <$ 0.001), location ($F_{3,96}$ = 44.73; $P <$ 0.001), and photoperiod ($F_{2,96}$ = 7.80; $P <$ 0.001). Survival was greater in low (52.8%) versus high densities (36.2%), and in the SD (52.9%) versus either LS (40.1%) or SS (40.5%) photoperiods. Locations also differed considerably in survival, with the highest survival in the
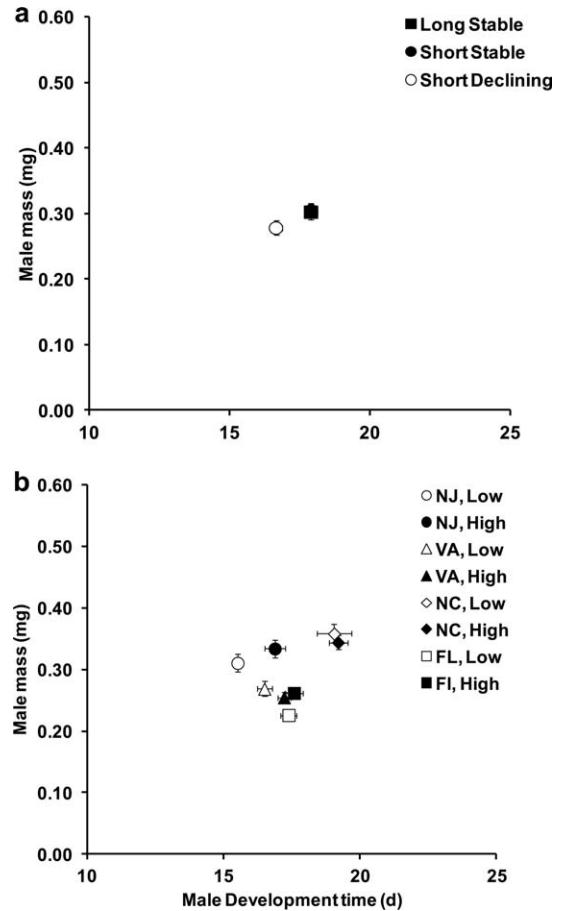
populations from FL (66.2%) and VA (57.9%) and lowest in NJ (26.3%) and NC (27.6%). All interactions were nonsignificant at $P \gg 0.05$.

Discussion

To our knowledge, this is the first study to demonstrate trade-offs in life-history traits initiated during the larval phase for *Ae. albopictus* subjected to simulated seasonal photoperiods, and therefore demonstrates a level of phenotypic plasticity not noted elsewhere for this species. Although we did not investigate the potential interactive effects of temperature and photoperiod that likely occur in nature, our work found ample support that larval *Ae. albopictus* exhibit photoperiodic sensitivity in life history traits (hypothesis a), but did not find any evidence that photoperiodic sensitivity in life history traits varied with geographic locations (hypothesis b), and thus we cannot currently support the hypothesis of adaptive geo-
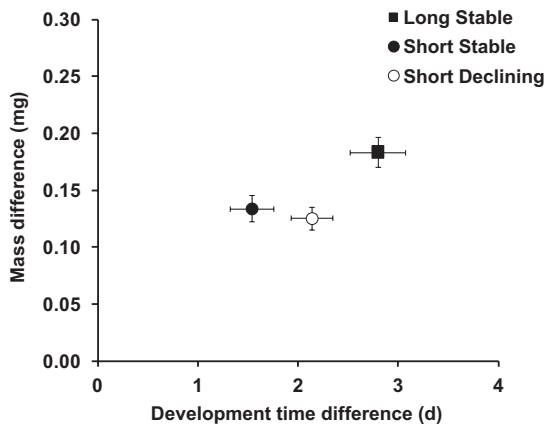
Fig. 3. Mean (±SE) values of sex dimorphism in mass and development time for adult *Ae. albopictus* for the significant effect of photoperiod. Values are means of males subtracted from females within each container.

graphic variation for *Ae. albopictus* across North America. We also identified strong differences between the sexes in their response to our treatments, and specifically we showed sexual dimorphism across photoperiods (Fig. 3), with differences between the sexes in mass-development trade-offs (Fig. 1a, 2b) likely explaining this result. Taken together, our results support sex-specific phenotypic plasticity in life-history traits for *Ae. albopictus* that matches variation in important environmental variables such as photoperiod. Given that temperature can mediate the response of mosquito life-history parameters to photoperiod (e.g., Holzapfel and Bradshaw 1981) this subject would benefit from future work to understand the implications for *Ae. albopictus* in natural environments.

As in our experiment, multiple recent studies have failed to find strong adaptive geographic variation of North American *Ae. albopictus* life history traits (e.g., Armbruster and Conn 2006, Leisnham et al. 2008, Kesavaraju and Juliano 2009). For our data, a number of potential explanations exist for a lack of life-history differences in response to simulated seasonal photoperiods across latitude. We used only one population per geographic location and this may have led to effects associated with specific populations rather than geography per se, with large variation among populations in life-history traits having been noted for *Ae. albopictus* in similar locations (Armbruster and Conn 2006). We did find that male development time displayed a trend suggestive of a photoperiod-location interaction (i.e., $P = 0.073$; Table 2), although this result was based on differences primarily between NC and all other locations (data not shown). This suggests that if this trend is real, it results from something site-specific, rather than being part of a large latitudinal pattern. Because many of our populations had been maintained in the laboratory through multiple generations, founder effects or inbreeding also could have affected the ability of populations to respond to our treatments, but these random effect are more

likely to have accentuated variation among populations, rather than reduce it. Relatively rapid evolution has been demonstrated in as few as 3–4 generations in another *Aedes* species, *Ae. triseriatus*, for egg diapause (Sims 1985) and for behavior (Juliano and Gravel 2002), although Kesavaraju and Juliano (2009) found no evidence for such rapid evolution of antipredator behaviors in *Ae. albopictus* under four generations of laboratory selection. Moreover, if differences in the amount of time populations were housed in the lab were the main determinant of responses, we might expect FL to be different from the other populations, a result that we did not find. Colony-specific rearing conditions (e.g., light regimes) may have inadvertently selected for modified photoperiodic responses. In particular, the population from NC did not fit along the latitudinal cline of populations used, as both males (Fig. 2b) and females (Fig. 1a) were larger and took longer to develop compared with other populations. In a preliminary run of this experiment, individuals from NC displayed low survival (6.4%), which was consistent with the low survival during this experiment (27.6%). Such overall low viability may arise either because of founder effects, or because of particular husbandry techniques used for colonizing this population. Statistical analysis without the NC population (not shown) did not change the final conclusions. Thus, taken together we conclude that outcomes observed here were likely reflective of natural population responses to photoperiods, although future studies using replicated populations from each geographic location would help to confirm this conjecture.

Although our data were largely consistent with our predictions, males and females displayed different levels of congruence with our expectations. Females showed a strong response of mass and development time to different photoperiods (Fig. 1b), with males showing less response in these traits, particularly with photoperiod (Fig. 2a). The apparent differences between males and females in response to photoperiod may be based on differences in the sexes in life-history trade-offs (protandry) or other mechanisms not well understood. Consistent with many studies on *Aedes*, females took longer to develop and were larger than males in long versus short photoperiods, although males did not appear to sacrifice size when decreasing development time as photoperiods decreased (Fig. 2a). Males have been shown to sacrifice mass to develop more quickly as a strategy to have access to virgin females (Kleckner et al. 1995). We should also not be surprised that males were less variable than females in response to different photoperiods, given that male mosquitoes often emerge days before females (Clements 2000), including male *Ae. albopictus* (e.g., Armbruster and Conn 2006, Yee et al. 2007), and thus may be less capable of accelerating development in response to seasonal cues. Male mass was less variable than development time across photoperiods, a result also obtained for *Ae. albopictus* across a similar geographic range by Armbruster and Conn (2006). Finding that male mosquitoes were able to accelerate development but emerge at similar sizes in

short versus long photoperiods is somewhat unexpected. One explanation is that a single measure (i.e., body mass) may obscure more subtle costs that were not quantified here. For instance, some insects that display accelerated life-history traits show associated costs in energy storage (Stoks et al. 2006a) and immune function (Rantala and Roff 2005, Stoks et al. 2006b) that ultimately may translate into reduced lifetime mating success (De Block and Stoks 2005). Few studies have shown long-term costs of small size in male mosquito mating success (e.g., Benjamin and Bradshaw 1994), but the fact that larger *Ae. aegypti* exhibit greater reproductive capacity and fitness via increased spermatogenesis (Ponlawat and Harrington 2007) may indicate a reproductive cost to small male size.

Beyond photoperiod effects, males displayed interactive effects of density and location, with the general result that development times were shorter, but masses similar for VA and NJ, but not for FL and NC, in low versus high densities. In addition, survival was significantly lower at high densities. Given that we maintained food resources at the same per capita level, it is unlikely that intraspecific competition for food is an explanation for poor performance of adults from high density beakers. Crowding could be an explanation for decreased survival and longer development times given that the densities we used (one larva/2.5–5.0 ml) were higher than in recent experiments that have showed strong intraspecific effects (Yee et al. 2007, Murrell and Juliano 2008). For a variety of reasons, increased larval density can negatively affect many aspects of mosquito life-history traits (Barbosa et al. 1972, Roberts and Kokkinn 2010).

Here we conclude that female and male *Ae. albopictus* larvae are sensitive to photoperiod, and that accelerated development and reduced body size in females, and to a lesser degree males, in response to short or declining photoperiods is potentially an adaptive life-history response to seasonal time constraints. Females, and to a lesser extent males, that experience short days as larvae may either accelerate development and potentially reproduce, or delay development to increase body size at the potential cost of death before emergence. Faced with such a scenario, selection likely favors genotypes that exhibit phenotypic plasticity that maximizes fitness across the range of environmental conditions encountered. Beyond the fact that this species is a superior resource competitor compared with many resident mosquito species across a wide variety of resource environments (e.g., Livdahl and Willey 1991, O'Meara et al. 1995, Juliano 1998, Daugherty et al. 2000, Costanzo et al. 2005, Yee et al. 2007), the results we present here also point to other attributes, such as phenotypic plasticity, that we must consider as part of the repertoire for the success of *Ae. albopictus* as a globally invasive species.

Acknowledgments

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