

# Impact of inter- and intra-specific competition among larvae on larval, adult, and life-table traits of *Aedes aegypti* and *Aedes albopictus* females

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**Abstract.** 1. Few studies have taken a comprehensive approach of measuring the impact of inter- and intra-specific larval competition on adult mosquito traits. In this study, the impact of competition among *Aedes aegypti* (L.) and *A. albopictus* (Skuse) was quantified over the entire life of a cohort.

2. Competitive treatments affected hatch-to-adult survivorship and the development time to adulthood of females for both species but affected the median wing length of females only for *A. albopictus*. Competitive treatments had no significant effect on the median adult female longevity nor were there any effects on other individual traits related to blood feeding and reproductive success.

3. Analysis of life table traits revealed no effect of competitive treatment on the net reproductive rate ( $R_0$ ) but there were significant effects on the cohort generation time ( $T_c$ ) and the cohort rate of increase ( $r$ ) for both species.

4. Inter- and intra-specific competition among *Aedes* larvae may produce individual and population-level effects that are manifest in adults; however, benign conditions may enable resulting adults to compensate for some impacts of competition, particularly those affecting blood-feeding success, fecundity, and the net reproductive rate,  $R_0$ . The effect of competition, therefore, affects primarily larva-to-adult survivorship and the larval development time, which in turn impacts the cohort generation time,  $T_c$ , and ultimately the cohort rate of increase,  $r$ .

5. The lack of effects of the larval rearing environment on adult longevity suggests that effects on vectorial capacity owing to longevity may be limited if adults have easy access to sugar and bloodmeals.

**Key words.** *Aedes aegypti*, *Aedes albopictus*, individual traits, larval competition, life table, population level traits.

## Introduction

Mosquitoes are efficient vectors of the world's most significant diseases, including, malaria, filariasis, and arboviral fevers. As insects that live in two distinct environments (larval – aquatic and adult – terrestrial), mosquitoes experience varied biotic

interactions that impact individual morphological, physiological, and behavioural traits (e.g. wing length, blood-feeding success, and lipid reserves; Schneider *et al.*, 2011), life-history traits closely associated with fitness (survival and fecundity) (Carrington *et al.*, 2013), and ultimately, population growth (Livdahl & Willey, 1991; Juliano, 1998). Any biotic or abiotic factors that affect adult mosquito phenotypes may also alter the transmission potential of associated pathogens (i.e. vectorial capacity) (Grimstad & Walker, 1991; Muturi *et al.*, 2011; Alto & Bettinardi, 2013).

Competitive interactions among mosquito larvae and their effects on population and individual traits are well studied. Intra- (Hawley, 1985; Briegel, 1990; Agnew *et al.*, 2000; Blackmore & Lord, 2000; Legros *et al.*, 2009) and inter-specific competition

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(Livdahl & Willey, 1991; Braks *et al.*, 2004; Costanzo *et al.*, 2005; Armistead *et al.*, 2008) among mosquito larvae affect the life history traits of resulting adults. Larval competition impacts egg-to-adult survivorship and the larval development time (Edgerly *et al.*, 1999; Gimnig *et al.*, 2002; Braks *et al.*, 2004; Leisnham & Juliano, 2009). The most frequently studied individual trait in resulting adults that is affected by competition is adult body size (smaller for individuals experiencing competitive stress as larvae; Braks *et al.*, 2004; Costanzo *et al.*, 2005). Size variation is associated with variation in lipid, protein, and carbohydrate reserves, that may directly impact the host-finding behaviour (Klowden *et al.*, 1988), feeding habits (Chadee & Beier, 1997), the number of bloodmeals taken (Feinsod & Spielman, 1980), blood consumption and fecundity (Briegel, 1990; Telang *et al.*, 2007), biting habits (Nasci, 1991), energy reserves (Nayar & Sauerman, 1970; Nasci, 1986), longevity under stress (Reiskind & Lounibos, 2009), immune traits (Telang *et al.*, 2012), and mortality (Hawley, 1985). Recent studies have evaluated the impact of larval competition on vector competence, with adults that have experienced intense inter-specific (Alto *et al.*, 2008; Bevins, 2008) or intra-specific (Grimstad & Walker, 1991) larval competition having a reduced ability to defend against ingested vector-borne viruses (Sindbis – Muturi *et al.*, 2011; Dengue – Alto & Bettinardi, 2013).

Although individual studies have focused on how competitive conditions at the larval stage can affect one or two adult traits, few studies have taken a comprehensive approach of measuring the impact of inter- and intra-specific larval competition on adult mosquito individual traits and population level traits. Both individual and population level effects are probably important in the assessment of the vectorial capacity of mosquitoes in specific ecological contexts. Further, most studies have evaluated the effects of competition by measuring adult traits for a specific, typically short, period (Reiskind & Lounibos, 2009) or one or two gonotrophic cycles (Farjana & Tuno, 2013) and have not followed cohorts of mosquitoes for their entire larval and adult lives. This situation leaves a knowledge gap concerning the full effects of competition among larvae on adult mosquito life histories. Further, although multiple studies have addressed the effects of competition among larvae on life table and population dynamic traits of cohorts of mosquitoes, most of these have estimated population rates of change (e.g., exponential growth rate  $r$ , or finite rate of increase  $\lambda$ ) based on data only from newly emerged adults (e.g. Livdahl & Sugihara, 1984; Livdahl & Willey, 1991; Juliano, 1998; Costanzo *et al.*, 2005; Armistead *et al.*, 2008), rather than trying to quantify these effects by taking a full life-table approach, following cohorts of mosquitoes from hatching, through adulthood, to the end of adult life. Only Chmielewski *et al.* (2010) have taken the latter approach to estimate effects of intra-specific density. The present study was designed to measure the impact of resource competition on population, life-history, and behavioural traits of adults, and to quantify over the entire life of a cohort, the life table and population vital rates of two important vector species. This was done by rearing larvae under conditions of varying intensities of inter- and intra-specific competition in a controlled laboratory setting, and following the resulting adults' survival and reproductive output in a favourable laboratory setting. We

then interpret those impacts in the context of vectorial capacity of adults and evaluate which of the individual traits are most strongly related to population-level effects on competitors. We investigated *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse), two well-studied container-dwelling competitors that encounter one another throughout the world (Braks *et al.*, 2004; Delatte *et al.*, 2011), and that compete intensely in nature (Juliano *et al.*, 2004).

## Materials and methods

### *Experimental design: larval competitive treatments*

A larval competition experiment was set up using *A. aegypti* and *A. albopictus*, both originating in Florida. One block consisted of one replicate cohort of six larval density treatments (numbers of *A. albopictus*: *A. aegypti*): low intra-specific density (20:0 and 0:20); high intra-specific density (40:0 and 0:40); low inter-specific density (10:10); and high inter-specific density (20:20). Replicates in each block were run together in the same environmental chamber and five blocks were run in sequence over a 9 month period in a randomised complete block design.

For each block, six 400-ml cups were prepared with 350 ml of deionized water and 0.7 g of live oak (*Quercus virginiana*) leaves and incubated at 26 °C at 90% RH for 3 days prior to adding larvae. One day prior to treatment group setup, ~200 eggs from each mosquito species were hatched synchronously in 0.15 g l<sup>-1</sup> suspension of yeast/lactalbumin (1:1) in 25-ml shell vials. Larvae hatching within 24 h was used in the experiment. On day 1, larvae from each mosquito species were counted and allotted to containers in the density treatments described above. Resource pulses of 0.7 g of live oak leaves from Florida were added to each treatment cup on days 14, 28, 42, 56, and 70 until all adults eclosed from each treatment group. The combination of this amount of leaf material, with densities of 20–40 larvae per 350 ml, and pulses of additional leaves at 2-week intervals was chosen to produce larval environments in which resource competition would occur, but in which survivorship would be relatively high, ensuring that we would have sufficient adults for the construction of the life table.

Eclosing females from treatment groups were placed into individual cages (600-ml, mesh-top plastic containers, with a 50-ml black plastic oviposition cup lined with a paper towel; a full description is given by Leisnham *et al.*, 2008), provided with continuous access to a sugar solution, and kept at 26 °C, 90%RH with a 14:10 LD cycle. Two males of the same species were put into each cage with one eclosed female to ensure mating. Females were offered an anaesthetised mouse for 15 min every 4 days and feeding success (yes/no) was recorded. We chose these conditions for adults to be benign (ample sugar, blood, and mates; high humidity) so that high adult mortality would not obscure any treatment effects on life table statistics. Cages were checked daily for eggs in oviposition cups (Leisnham *et al.*, 2008) and female survivorship. Eggs were counted every 4 days. At death, the wings of the females were removed, dried, and measured under a stereomicroscope. The protocol continued for

each cohort from a replicate container within a block until all adult females died.

### Statistical analysis

**Larval–adult transition traits.** We used randomised complete block ANOVA, with block and treatment as independent variables (PROC GLM; SAS Institute Inc., 2002–2005). We analysed each species separately so that there were four treatments in the experiment (low and high intra-specific, and low and high inter-specific). Three population growth correlates were used as dependent variables to quantify the effects of intra- and inter-specific competition on the production of adult mosquitoes: larval survivorship (percent of the initial number of larvae reaching adulthood; both sexes), the median size at adulthood (wing length of females), and the median female time to eclosion. For size, one wing was removed from each female after death and measured under a dissecting microscope with an ocular micrometer (Braks *et al.*, 2004). We arcsine square-root transformed proportions surviving and log-transformed time data to meet assumptions of normality and homogeneity of variances. The median wing length and the median time from hatch to adulthood of females within a container were used because of non-normal distributions of these variables within cohorts. The development time was calculated as the number of days from hatching to adulthood. Ideally, female size would be measured for all females emerging from the same container; however, because of the long adult life span, some wings (47 out of 230 adult females, or about 20%) were so damaged at the time of death that they could not be measured.

**Mean traits of adults.** For each cohort of adult females, the mean blood-feeding frequency (= mean of total number of meals each female took divided by the number of meals each female was offered), the mean number of bloodmeals, the mean lifetime fecundity for those females laying eggs, the mean length of the first egg production cycle, the and mean fecundity in the first cycle for those females laying eggs, were analysed using randomised complete block ANOVA with replicate and treatment as variables (PROC GLM; SAS Institute Inc., 2002–2005). Adult longevity (=days from eclosion until death) was analysed using Cox's proportional hazards survival analysis (SAS PROC PHREG; SAS Institute Inc., 2002–2005) including block, and treatment as class variables and female wing length as a covariate. We also included a cumulative reproductive output in the model as time-dependent covariates (i.e. their values can change over the course of the experiment, See Leisham *et al.*, 2008). This time-dependent covariate tests for a physiological cost of reproduction in our laboratory environment. The estimated age-specific hazard rate was compared among treatments using contrasts in this proportional hazard model. Hazard functions (mean hazard rate at age  $x$ ) were calculated for each treatment in PHREG using the BASELINE statement.

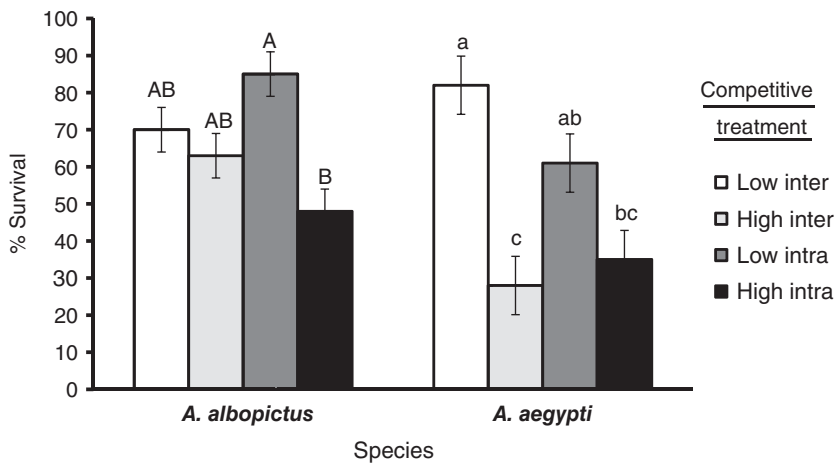
**Life table traits.** We used a life table approach to assess the treatment effects on population-level performance. We

estimated life table statistics for each replicate cohort and analysed them by randomized block ANOVA if assumptions of normality were met, and by non-parametric tests when the data were obviously non-normal due to outlying observations (see below). Quantification of cohort rates of change and other life table statistics for mosquitoes in these kinds of experiments is often achieved using approximations developed by Livdahl and Sugihara (1984) and as modified by Juliano (1998). These estimates ( $r'$ ,  $\lambda'$ ) assume that fecundity in the first reproductive cycle can be predicted by female size, that females only produce one batch of eggs, and that all females require the same amount of post-eclosion time to reach reproduction (Livdahl & Sugihara, 1984), and have been shown to be consistent estimators, but with a slight bias toward overestimation of  $r$  (Chmielewski *et al.*, 2010). In our experiment, because we followed survival and reproduction of females for their entire lives, we can quantify these life table statistics directly without these assumptions and estimation procedures. We calculated these values for each cohort of mosquitoes of each species. For  $R_0$ , the net reproductive rate or the number of offspring left, on average, by newborn females over her entire life, taking into account mortality throughout the lifespan, we determined the total number of eggs laid by a single cohort of a species and divided by the initial number of eggs (i.e. newly hatched larvae, 10, 20, or 40 depending on the treatment) of that species in the cohort. This approach of counting eggs ignores the (unknown) sexes of the eggs both at the start of the experiment and laid by females produced from a cohort. The same  $R_0$  estimate would be obtained if we divided both the initial number of eggs and the final number of eggs by 2 (i.e. assumed a 1 : 1 sex ratio). The cohort generation time,  $T_c$ , is defined as the mean time from birth of a mother to birth of her offspring, and we calculated this as the mean day of oviposition (measured from the day of hatching of the larvae entering the competition experiment) of all eggs from a cohort across all females in the cohort. As with  $R_0$ , we ignored the sexes of the eggs. The same estimated  $T_c$  would be obtained if we assumed half of the initial eggs and half of the laid eggs were females. We calculated  $r$ , the cohort rate of increase by solving the Euler–Lotka equation;  $\sum e^{-rx} l_x m_x = 1.0$  using the solver add-in in Excel 2010. In this equation,  $x$  is the time from hatch in days,  $l_x$  is proportion surviving on day  $x$ , and  $m_x$  is the mean number of eggs laid per surviving female on day  $x$  (Gotelli, 1995). One cohort of *A. aegypti* in the 20 *A. albopictus* 20 *A. aegypti* treatment produced no surviving females, yielding  $R_0 = 0$  and undefined values of  $T_c$  and  $r$  ( $T_c = \infty$  and  $r = -\infty$ ). For these variables, for analysis we substituted large positive and large negative values for  $T_c$  and  $r$ , respectively, and used non-parametric randomized complete block analyses (Friedman's test based on ranks) to compare  $T_c$  and  $r$  among treatments.

## Results

### Larval–adult transition

**Survivorship.** For both *A. albopictus* ( $F_{3,12} = 4.89$ ,  $P = 0.0190$ ) and *A. aegypti* ( $F_{3,12} = 7.61$ ,  $P = 0.0041$ ), survivorship was significantly affected by treatment (Fig. 1). For



**Fig. 1.** Proportion survivorship to eclosion of larvae for inter- and intra-specific competitive treatments of *Aedes albopictus* and *A. aegypti*. Values plotted are means  $\pm$  SE (pooled SE based on overall analysis and Error Mean Squares). For each species, treatment means associated with the same letters are not significantly different (pairwise comparisons after ANOVA, sequential Bonferroni).

**Table 1.** Effect of competitive treatments at larval stages on time to adult eclosion and wing size of adult females.

Species treatments	Time to adult eclosion (median days $\pm$ SE)*	Wing size (mm $\pm$ SE) *
<i>A. aegypti</i>		
Low inter	29 $\pm$ 4.1 (C)	2.43 $\pm$ 0.05 (A)
High inter	45 $\pm$ 4.1 (AB)	2.37 $\pm$ 0.05 (A)
Low intra	33 $\pm$ 4.1 (BC)	2.44 $\pm$ 0.05 (A)
High intra	55 $\pm$ 4.1 (A)	2.23 $\pm$ 0.05 (A)
<i>A. albopictus</i>		
Low inter	25 $\pm$ 1.9(A)	2.26 $\pm$ 0.03 (A)
High inter	37 $\pm$ 1.9(B)	2.15 $\pm$ 0.03 (AB)
Low intra	27 $\pm$ 1.9 (A)	2.22 $\pm$ 0.03 (AB)
High intra	42 $\pm$ 1.9(B)	2.11 $\pm$ 0.03 (B)

\*Within columns, competitive treatment means associated with different letters are significantly different (pairwise comparisons after ANCOVA, sequential Bonferroni).

All SE are pooled SE based on overall analysis and Error Mean Squares.

both species, survivorship was lower for both the high intra- and inter-specific densities compared with the low-density treatments (Fig. 1). However, *A. albopictus* survivorship was significantly reduced by high density only in the intra-specific competition treatments (Fig. 1), whereas *A. aegypti* survivorship was significantly reduced by high density only in the inter-specific competition treatments (Fig. 1).

**Developmental time.** For both *A. albopictus* ( $F_{3,12} = 17.34$ ,  $P = 0.0001$ ) and *A. aegypti* ( $F_{3,12} = 8.23$ ,  $P = 0.0030$ ), treatment significantly affected the median time to emergence of female adults, with median times for high-density treatments usually greater than those of the low-density treatments (Table 1).

**Size of female adults.** Competition significantly affected the median wing length of female *A. albopictus* ( $F_{3,12} = 3.89$ ,  $P = 0.0374$ ) and was nearly significant for female *A. aegypti* ( $F_{3,12} = 3.07$ ,  $P = 0.0690$ ) (Table 1). There were no significant

differences between intra- and inter-competitive treatments (Table 1). However, *A. albopictus* females from the low inter-specific competitive treatment were significantly larger than those in the high intra-specific treatment (Table 1).

#### Adult life-history traits

**Adult longevity.** Several females in each replicate/treatment group had a very long adult lifespan (>100 days). Competitive treatments did not significantly affect the median adult female longevity for either *A. albopictus* ( $F_{3,12} = 0.37$ ,  $P = 0.7731$ ) or *A. aegypti* ( $F_{3,12} = 0.80$ ,  $P = 0.5168$ ) (Table 2). Means of the cohort median longevity of adult females for *A. albopictus* (range inter-specific treatment 56–63 days) tended to be greater than those for *A. aegypti* (range inter-specific treatment: 36–48 days). Cox's Proportional Hazard model incorporating effects on longevity of treatment and run with female size and cumulative egg production as covariates was used to evaluate whether the competitive effects experienced by larval mosquitoes are evident after accounting for these other sources of variation. In all cases, effects involving treatments were not significant (*A. albopictus*  $\chi^2 = 2.8346$ , d.f. = 3,  $P = 0.4178$ ; *A. aegypti*,  $\chi^2 = 2.0099$ , d.f. = 3,  $P = 0.57033$ ).

**Blood feeding and reproductive success.** Treatments did not affect the percentage of females that took bloodmeals when offered (*A. albopictus*:  $F_{3,12} = 0.28$ ,  $P = 0.8402$ ; *A. aegypti*:  $F_{3,12} = 0.58$ ,  $P = 0.6385$ ), or that successfully laid eggs after taking blood meals (*A. albopictus*:  $F_{3,12} = 0.36$ ,  $P = 0.7811$ ; *A. aegypti*:  $F_{3,12} = 0.53$ ,  $P = 0.6710$ ) (Table 2). Similarly, treatment did not affect the mean number of bloodmeals taken (*A. albopictus*:  $F_{3,12} = 0.77$ ,  $P = 0.5301$ ; *A. aegypti*: ( $F_{3,12} = 0.38$ ,  $P = 0.7698$ ) or the mean number of eggs produced in a lifetime (*A. albopictus*:  $F_{3,12} = 0.52$ ,  $P = 0.6741$ ; *A. aegypti*:  $F_{3,12} = 0.61$ ,  $P = 0.6229$ ) (Table 2).

**Median time for the first gonotrophic cycle.** The only significant treatment effect was for *A. albopictus* for a median

**Table 2.** Effect of larval competitive treatments on adult median longevity, bloodmeals, and oviposition success.

Species treatment	Adult longevity* (mean of replicate median days $\pm$ SE)	Percent of offered bloodmeals taken <sup>†</sup> (% $\pm$ SE)	Number of bloodmeals taken <sup>‡</sup> (mean $\pm$ SE)	Percent of successful reproductive opportunities <sup>§</sup> (% $\pm$ SE)	Mean lifetime fecundity <sup>¶</sup> (mean $\pm$ SE)
<i>A. aegypti</i>					
Low inter	43.3 $\pm$ 7.63	74.7 $\pm$ 7.2	3.86 $\pm$ 0.93	74.7 $\pm$ 7.9	178 $\pm$ 38.9
High inter	51.8 $\pm$ 7.63	81.0 $\pm$ 7.2	5.16 $\pm$ 0.93	69.3 $\pm$ 7.9	247 $\pm$ 38.9
Low intra	35.9 $\pm$ 7.63	76.7 $\pm$ 7.2	4.69 $\pm$ 0.93	70.0 $\pm$ 7.9	231 $\pm$ 38.9
High intra	47.9 $\pm$ 7.63	87.1 $\pm$ 7.2	4.98 $\pm$ 0.93	81.8 $\pm$ 7.9	205 $\pm$ 38.9
<i>A. albopictus</i>					
Low inter	55.7 $\pm$ 5.38	88.3 $\pm$ 4.1	2.97 $\pm$ 0.4	83.3 $\pm$ 5.0	181 $\pm$ 27.4
High inter	58.6 $\pm$ 5.38	88.1 $\pm$ 4.1	3.72 $\pm$ 0.4	88.1 $\pm$ 5.0	160 $\pm$ 27.4
Low intra	62.6 $\pm$ 5.38	92.7 $\pm$ 4.1	3.63 $\pm$ 0.4	81.5 $\pm$ 5.0	150 $\pm$ 27.4
High intra	55.6 $\pm$ 5.38	88.6 $\pm$ 4.1	3.72 $\pm$ 0.4	81.8 $\pm$ 5.0	133 $\pm$ 27.4

\***Adult longevity:** Median days survived for a replicate cohort, averaged over the treatment group.

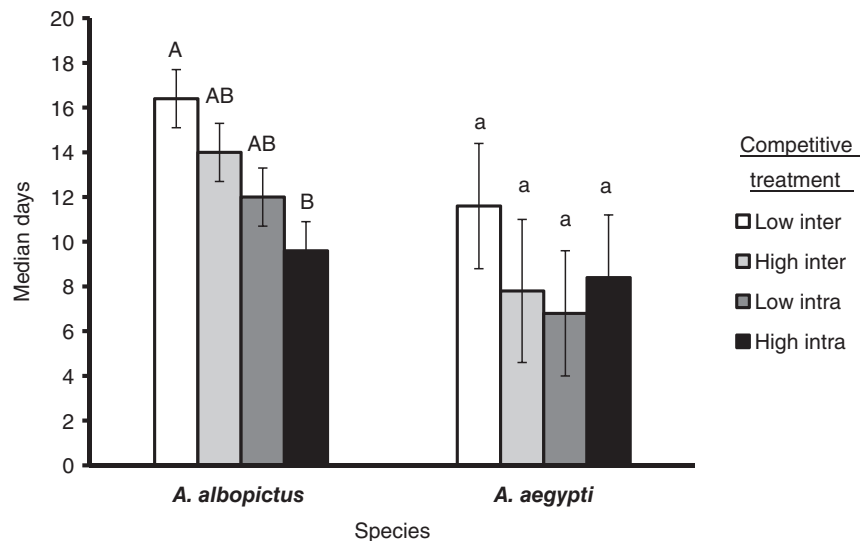
<sup>†</sup>**Per cent of offered bloodmeals taken:** Percent of bloodmeals offered to members of a replicate cohort that were taken, averaged over the treatment group; 100% would indicate that all females in a particular treatment took bloodmeals every time they were offered.

<sup>‡</sup>**Number of bloodmeals taken:** Mean number of bloodmeals taken per female within each replicate cohort, averaged over the treatment group.

<sup>§</sup>**Percent of successful reproductive opportunities:** Percent of bloodmeals offered to members of a replicate cohort that resulted in oviposition, averaged over the treatment group; 100% would indicate that all females in a particular treatment laid eggs every time they were offered a blood meal; if this percent is equal to the percent of offered bloodmeals taken, then all females oviposited every time they took a blood meal.

<sup>¶</sup>**Mean lifetime fecundity:** Mean number of eggs produced in a lifetime by the females in a cohort with opportunity to feed, averaged over the treatment group.

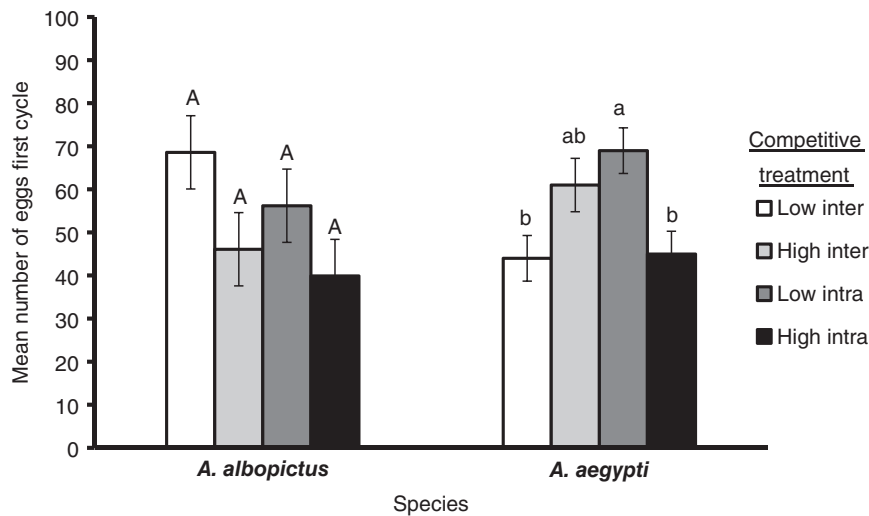
All analyses based on means or medians for the entire replicate cohort. No significant differences were observed among any of the competitive treatments. All SE are pooled SE based on overall analysis and Error Mean Squares.



**Fig. 2.** Effects of larval competition on time from feeding to oviposition in the first gonotrophic cycle of adult *Aedes albopictus* and *A. aegypti* females. Results are presented as the mean of cohort median days to produce the first clutch of eggs + SE (pooled SE based on overall analysis and Error Mean Squares). For each species, treatment means associated with the same letters are not significantly different (pairwise comparisons after ANOVA, sequential Bonferroni).

time from first feeding to oviposition ( $F_{3,12} = 5.21$ ,  $P = 0.0156$ ; Fig. 2). *Aedes albopictus* from the low inter-specific treatments (10 *A. albopictus* 10 *A. aegypti*) took significantly longer to complete a gonotrophic cycle, than did *A. albopictus* from the high intra-specific treatments (40 *A. albopictus*) (Fig. 2). This result was not observed for *A. aegypti* ( $F_{3,12} = 0.54$ ,  $P = 0.6661$ ).

*Fecundity in the first gonotrophic cycle.* Treatments did not significantly affect the mean numbers of eggs laid in the first gonotrophic cycle for *A. albopictus* ( $F_{3,12} = 2.13$ ,  $P = 0.1492$ ) but did so for *A. aegypti* ( $F_{3,11} = 5.10$ ,  $P = 0.0188$ ) (Fig. 3). For *A. aegypti*, density did not affect first-cycle fecundity with inter-specific competition, but a high density resulted in



**Fig. 3.** Effect of larval competitive treatment on the fecundity of the first female cycle for adult *Aedes albopictus* and *A. aegypti* females. Results are presented as the mean number of eggs produced in the first gonotrophic cycle + SE (pooled SE based on overall analysis and Error Mean Squares). For each species, treatment means associated with the same letters are not significantly different (pairwise comparisons after ANOVA, sequential Bonferroni).

**Table 3.** Results of Friedman's test (d.f. = 3 in all cases) based on ranks for median basic reproduction rate ( $R_0$ ), cohort generation time ( $T_c$ ) and cohort rate of increase ( $r$ ) for *Aedes aegypti* and *A. albopictus* in response to competitive species-density treatments.

Species treatment	Basic reproductive rate ( $R_0$ ) Median (max, min)	Cohort generation time ( $T_c$ ) Median (max, min)	Cohort rate of increase ( $r$ ) Median (max, min)
<i>Aedes aegypti</i>			
Low inter	53.0 (115.5, 20)	A 59.97 (66.37, 32.5)	A 0.0788 (0.1013, 0.05837)
High inter	54.2 (70.55, 0)	B 70.56 ( $\infty$ , 69.63)	B 0.0505 (0.0739, $-\infty$ )
Low intra	42.05 (172.4, 9.6)	A 63.65 (74.60, 43.97)	A 0.0679 (0.0929, 0.0543)
High intra	38.18 (46.38, 14.9)	B 85.72 (91.73, 85.64)	B 0.0443 (0.0514, 0.0333)
Friedman's test	H = 1.32, $P = 0.7244$	<b>H = 10.9, <math>P = 0.0122</math></b>	<b>H = 9.96, <math>P = 0.0189</math></b>
<i>Aedes albopictus</i>			
Low inter	42.7 (63.2, 10.9)	A 59.26 (63.58, 51.47)	AB 0.0672 (0.0938, 0.0438)
High inter	34.2 (84.25, 17.55)	B 76.60 (86.67, 60.37)	AB 0.0639 (0.0654, 0.0344)
Low intra	60.45 (83.7, 30.1)	A 60.74 (70.51, 58.83)	A 0.0778 (0.0922, 0.0633)
High intra	36.98 (58.52, 6.68)	B 78.70 (80.91, 74.04)	B 0.0499 (0.06250, 0.0261)
Friedman's test	H = 1.56, $P = 0.6685$	<b>H = 9.96, <math>P = 0.0189</math></b>	<b>H = 8.76, <math>P = 0.0327</math></b>

Significant Friedman's tests indicated in **bold-faced type**. For each response variable, within a species, medians associated with the same letter are not significantly different at experiment wise  $\alpha = 0.05$ .

a reduced first-cycle fecundity with intra-specific competition (Fig. 3).

*Life table traits.* All life table traits were analysed by non-parametric Friedman's tests (Real Statistics Resource Pack software; Zaiontz, 2015) for consistency.

*Net reproductive rate,  $R_0$ .* Treatments did not affect  $R_0$  for either *A. albopictus* or *A. aegypti* (Table 3).

*Cohort generation time  $T_c$ .* Treatment significantly affected  $T_c$  for *A. albopictus* and for *A. aegypti* (Table 3). For both species, the median  $T_c$  was significantly longer for the high-density treatments with one or both species than for

the low-density treatments with one or both species, and within a density, there were no differences owing to the effects of inter- or intra-specific competition (Table 3).

*Cohort rate of increase  $r$ .* Treatment significantly affected  $r$  for both *A. albopictus* and *A. aegypti* (Table 3). For *A. albopictus*, the low-density intra-specific treatment (20 *A. albopictus*) yielded significantly greater  $r$  than did the high-density intra-specific treatment (40 *A. albopictus*), but the two inter-specific treatments did not differ and were not significantly different from the two intra-specific treatments (Table 3). For *A. aegypti*, both low-density treatments yielded significantly greater  $r$  than did both high-density treatments (Table 3), and there were no differences between inter- and intra-specific competition treatments within either density (Table 3).

## Discussion

The purpose of this study was to address the knowledge gap concerning the effects of larval competitive interactions on adult mosquito life history traits. Instead of estimating population rates of change using data from newly emerged adults (Livdahl & Sugihara, 1984; Livdahl & Willey, 1991; Juliano, 1998; Costanzo *et al.*, 2005; Armistead *et al.*, 2008), we quantified these effects by following cohorts of mosquitoes from hatching, through adulthood, and to the end of adult life. As such, we have quantified the effects of competition among larvae as they are propagated throughout the adult stage, providing a comprehensive assessment of the impact of competition on life table traits. We will use the Discussion to evaluate how effects of competition on individual behavioural and life history traits (e.g. survivorship, development time, reproductive cycle length, blood-feeding success, and fecundity) are translated into effects at the population level.

Within the life table analysis, changes in  $r$  (the cohort rate of increase) are affected by variables that impact  $R_0$  (net reproductive rate) or  $T_c$  (cohort generation time). In the present study, the lack of effect on measurable adult blood-feeding traits or  $R_0$  suggests that larval conditions primarily influence  $T_c$  which then has an effect on  $r$ . These changes in the life table traits most likely reflect the effect of competitive treatments on larval development time to adulthood and not on size or fecundity of resulting adult females. Even the impact of competition on per cent survivorship to adulthood, although significant, does not appear to be strongly associated with impacts on  $r$  and  $T_c$  (compare Fig. 1 and Table 3). Competition among mosquito larvae, then, has strong effects at the cohort or population level, and those effects are largely dependent on the effects on development time. Although our data demonstrate the significant effects of inter- and intra-specific competition on survivorship, size, and developmental time, size effects, in particular, do not seem to translate into strong effects on individual fecundity (Table 2), adult longevity (Table 2), nor on  $R_0$  (Table 3). This may be a result of high variability in size, longevity, and fecundity among surviving females. This lack of effect on fecundity suggests that even in situations of competition within a single cohort of larvae, some resulting females achieve high fecundity, which may compensate for low reproductive output by females from that cohort that died or were severely debilitated by larval competition. This leads to a prediction that egg production per female within a treatment may be both more variable and more strongly right skewed in the higher density treatments. Our data showed that within-treatment egg production was, indeed, always right skewed (coefficients of skewness all  $>0$ ) and within all treatments there were usually a 1–5 females that produced  $>500$  eggs each and 6–9 females that produced 0 eggs. There were, however, no obvious trends in variance or skewness with larval density. These few highly fecund females typically lived a long time ( $>100$  days in some cases). This reproductive skewness thus appears to be a general property of these mosquitoes, rather than an effect of density. This potential for a few females to dominate cohort reproduction may have depended, in our experiment, on reliable availability of sugar and blood. The general lack of any significant differences in mean individual traits of

surviving adults suggests that average adult traits may have limited, or even no influence on cohort life table traits. The heterogeneity among individual adults eclosing from similar larval environments may be a more important influence on population dynamics.

Our data demonstrate that competition among larvae has multiple strong effects on the production of resulting adult mosquitoes, inducing lower survivorship to adulthood, longer larval developmental times, and smaller adult size. These effects on production of adults have been demonstrated by many others (e.g. Livdahl & Willey, 1991; Braks *et al.*, 2004; Costanzo *et al.*, 2005). However, when resulting females of both species from both competitive treatments are provided regular access to sugar and blood, those significant effects of the larval environment do not translate to impacts on adult traits such as average longevity, fecundity, and blood-feeding success. Females resulting from high-density, competitive larval environments appeared to compensate for initial decreased metabolic reserves and on average, lived as long, took as many bloodmeals, laid as many eggs, and had the same median time for the first gonotrophic cycle as females that developed in low-density non-competitive environments. At the life table level, this lack of a significant difference among competitive treatments during the adult lives of female mosquitoes suggests that observed effects on population level traits depend primarily on effects evident at the completion of larval development (e.g. development time to adulthood).

Compensation by adults raised in nutritionally challenged larval environments has been documented (Feinsod & Spielman, 1980; Briegel, 1990; Blackmore & Lord, 2000; Joy *et al.*, 2010). Teneral reserves are always reflected in body size of eclosing female *A. aegypti* (Briegel, 1990) and *A. albopictus* (Blackmore & Lord, 2000) irrespective of larval crowding, food supply, or temperature. Smaller females from stressful larval conditions manifest symptoms of nutritional deprivation, including lower lipid, protein, and carbohydrate reserves, and these symptoms directly impact their blood consumption and fecundity (Briegel, 1990; Telang *et al.*, 2006). Briegel (1990) showed that large females, when offered blood until repletion, ingested more than twice the blood volume of smaller females and their fecundity was almost quadrupled; their metabolism during the gonotrophic cycle thus appears to be more efficient than that of smaller females from more competitive larval-rearing environments. In the present study, the effects of competitive treatment on the female mean size were either subtle or absent (*A. albopictus* and *A. aegypti*, respectively; Table 1). Our high-density treatments did, however, result in a significantly lower survival to adulthood for both species, in both inter- and intra-specific competition treatment (Table 1), and the reductions of density owing to the mortality of larvae may have reduced the potential effects of competition on adult size among the survivors.

This experiment was carried out using ideal rearing conditions for the adults, with continuous access to sugar, regular access to blood, and benign temperature and humidity conditions. Thus, there was minimal nutritional or environmental stress to adult females. This was done to provide a baseline from which to compare future studies. The provision of continuous sugar, specifically, significantly influences adult female mosquito survival, especially for *A. albopictus* (Xue *et al.*, 2010). As such,

the provision of both sugar and easily acquired blood may have enabled females from high-density competitive environments to compensate for low metabolic reserves caused by challenging conditions for larvae. The net effect of these benign conditions for adults may have minimised the impacts of the larval-rearing environment on fecundity and longevity of females. Nevertheless, competition among larvae had clear and significant effects on life-table estimates of a cohort rate of increase and cohort generation time, indicating competition would have important population-level effects on both species. An important result of this experiment is that the impact of competition among larvae seems to be driven largely by effects on cohort generation time, rather than net reproductive rate, and the implication that competitive effects on larval development time can be a very important component of population dynamics of these *Aedes*. Future studies need to evaluate what would happen with more harsh or more complex conditions for adults, such as more stressful temperatures or humidity, or more limited availability of sugar or blood, as would be experienced in natural field situations (Steinwascher, 1982; Lounibos *et al.*, 2002; Ma & Roitberg, 2008; Reiskind & Lounibos, 2009; Xue *et al.*, 2010; Alto & Bettinardi, 2013; Carrington *et al.*, 2013).

In conclusion, our results indicate that while inter- and intra-specific competition among *Aedes* larvae produced important individual and population-level effects, benign conditions for adults may enable resulting adults to compensate for some impacts of competition, particularly those affecting blood-feeding success, fecundity, and net reproductive rate,  $R_0$ . The effect of competition appears to be manifest primarily in survivorship of larvae to the adult stage and in the larval development time, which in turn impact the cohort generation time,  $T_c$ , and ultimately cohort rate of increase,  $r$ . It remains to be studied how altering the environment or resources for adults from competitive environments could impact components of vectorial capacity. The lack of effects of a larval-rearing environment on adult longevity does suggest that the effects on the vectorial capacity owing to longevity may be limited, at least when adults have access to ample sugar and blood. However, physiological competence determined by the metabolic, biochemical, and immunological traits that may be derived from resource competition among mosquito larvae (Telang *et al.*, 2007, 2012; Alto *et al.*, 2008) needs further examination. Such information will further our understandings of how larval environments shape vector competence to transmit pathogens and ultimately to affect the vectorial capacity of these vector mosquitoes. The apparent importance of reproductive output of a few, long-lived adult females, even when competition among larvae affects survivorship to adulthood, suggests that heterogeneity among adults may also have important effects on vectorial capacity, so that a small number of individuals may make a dominant contribution to vectorial capacity as they do to cohort rate of increase.

## Acknowledgements

We thank E.G. Murrell and P.T. Leisnham for useful discussion during the planning and execution of this study, and two anonymous referees for useful suggestions. This research was

supported by NIAID grants R15 AI075306-01 and R01 AI44793 (ISU subaward) to S.A.J. Partial financial support for B.H.N. during the analysis and writing stages came from the Oklahoma Agricultural Experiment Station (OKL-02909). B.H.N. and S.A.J. designed the study. B.H.N., P.A.O., and J.E.F. collected the data. B.H.N. and S.A.J. analysed the data. All authors were involved in the writing of the manuscript and agreed on the final version.

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Accepted 21 October 2015

First published online 8 December 2015

Associate Editor: Mark Fellowes