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Population impacts of *Wolbachia* on *Aedes albopictus*

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Abstract. Prior studies have demonstrated that *Wolbachia*, a commonly occurring bacterium capable of manipulating host reproduction, can affect life history traits in insect hosts, which in turn can have population-level effects. Effects on hosts at the individual level are predicted to impact population dynamics, but the latter has not been examined empirically. Here, we describe a biological model system based on *Aedes albopictus* (Asian tiger mosquito) that allows for measurement of population dynamics, which has not been accomplished in prior field trials or laboratory designs. The results demonstrate the studied populations to be robust and allow for persistent, closed populations with overlapping generations, which are regulated solely through density-dependent, intraspecific competition for limited resources. Using a novel experimental design, we compare populations that are either uninfected or infected with *Wolbachia*. The results show differences that include population size, eclosion rates, adult survivorship, and fecundity. The aposymbiotic populations were generally larger and adults longer lived relative to the infected populations. The outcome is discussed in context with naturally occurring *Wolbachia* invasions, proposed autocidal strategies, and the utility of the developed system as a biological platform for hypothesis testing and improved parameterization.

Key words: Aedes albopictus; *cytoplasmic incompatibility; dengue virus; endosymbiont; genetic control; mosquito control; population replacement; sterile insect technique; vector control;* Wolbachia pipientis.

Introduction

The intracellular, maternally inherited α-proteobacteria *Wolbachia pipientis* is one of the most widespread animal endosymbionts and is estimated to infect a majority of insect species (Hilgenboecker et al. 2008). In arthropods, *Wolbachia* behaves as a reproductive parasite by manipulating host reproduction to enhance its vertical transmission (Werren et al. 2008). The most common reproductive modification caused by *Wolbachia* in insects is cytoplasmic incompatibility (CI). CI occurs when a *Wolbachia*-infected male mates with an uninfected female, causing developmental arrest of the embryo. In contrast, *Wolbachia*-infected females can mate with either an uninfected male or a male infected with the same *Wolbachia* strain. This pattern of incompatibility can provide *Wolbachia*-infected females with a reproductive advantage because they can mate with all males in the population, leading to *Wolbachia* spread (Turelli and Hoffmann 1991, Werren 1997, Dobson et al. 2002*a*, Xi et al. 2005, Werren et al. 2008).

Prior studies show that *Wolbachia* can affect the fitness of its host, including examples that range from minor fitness costs (Calvitti et al. 2009, 2010, Brelsfoard and Dobson 2011) to fitness benefits (Hedges et al. 2008, Teixeira et al. 2008) to strongly maladaptive effects on host fitness (Fry et al. 2004, Suh et al. 2009, Yeap et al. 2011, Graham et al. 2012). Models predict conditions under which fitness-decreasing infections can invade and stably persist within an insect population (Crain et al. 2011, Hancock et al. 2011). Population-level impacts such as carrying capacity (maximum sustained population size due to limiting factors) and adult sex ratios are predicted following the invasion of *Wolbachia* that affect host fitness, but there has not been a method to empirically examine model predictions (Dobson et al. 2002*a*, Hancock et al. 2011).

Here, we examine laboratory populations of *Aedes albopictus* Skuse (Asian tiger mosquito), a medically and economically important, globally invasive pest and disease vector (Gratz 2004, Benedict et al. 2007). The goals of this work are (1) to test whether density-dependent regulation occurring at the larval stage of *A. albopictus* populations would allow for persistent closed populations, without the direct regulation of population size, and (2) to examine for predicted population-level effects of *Wolbachia* in *A. albopictus* mosquitoes.

While the study of field populations is unarguably the golden standard, there are valid reasons not to rely upon natural populations for early hypothesis testing. For example, there may be regulatory, ethical, or community concerns that discourage open release experiments

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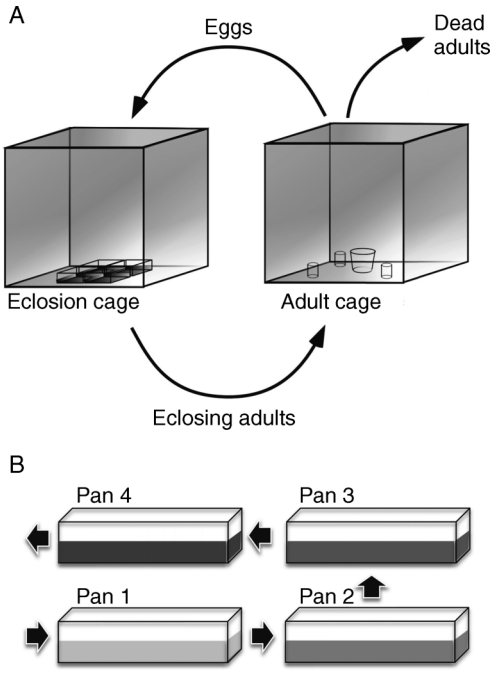

FIG. 1. (A) Schematic of the population cage design utilizing dual cages for *Aedes albopictus*, the Asian tiger mosquito. (B) Rotation pattern for the system of four larval pans within the eclosion cage, with levels of organic matter symbolized by pan shading. Darker colors represent increasing age of the pans and higher levels of pollution.

(Benedict et al. 2008, Lavery et al. 2008, Beech et al. 2009). Additionally, it can be difficult to distinguish the source of variation in natural populations, since the dynamics observed are driven by both exogenous and endogenous factors (Turchin 1991, Turchin and Taylor 1992). Furthermore, the containment provided by artificial systems allows a level of measurement and testing of scenarios that would not be feasible to test in the field.

With mosquitoes, larval survival and development are directly affected by density-dependent intraspecific competition (Seawright et al. 1977). In contrast, density-dependent effects are less obvious at the mosquito adult stage. Models predict that density-dependent effects at the immature stages can impact the resulting adults in the population, and this has the potential to affect pathogen transmission. For example, reduced intraspecific competition, such as that resulting from an autocidal suppression approach, may result in an increase in fitness or longevity (Dobson et al. 2002*a*). More robust, longer-lived female mosquitoes can increase pathogen transmission rates, since females are more likely to accomplish the extrinsic incubation period required for transmission. But the hypothesized effects should be examined empirically.

Systems for studying closed populations with overlapping generations have been developed for other insects; however, until recently, mosquito population cage studies have dealt with discrete cohorts (James et al. 2011). Nicholson pioneered the idea that negative feedback or density-dependent regulation can play an important role in population stability (Nicholson 1933). The persistence of a closed population implies a balance between reproduction and mortality, which can be affected by biotic (e.g., competition, predation) and abiotic factors. The importance of understanding these relationships in mosquitoes is heightened with the prospect of new autocidal approaches. For example, empirical tests can examine for predicted potential population-level effects of applied strategies that introduce fitness load, sterilize the population, abbreviate longevity, and/or manipulate symbiotic associations. Here we examine for potential population-level effects of a CI-inducing *Wolbachia* infection, including impacts on the host carrying capacity, sex ratio, and longevity.

MATERIALS AND METHODS

Population cage procedures

Four separate populations of *A. albopictus* were maintained, based on a dual cage design. Two of the populations (IHA and IHB) consisted of a wild-type *A. albopictus* strain that is naturally infected (*w*AlbA and *w*AlbB) with *Wolbachia* (IH), while the remaining two populations (UTA and UTB) consisted of an aposymbiotic strain (UT) in which the *Wolbachia* infection was removed by tetracycline treatment (Dobson and Rattanadechakul 2001). Both the IH and UT strains share a similar genetic background (Dobson et al. 2004).

Each population consisted of two cages: an adult cage and an eclosion cage for immature development (Fig. 1). Both the adult and eclosion cages had volumes of 30 cm^3 and were lined with lumite mesh (Bioquip Products, Rancho Cominguez, California, USA). One side of each cage consisted of a stockinette cotton sleeve that allowed for manipulations within the cage, while preventing escapes. Each adult cage contained three 20-mL glass vials containing a cotton wick and a 10% sucrose solution for adult nutrition. A 120-mL specimen cup lined with heavy seed germination paper and 60 mL of distilled water (Anchor Hocking Paper, St. Paul, Minnesota, USA) was provided for female oviposition. Eclosion cages consisted of four plastic pans ($10.5 \times 7.5 \times 4.5$ cm) containing 200 mL of distilled water. Environmental conditions were maintained at 28° ± 2°C, 75% ± 3% relative humidity and 16:8 light : dark cycle throughout the experiment.

Populations were initiated by introducing 100 female and 100 male adults into each of the adult cages. Weekly, an anesthetized mouse was placed inside the adult cage for 20 minutes for female blood feeding (following Institutional Animal Care and Use Committee [IACUC] No. 00905A2005). Sucrose bottles and oviposition cups were replaced weekly. Three times per week dead adults were removed from the cage, counted, and identified to sex.

Eggs removed from the adult cage were allowed to embryonate for seven days. Prior to hatching, the number of eggs was estimated by digital analysis using ImageJ 1.37v software (*available online*)[4] (Mains et al. 2008). In brief, a linear regression based on prior correlations between the area (pixels squared) taken up by oviposited eggs estimated by ImageJ and the number of manually counted eggs was used to estimate egg number.

To estimate the number of eggs oviposited per female, the number of estimated eggs was divided by the number of females present in the adult cage five days prior to oviposition cup removal. A period of five days was chosen since most *A. albopictus* females oviposit between three and five days following a blood meal (Hawley 1988). Prior to hatching, each egg paper was divided into three sections, based upon egg number. The eggs were then submerged in larval pans 1–3 (Fig. 1), which exposed newly hatched larvae to a varying range of immature environments. After five days, egg papers were removed from the larval pans and egg hatch was estimated. To estimate egg hatch, a method of "patch counting" was used in which hatched and unhatched eggs were counted using a dissecting microscope (Leica MZ75) from three randomly selected fields of view (44.17 mm^2). Fields with <20 eggs were not counted, and a new random field was selected. The "patch count" method was used since it was less time intensive and remained predictive of whole-egg paper counts. Hatched eggs were defined based upon the appearance of the operculum. The portion of egg paper used for hatch rate estimation was selected at random from the three sections previously defined.

Three times per week (Monday, Wednesday, and Friday), each of the four pans was provided with 0.6 mL of liver powder solution (60g/L) (ICN Biomedicals, Aurora, Ohio, USA) for larval development. Newly eclosed adults in the eclosion cage were collected using a hand-held aspirator (Clarke Mosquito, Roselle, Illinois, USA) and immobilized by chilling on wet ice, identified to sex, counted and released into the adult cage.

As diagrammed in Fig. 1, larval pans were rotated each Wednesday. The fourth larval pan (Pan 4) was removed from the eclosion cage, and the remaining pans were reassigned (e.g., Pan 1 becomes Pan 2, etc.). A new pan was introduced and assigned as the new Pan 1. At the same time, deionized water was added to offset evaporation in the older three pans.

For all populations, the initial period following the start of populations has been excluded from analyses, due to changes made to the food amount, which resulted in variation in the population size.

Population cage monitoring

The number of adults within the adult cage was monitored using a running population count calculated by:

$$F_t = F_{t-1} + E_{f,t} - D_{f,t}$$

where the number of females (F_t) at time t was calculated from the number of females in the previous count (F_{t-1}), the number of newly eclosed females ($E_{f,t}$), and the number of dead females ($D_{f,t}$). A similar formula was used to monitor the number of adult males (M_t):

$$M_t = M_{t-1} + E_{m,t} - D_{m,t}.$$

The total number of adults in the adult cage (A_t) was calculated as

$$A_t = F_t + M_t.$$

To periodically calibrate and prevent the accumulation of counting errors, a full count of the entire adult population within the adult cage was conducted every third week. Adults were aspirated in groups of ~50, immobilized on wet ice, and then counted and the sex determined under a dissecting microscope. Total counts were then used to recalibrate the running counts.

Estimates for weekly survival rates (S) were calculated using the "running count" method as

$$S = 1 - (G \mid H)$$

based upon the mean number of adults in the population (H) and the mean number of newly eclosed adults (G).

Population dynamics

To predict population dynamic parameters of both infected and aposymbiotic cages, we fit our data to a previously published model by Dye for mosquito population dynamics (Dye 1984):

$$A^* = \frac{[\ln(P/\delta)/\alpha]^{1/\beta}}{E}.$$

Estimates for three of the six parameters were derived. Per capita daily mortality (δ) was calculated by dividing estimated weekly survival by seven. Adult egg production rate (E) was calculated by dividing the number of adults at time $t-1$ by the number of eggs oviposited at time t. New adult production rate (P) was calculated by dividing the average number of newly eclosed adults by the average adult population size. The larval survival coefficient and exponent (α, β) could not be estimated from the data collected, but was estimated using data from a previously published report using the same mosquito strains (Gavotte et al. 2009). Each of the parameters was used to estimate the equilibrium adult population size (A^*). To estimate A^*, we assumed that the average standing adult population was the equilibrium population size. The predicted values for model parameters were calculated by solving the equilibrium expression (Dye 1984) using the values defined in Table 1. In this analysis, we did not estimate generation time (T), but instead used a constant defined in previous work (Yakob et al. 2008). Importantly, changes to generation time estimates do not affect A^* (Dye 1984).

[4] http://rsbweb.nih.gov/ij/

TABLE 1. Comparison of empirical results from populations and predictions of the Dye model (Dye 1984), showing score (t) and P value.

Parameter	Aposymbiotic				Infected			
	Empirical estimate	Model prediction	t test	P	Empirical estimate	Model prediction	t test	P
δ	0.0572	0.0442	−5.52	0.1141	0.0710	0.0682	2.44	0.248
P	0.3967	0.5133	−10.64	0.0596	0.4978	0.5184	−2.42	0.2498
T	27				27			
α	0.06564	0.05793			0.06564	0.06430		
β	0.4308	0.4155			0.4308	0.4282		
E	13.1	9.8	9.53	0.0666	17.4	16.6	1.96	0.3008
A^*	263.4	197.1	3.51	0.1767	157.7	150.3	1.12	0.465

Notes: Parameters are per capita mortality (δ), new adult production rate (P), generation time (T), larval survival coefficient (α), larval survival exponent (β), adult egg production rate (E), and adult population size equilibrium (A^*).

Wolbachia confirmation

PCR assays to confirm the presence of *Wolbachia* in the populations were conducted at least every 20 weeks. The general *Wolbachia* primers 438F (5′-CATACCTATTC-GAAGGGATAG-3′) and 438R (5′-AGCTTCGAGT-GAAACCAATTC-3′) were used to test for infection status. PCR conditions were as previously described (Dobson et al. 2004). The presence of *Wolbachia* was not detected in adults from aposymbiotic populations. All adults tested from the infected populations were *Wolbachia* positive. Thus, the infection status of each population did not change over the course of the experiment.

Statistical analysis

All statistical analysis was performed using JMP 9.0.016 (SAS Institute 2010). The normality of the life history traits was tested using a Shapiro-Wilk test. Proportional data (i.e., hatch rate, survival rate) were transformed using an arcsine square-root transformation to insure normality. Comparisons between and among infection types were performed using a repeated-measures, one-way analysis of variance (ANOVA). When appropriate, data were characterized by two factors (i.e., infection status and sex) and a full-factorial, repeated-measures ANOVA design. The full factorial design was used to analyze adult populations, eclosing adults, dead adults, adult net change, and estimated adult survival. Sex ratio was analyzed by a full-factorial, repeated-measures ANOVA design with infection status and adult stage (i.e., adult, eclosion, dead) as factors. For all other parameters (i.e., egg number, fecundity, hatch rate, hatching larvae and fourth pans), infection status was the only factor. A t test was used to compare net change from a theoretical mean of zero. To compare whether the population dynamic parameter values reported here were equal to predictions made using Dye's model (Dye 1984), we performed a one-sample t test. For each parameter, the predicted parameter value taken from the population cage data was compared to the model's predicted value (i.e., a constant).

RESULTS

Population cage results

Four *A. albopictus* populations were studied in parallel to test for an ability of populations to persist, to assess replicability and to compare the dynamics of *A. albopictus* populations that differ in their *Wolbachia* infection type. The dampening effect of density dependence on exponential growth does not always result in stable populations, and possible outcomes included: (A) fluctuations in population size would lead to periodic extinctions, or (B) large variation between replicate populations would occur, which could complicate downstream comparisons. With the experimental design described, we did not observe extinction for any of the populations. Each population was maintained for more than one year prior to stopping the experiment. Due to fluctuations resulting from protocol changes early in the experiment, statistical comparisons were made using only data from the final 45 weeks of the experiment, which were designated as Weeks 1–45 in our analysis.

During the 45-week period illustrated in Fig. 2, we observed a stable number of adults in each population, varying around a point of equilibrium. For stable populations, the net change (i.e., adult eclosion minus adult mortality) should be approximately zero. Analysis of each population demonstrated that the net change for females and males do not differ significantly from zero ($t(42)$, $P > 0.21$) (Tables 2 and 3). Comparisons between the four populations demonstrate that the net change does not differ for sex, infection type, or their interaction.

Comparisons of the additional population dynamics demonstrate significant differences between the infected and uninfected populations. Significantly more adults were observed in the aposymbiotic populations (Fig. 3, Table 2), and while the adult sex ratio (i.e., percent female) was female biased in all populations, aposymbiotic populations had a significantly higher sex ratio, relative to infected populations (Table 2). Higher numbers of eclosing and dying adults were observed

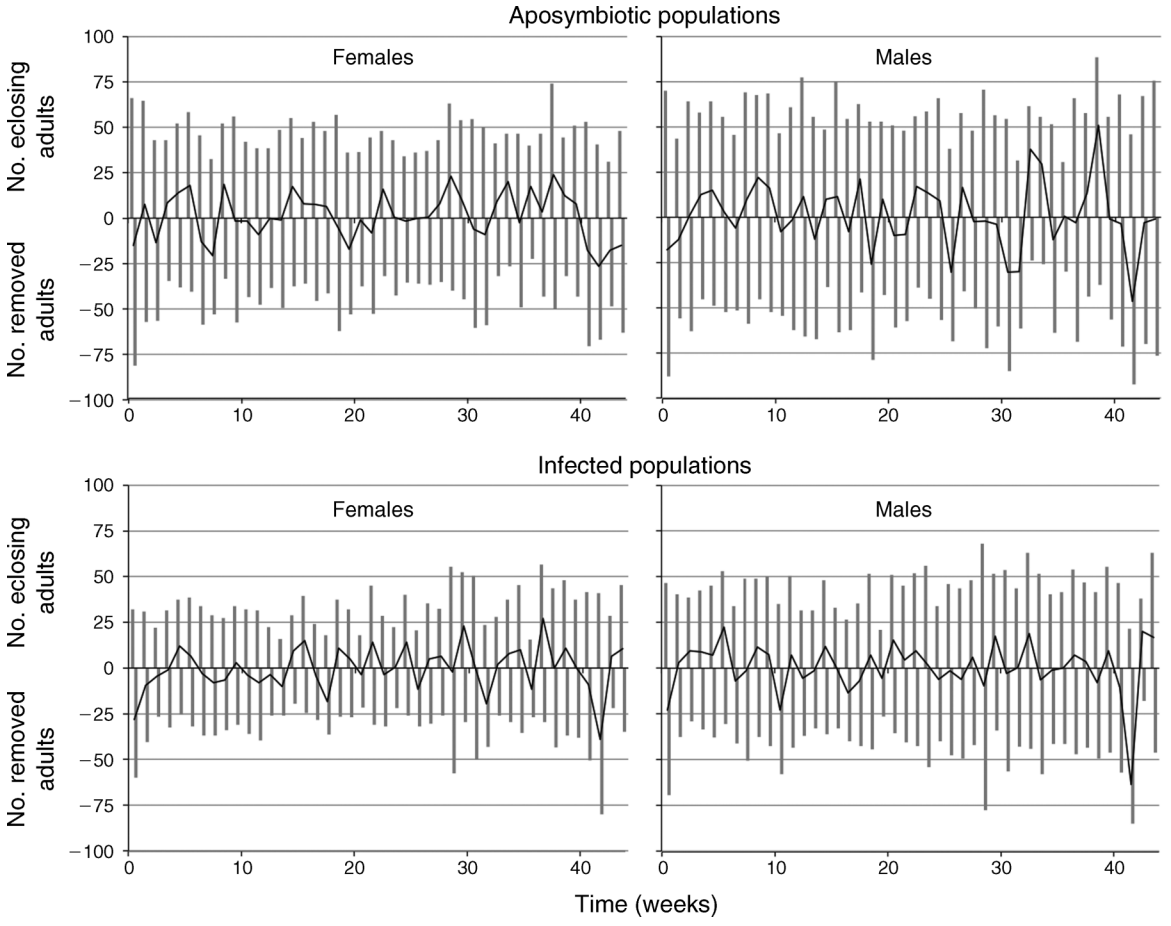

FIG. 2. Weekly mean values for adult eclosion, mortality (i.e., removed adults), and net change for each sex and *Wolbachia* bacterial infection type (aposymbiotic vs. infected). Eclosion and mortality rates are displayed as gray bars (above and below the *x*-axis) and net change is shown as a black trace extending from left to right.

for aposymbiotic populations (Table 2). Compared to the sex ratio of the standing adult population, the sex ratios of both eclosing and dying adults differed significantly and were male biased (Table 2). Interactions between infection type and sex were not observed for standing adult populations, adult eclosion, and adult mortality. Interactions between infection type and adult stage were not observed for sex ratio. The estimated survival probability differed significantly between the populations, for both infection type and sex but not their interaction (Table 3). Specifically, females were longer lived than males, and longer survivorship was estimated in aposymbiotic populations.

The mean egg number per week was observed to differ significantly between the infection types (Table 4), with the aposymbiotic populations producing a greater number of eggs relative to the infected populations. Since the aposymbiotic populations had significantly more adult females, the number of eggs per adult female was estimated. Females from the infected populations were calculated to produce significantly more eggs per female relative to aposymbiotic populations (Table 4).

TABLE 2. Adult population dynamics within replicate biological model systems.

Population dynamic	Sex	Population type	
		Aposymbiotic	Infected
Standing adult population	Female	151.8 ± 30	86.7 ± 18
	Male	110.5 ± 21	71.5 ± 16
	Total	263.4 ± 43	157.7 ± 29
Adult eclosion per week	Female	46.8 ± 9	33.9 ± 10
	Male	57.7 ± 12	44.6 ± 10
	Total	104.5 ± 14	78.6 ± 17
Adult mortality per week	Female	45.1 ± 11	33.5 ± 10
	Male	56.1 ± 15	43.5 ± 10
	Total	101.2 ± 24	77.0 ± 21
Sex ratio	Adult	57.5% ± 5%	55.0% ± 6%
	Eclosion	44.9% ± 7%	42.7% ± 8%
	Mortality	44.8% ± 5%	43.3% ± 5%
Net change	Female	1.6 ± 16	0.4 ± 15
	Male	1.5 ± 23	1.1 ± 16
	Total	3.2 ± 34	1.5 ± 27
Weekly survival probability (%)	Female	69.1 ± 1	60.9 ± 2
	Male	47.6 ± 2	37.4 ± 1
	Total	60.2 ± 1	50.2 ± 1

Notes: All values are mean ± SD per week. Sex ratio is the number of females/total number of adults. Net change is the adult population size (adult eclosion − adult mortality).

TABLE 3. ANOVA results for adult population dynamics within replicate biological model systems, using values in Table 2.

Population dynamic	Infection		Sex		Infection × sex		Life stage (adult vs. eclosion)		Infection × life stage	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Standing adult population	14.0	<0.001	4.1	<0.02	0.8	0.1				
Adult eclosion per week	6.2	<0.007	4.3	<0.02	0.0002	0.9				
Adult mortality per week	7.1	<0.006	5.3	<0.01	0.009	0.8				
Sex ratio	1.3	<0.02					34.7	<0.0001	0.1	0.7
Net change	0.2	0.3	0.03	0.7	0.05	0.6				
Weekly survival probability	52.7	<0.002	307.6	<0.0001	0.4	0.5				

Note: For all ANOVAs, df = 1, 42, except for sex ratio, where df = 2, 42 for the factors life stage and the interaction of infection status and life stage.

The observed hatch rates did not differ between uninfected and infected populations (Table 4). The estimated number of first-instar larvae was calculated as a multiple of the egg number and hatch rate. No difference was observed between infection types in the estimated first-instar larval numbers (Table 4). Similarly, no differences were observed in comparisons of older immatures (i.e., larvae and pupae in Pan 4).

Population dynamics

Parameter estimates from empirical data were compared to predictions of the Dye model (Dye 1984), showing similar population dynamics (Table 1). For each parameter, model-derived estimates did not differ significantly from predictions of the Dye model. For both aposymbiotic and infected populations, our estimates of adult numbers were similar to the predicted equilibrium size. Likewise, parameter estimates for adult mortality, fecundity, and population growth were similar to the predicted values. The overall population dynamics are summarized in Fig. 4.

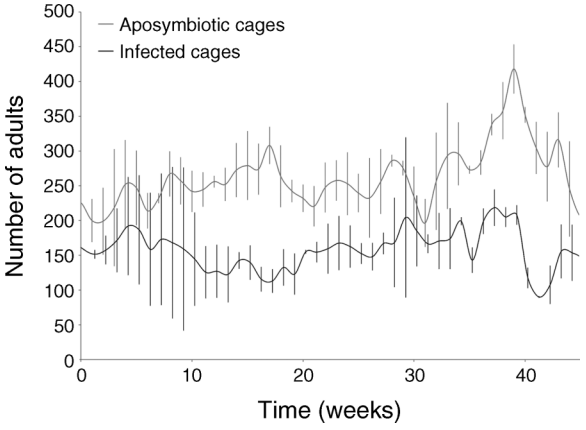

FIG. 3. Total adults (mean ± SD) for each of the replicate population types (aposymbiotic and *Wolbachia* infected) maintained using a model laboratory system with overlapping generations. The number of adults differed significantly ($F_{1,42} = 14.01$, $P < 0.001$) between the population types during the 45-week experiment.

DISCUSSION

With a renewed interest in autocidal control measures, it is important to develop tools to empirically examine for population-level effects. Here we have examined *Wolbachia* endosymbionts using a laboratory-based model system, but the system can be used for additional measures (e.g., traditional SIT and more recent transgenic strategies). Overall, the empirical findings here are consistent with mathematical predictions and show the populations to differ significantly in density and longevity. As an example, a prior model predicts that the carrying capacity of an uninfected population can be higher than that of an identical population infected with *Wolbachia* (Dobson et al. 2002*a*). Improved models that include more detail (e.g., simulating immature dynamics) are now available, including models with a focus on *Wolbachia* infections in mosquitoes (Bossan et al. 2011, Crain et al. 2011, Hancock et al. 2011).

Consistent with model predictions, the population dynamics differ significantly between the infected and uninfected populations. The number of female and male adults is higher for the aposymbiotic populations, which corresponds with lower eclosion and adult survival rates for the *Wolbachia*-infected populations (Fig. 4). Despite the difference between population types, the eclosion and mortality rates were balanced within populations, resulting in stable, standing populations, with no net change over time.

The ratio of female to male adults (sex ratio) did not differ between aposymbiotic and infected populations.

TABLE 4. Population dynamics of immatures within replicate biological model systems.

Parameter	Population type		Statistical analysis	
	Aposymbiotic	Infected	*F*	*P*
Egg number	2072.6 ± 464	1444.8 ± 390	86.7	<0.01
Eggs per female	13.1 ± 2	16.7 ± 3	11.6	<0.01
Egg hatch (%)	66.5 ± 14	60.3 ± 17	7.07	0.06
First-instar larvae	1346.3 ± 353	863.9 ± 380	0.5	0.4
Fourth pan	199.2 ± 56	200.4 ± 48	<0.01	0.9

Notes: All values are mean per week ± SD. For all *F* tests, df = 1, 42.

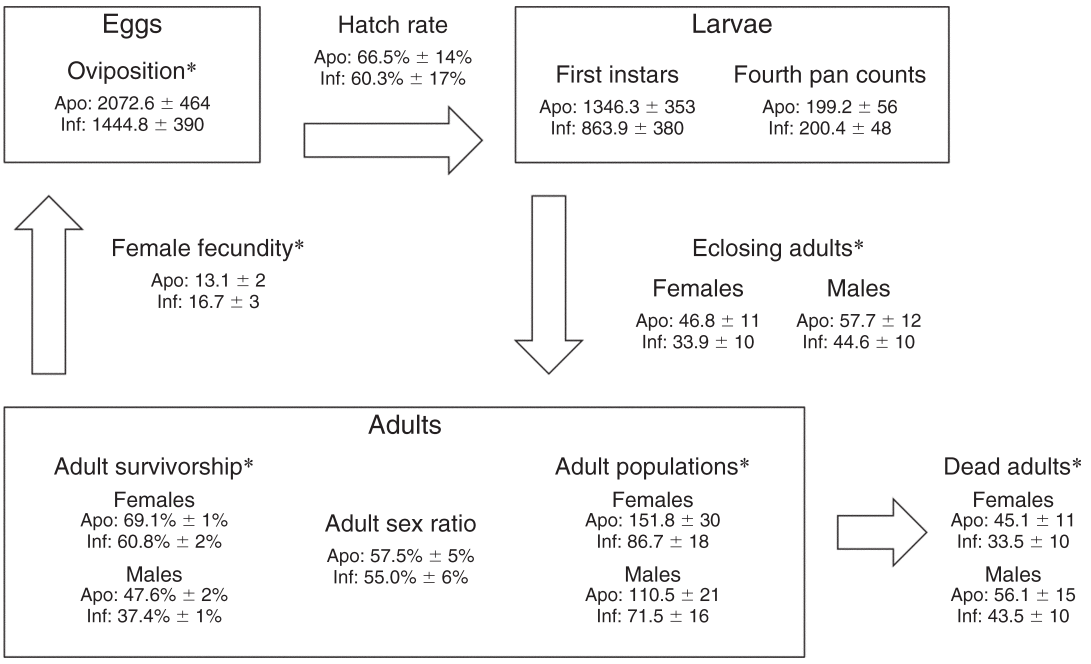

FIG. 4. Summary comparison of mean values of measured parameters for both infection types (Apo, aposymbiotic; Inf, *Wolbachia* infected) for parameters collected during the 45-week experiment. Single asterisks indicate significant differences ($P < 0.05$) between some infected and uninfected populations.

However, the male bias observed in newly eclosed and dying adults differed significantly from the female bias observed in the standing population. This contrast reflects the greater female longevity, which is consistent with observations in both laboratory and field studies (Hawley 1988, Dobson et al. 2004). Based upon the standing adult population size and the eclosion/mortality rates, adult survivorship was estimated for females and males of each population type. Females are longer lived than males in both population types, and aposymbiotic adults were longer lived than individuals of the same sex in the infected populations. This result differs from prior reports that show longer-lived, infected *A. albopictus* females (Dobson and Rattanadechakul 2001, Dobson et al. 2004), which may reflect differences between the experiments in the immature developmental conditions. Specifically, in the prior studies, tests were of adults reared under optimal conditions of low density and excess food. Thus, additional experiments are required to examine for an interaction between immature stress and *Wolbachia* infection type in the longevity of the resulting adults.

Significantly more eggs were produced from the aposymbiotic populations. However, when considered on a per capita basis, females from infected populations were estimated to produce significantly more eggs relative to females from aposymbiotic populations. This observation is consistent with those from prior empirical studies showing higher egg production by infected *A. albopictus* females (Dobson et al. 2002*b*). No difference was observed in egg hatch rates, which differs from prior studies that have shown higher hatch from *Wolbachia*-infected individuals (Dobson et al. 2002*b*, 2004). In a similar way, this difference may reflect the impact of elevated competition levels, which represents a future area of study. While the greater number of eggs from aposymbiotic populations were estimated to result in more larvae, the differences were nonsignificant. This is consistent with expectations for a hypothesized carrying capacity for the immature number, which results from density-dependent effects as larvae compete for limited resources. Based upon the average weekly egg number and eclosing adults, an approximate 5% immature survival rate is estimated for both the aposymbiotic and infected populations.

Aposymbiotic populations generally experienced greater or equal numbers through all life stages relative to infected populations (Fig. 4). An exception is the estimated per capita egg production, which was significantly higher in the infected populations. This difference in egg production could result from an overall younger age of females in the infected cages. As previously shown, older females produce fewer eggs (Hawley 1988). An additional explanation may be that infected females are more fecund, relative to uninfected females (Dobson and Rattanadechakul 2001, Dobson et al. 2004). Regardless, prior models demonstrate the importance of fecundity to the ability of *Wolbachia* to establish and invade naive populations (Egas et al. 2002, Crain et al. 2011). In the work described here, cages were uniformly infected/uninfected. Thus, the ability of *Wolbachia* to invade under these conditions and the

population dynamics that result during an invasion should be a focus of future experiments.

Using a previously published mosquito population dynamics model, we determined that the populations were at equilibrium, which is supported by a zero net change in each population (Fig. 2). Furthermore, parameters derived from the model system did not differ significantly from predictions made in a prior model (Dye 1984). For example, consistent with observations here, the Dye model predicts that aposymbiotic populations should have a larger population equilibrium compared to infected cages, due to lower daily mortality (δ, Table 1).

Of the two population types, Dye's predictions best fit with the dynamics observed in the infected populations, which is the natural state of *A. albopictus* populations (Dobson and Rattanadechakul 2001). While the observed dynamics do not differ significantly, the model's predictions for aposymbiotic cages were less accurate (Table 1). The parameter that most influences the adult population equilibrium in the Dye model is the larval survival exponent β, and an intriguing potential explanation is that *Wolbachia* affects larval responses to competition. This would be consistent with results from a previous study that reported significant differences in larval responses to differing levels of competition between aposymbiotic and infected individuals (Gavotte et al. 2010).

Applications for Insect Management

The need to study and discuss population-level impacts is made even more urgent by proposed and ongoing open-release field trials of autocidal strategies against mosquitoes. This new technology is drawing attention from industry, public health policy makers, environmental regulators, and scientists. For example, some models predict that strategies designed to suppress and replace vector populations may reduce intraspecific competition levels, resulting in more robust, longer-lived mosquitoes, which could have an undesired impact on pathogen transmission. In addition to the results presented here, the demonstrated system provides a platform by which additional population-level hypotheses and applied strategies (e.g., female killing) can be tested and contrasted.

A key design difference between prior model systems and that described here is our reliance on density dependence as the sole regulator of population size. Prior studies are based upon discrete generations or the artificial control of the population size by the experimenter (Xi et al. 2005, James et al. 2011). A concern of autocidal approaches, such as Sterile Insect Technique, is that compensatory density-dependent effects will mitigate and complicate the strategies (Alphey et al. 2010). The design described here can be used for tests of autocidal approaches and measuring for resulting changes in the dynamics of the treated population. Given that multiple groups are targeting *A. albopictus* and that systems are available for classical sterilization via irradiation (Bellini et al. 2007), new transgenic approaches (Alphey and Andreasen 2002, James et al. 2011), and *Wolbachia*-based suppression (Brelsfoard et al. 2008), the system can be used as a common platform to compare each of these approaches and the response of the targeted population.

Lower adult numbers and abbreviated longevity of the infected populations could lend support to the strategy of *Wolbachia*-based population replacement. Specifically, reduced disease transmission is anticipated if there are fewer vectors and if these females have a reduced life expectancy. However, prior studies demonstrate that different *Wolbachia* types can induce different individual-level effects, and that these can vary depending upon host species (Dobson et al. 2004, Engelstadter et al. 2004). Thus, the results here are not necessarily indicative of all *Wolbachia* infections and mosquitoes. Downstream experiments should include examining additional species and infection types using a similar model system. Since *A. albopictus* populations are naturally infected with *Wolbachia* (Armbruster et al. 2003), applied strategies for *Wolbachia*-based replacement and suppression would be based upon populations of differing infection types (i.e., not an aposymbiotic population), which provides another example of a needed downstream test.

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