Journal of Applied Ecology

British Ecological Society

*Journal of Applied Ecology* 2016, **53,** 785–793 doi: 10.1111/1365-2664.12620

# Density-dependent population dynamics in *Aedes aegypti* slow the spread of *w*Mel *Wolbachia*

**Penelope A. Hancock[1]*, Vanessa L. White[2], Ashley G. Callahan[2], Charles H. J. Godfray[1], Ary A. Hoffmann[2] and Scott A. Ritchie[3]**

[1]*Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK;* [2]*Bio 21 Institute, School of Biosciences, University of Melbourne, Parkville, Vic. 3010, Australia; and* [3]*Australian Institute of Tropical Health and Medicine, James Cook University, Townsville, Qld 4878, Australia*

## Summary

**1.** Field release of endosymbiotic *Wolbachia* bacteria into wild *Aedes aegypti* mosquito populations is a promising strategy for biocontrol of dengue. This strategy requires successful *Wolbachia* invasion through the mosquito vector population. Natural variation in mosquito fitness due to density-dependent competition for limited food resources may influence *Wolbachia* invasion. We know little about these effects, largely because our understanding of density-dependent dynamics in mosquito populations is limited.

**2.** We developed an empirical model of *A. aegypti–Wolbachia* dynamics where food resources available to the developing larvae are limited. We assessed the extent of density-dependent regulation in our *A. aegypti* population using a Bayesian statistical model that estimates the temporal variation in mosquito fitness components. We monitored the spread of *Wolbachia* and assessed the effect of the bacteria on larval fitness components.

**3.** We demonstrate that mosquito population growth is regulated by strong larval density-dependent variation in mosquito fitness components. *Wolbachia* spread was slowed by this heterogeneity in mosquito fitness, which reduces the capacity of the bacteria to invade. However, we found no evidence that *Wolbachia* affects larval fitness components.

**4.** *Synthesis and applications*. We demonstrate that the extent and form of density-dependent dynamics in the host population can have a major influence on *Wolbachia* invasion. These findings help explain slow *Wolbachia* invasion rates and indicate that the success of field release strategies for dengue control can depend on attaining high *Wolbachia* frequencies in the mosquito population.

**Key-words:** Bayesian statistical model, biocontrol, demography, dengue, density dependence, fitness, invasion, population dynamics, vector-borne, *Wolbachia*

## Introduction

The transmission of parasites, pathogens and other infectious organisms can depend strongly on the fitness of their hosts (Lipsitch, Siller & Nowak 1996) and can be affected by heterogeneity in the hosts' fitness driven by environmental variation (Reeson *et al*. 2000). The extent to which the mean fitness of populations is regulated by their density is central to ecology (Sibly *et al*. 2005). However, there are few studies of how density-dependent fluctuation in the hosts' fitness interacts with the spread of infections (but see Washburn, Mercer & Anderson 1991; Altizer & Augustine 1997), and these interactions can be complex and depend on the form of density-dependent regulation (Washburn, Mercer & Anderson 1991; Altizer & Augustine 1997). The transmission rate of infectious organisms that can only spread between hosts by vertical transmission depends directly on their host's fitness and they commonly confer a fitness advantage to their host in order to spread and persist (Lipsitch, Siller & Nowak 1996; Haine 2008). The dynamics of vertically transmitted infections are therefore likely to be sensitive to heterogeneity in the hosts' fitness. Theoretical studies show a number of mechanisms by which density-dependent dynamics in the host population can alter the invasion potential of vertically transmitted micro-organisms (Altizer & Augustine 1997; Hancock, Sinkins & Godfray 2011) but these interactions have not been empirically demonstrated.

*Correspondence author. E-mail: penelope.hancock@zoo.ox.ac.uk

*Wolbachia* are maternally transmitted endosymbiotic bacteria that are widespread in insects and some other arthropods (Hilgenboecker *et al.* 2008; Zug & Hammerstein 2012). *Wolbachia* increase their frequency through successive generations of the host population by manipulating the reproductive system of their host, most commonly using a mechanism known as cytoplasmic incompatibility (CI) (Laven 1956). CI causes inviability of offspring from matings between uninfected females and infected males, conferring a relative fitness advantage to infected mothers who are able to reproduce successfully with infected and uninfected males (Caspari & Watson 1959). The strength of this advantage depends on the *Wolbachia* frequency in the (male) insect population. If the *Wolbachia* frequency is lower than a critical threshold, the benefits conferred by CI can be outweighed by fitness costs of *Wolbachia* infection on the host insect which may include reductions in important fitness components, such as the fecundity, survival and juvenile development rate (Turelli 1994; Hancock, Sinkins & Godfray 2011). Thus, *Wolbachia* invasion depends on an interrelationship between the hosts' fitness and the *Wolbachia* frequency in the host population.

The application of *Wolbachia* to biocontrol of the dengue virus is currently of great interest (Hoffmann *et al.* 2011; Walker *et al.* 2011). The *Aedes aegypti* mosquito is the primary vector of dengue, which is the leading cause of insect-vectored viral (arboviral) disease in humans, infecting an estimated 390 million people per year across over 100 countries (Bhatt *et al.* 2013). The infection can cause severe illness and in some cases death, usually in children and young adults (Simmons *et al.* 2012). *Wolbachia* infection has been shown to greatly reduce dengue transmission by *A. aegypti* by inhibiting the development of the virus within the mosquito (Moreira *et al.* 2009; Bian *et al.* 2010). *Wolbachia* do not occur naturally in *A. aegypti* (Xi, Khoo & Dobson 2005); however, certain *Wolbachia* strains have been established in laboratory *A. aegypti* colonies following transfer from their natural hosts *Drosophila melanogaster* and *Aedes albopictus* (Xi, Khoo & Dobson 2005; McMeniman *et al.* 2009; Walker *et al.* 2011). In north-eastern Australia, the *Wolbachia* strain *w*Mel has been successfully established in wild *A. aegypti* populations by releasing infected mosquitoes to drive the infection to near fixation (Hoffmann *et al.* 2011). Further releases are currently underway in high dengue incidence regions of South-East Asia and South America (see www.eliminatedengue.com).

We currently know very little about the ecological factors that affect *Wolbachia* dynamics in wild *A. aegypti* populations, which limits our ability to predict the invasion dynamics of *Wolbachia* in these field trials. A critical knowledge gap is our lack of understanding of the extent and form of density-dependent regulation in wild *A. aegypti* populations. *A. aegypti* population dynamics are thought to be significantly influenced by density-dependent competition for the limited food resources available to the developing larvae (Dye 1984; Legros *et al.* 2009; Walsh *et al.* 2013). *A. aegypti* rely on human blood to reproduce, and their larval habitat consists of water containers close to human dwellings, such as water storage jars and pot plant plates (Southwood *et al.* 1972). Experimental studies on *A. aegypti* have demonstrated that multiple fitness components respond to changing larval density, with higher densities resulting in lower survival and prolonged development of larvae, and smaller body size of surviving adults which reduces their potential fecundity (Barrera 1996; Walsh *et al.* 2011, 2013). However, these experiments studied sets of larval cohorts in isolation from the wider mosquito population. Wild *A. aegypti* populations have continuous and overlapping generations, which has made assessment of effects of natural variation in larval density on mosquito fitness components difficult and limited, particularly given our lack of knowledge of mosquito life-history parameters (Southwood *et al.* 1972; Dye 1984; Legros *et al.* 2009). This inhibits our ability to predict the impact of a range of mosquito vector control strategies (Phuc *et al.* 2007) including *Wolbachia* release strategies (Hancock, Sinkins & Godfray 2011).

Moreover, in *A. aegypti,* fitness costs of *Wolbachia* are typically considered under only low-density larval conditions with unlimited resources being available to all life stages (Xi, Khoo & Dobson 2005; McMeniman *et al.* 2009; Walker *et al.* 2011). We therefore know little about the fitness costs of *Wolbachia* under more stressful conditions arising from larval resource competition (Ross *et al.* 2014). It is important to better understand density-dependent dynamics in *A. aegypti* in order to predict the natural variation in mosquito fitness components that are critical to *Wolbachia* invasion and spread (Hancock, Sinkins & Godfray 2011; Walsh *et al.* 2011).

Here, we developed an empirical and mathematical model to assess the extent of density-dependent regulation in an *A. aegypti* population where food resources available to the developing larvae are limited. We focused on three mosquito fitness components, larval survival and development time and adult female fecundity, which we estimated over time using a Bayesian statistical model. We assessed the relationship between these fitness components and the intrinsic variation in larval density that occurs in our *A. aegypti* population, and find strong evidence for larval density-dependent variation in all three fitness components. We then monitored the spread of a *Wolbachia* strain (*w*Mel) in our *A. aegypti* population and assess the effects of the *Wolbachia* on two fitness components, larval survival and development time, as larval density varies over time. We found that over most larval density conditions *Wolbachia* did not significantly affect these fitness components. However, density-dependent heterogeneity in these fitness components greatly slowed *Wolbachia* spread, which prevented the bacteria from gaining the strong frequency-dependent fitness advantage afforded by CI. The results show how density-dependent dynamics in the host insect population can be a crucial determinant of *Wolbachia* invasion success.

 

## Materials and methods

### AN EMPIRICAL MODEL *A. AEGYPTI* POPULATION

Our model population of *A. aegypti* was housed in a semi-field cage of dimensions 5 × 7 × 4 m designed to simulate the natural habitat of *A. aegypti* in north-eastern Australia (Darbro *et al.* 2012). The population was seeded from a cohort of 100 first-instar larvae that was hatched on 20 December 2013 from wild-caught eggs of an *A. aegypti* population located in Cairns, Australia. The population was maintained for a period of 194 days (about 6·5 months) by the regular schedule of activities described in Appendix S1 in Supporting Information. In summary, adult females were allowed to feed on blood from a live human three times a week. A single container (a 5-L bucket filled with 2 L of water) provided an oviposition site for gravid females and habitat for their larvae. The larval container received regular addition of a fixed amount of food (0·32 g ground lucerne). The part of the container above the waterline was lined with red felt strips that collected the oviposited eggs. The egg strips were removed from the cage three times a week and after a 2 day period of incubation were submerged in a yeast solution to stimulate hatching. All newly hatched larvae in the cohort were counted, a sample of 30 was retained, and the remaining individuals were placed in the semi-field cage larval container. After approximately 5 months (147 days) we ceased the recruitment of new larvae to the population and the remaining individuals either matured or died. *A. aegypti* population dynamics were monitored throughout the time period by daily counts of all newly eclosed pupae and counts three times a week of all larvae (categorized as first, second, third or fourth instar). A sample of 20% of the pupae that eclosed on each day was retained (see below).

### INTRODUCING *W*MEL *WOLBACHIA*

*A. aegypti* larvae infected with *w*Mel *Wolbachia* were obtained from the eggs of a semi-field cage colony which has fixed *w*Mel infection and is regularly backcrossed with wild-caught *A. aegypti* (Walker *et al.* 2011). We reared the *w*Mel-infected larvae under similar conditions of larval density, age-structure and food availability to those experienced in our semi-field cage population using the following procedures. The infected larvae were reared in a Controlled Temperature (CT) room at 26°C in a 5-L bucket containing the same amount of water and receiving the same food supply regime as the semi-field cage larval container habitat. A new cohort of *w*Mel-infected first-instar larvae was hatched and added to the CT room rearing bucket on the same days that first-instar larvae were hatched in the semi-field cage population. The size of each new cohort of infected first-instar larvae was set to 700, which is close to the average first-instar cohort size of the semi-field cage population over the study period (524).

Starting from day 68, we began regular introductions of *w*Mel-infected pupae into our semi-field cage *A. aegypti* population (see Appendix S1). Three times a week, 16 infected pupae were randomly selected from the rearing bucket and placed in the semi-field cage. This rate of introduction is equal to approximately one-third (28·9%) of the average rate of pupal eclosion in the semi-field cage population over the study period, and was chosen to approximate a rate of immigration that may plausibly occur in wild *A. aegypti* populations. The *Wolbachia* introductions continued for 12 weeks. This time period was considered a plausible time-scale for natural *Wolbachia* immigration into a wild (uninfected) *A. aegypti* population.

We monitored the *Wolbachia* frequency over time in both the first-instar larvae and pupae by testing all individuals in the samples of first-instar larvae and eclosed pupae for *Wolbachia* infection using real-time PCR (see Appendix S1; Lee *et al.* 2012). The *Wolbachia* frequency in each sample of first-instar larvae was used to estimate the *Wolbachia* frequency in each cohort of newly hatched first-instar larvae. The *Wolbachia* frequency in the eclosed pupae was estimated on a weekly time step, based on the frequency in the sampled pupae pooled over each week, in order to reduce the sampling error.

### ASSESSING DENSITY-DEPENDENT POPULATION REGULATION IN *A. AEGYPTI*

We focused on three main *A. aegypti* demographic variables: first instar-to-pupal survival, larval development time and adult female fecundity. These fitness components are thought to be potential drivers of density-dependent population regulation in *A. aegypti* (Walsh *et al.* 2013), and are also critical determinants of the invasion potential of *Wolbachia* (Turelli 1994; Hancock, Sinkins & Godfray 2011). We aimed to assess the relationship between these three variables and the intrinsic variation in larval density that occurred in our *A. aegypti* population over time. This required estimation of these variables over time, which was performed using the following procedures.

#### *Estimating larval survival and development time*

Due to the overlapping generations in our *A. aegypti* population, larval development time and first instar-to-pupal survival could not be directly observed. Therefore, we developed a statistical demographic model informed by our observed larval and pupal abundances to estimate their values over time. This involved inference of the full distributions of the development times of the individuals within each larval cohort using a Bayesian Markov chain Monte Carlo (MCMC) Metropolis–Hastings algorithm, which is detailed in Appendix S2. In summary, for all larvae hatched on day $c$ (cohort $c$) the probabilities of pupating on different days $i$, $p_{i,c}$, were assumed to follow a gamma distribution:

$$p_{i,c} = \Gamma(i - c - T_{\min}, \eta_c, \theta_c) \qquad \text{eqn 1}$$

where $\eta_c$ and $\theta_c$ are the shape and scale parameters and $T_{\min}$ is the minimum development time, set to 5 days. The number of larvae in a cohort hatched on day $c$ that eclose as pupae on day $i$, $P_{i,c}$, was estimated by:

$$P_{i,c} = H \prod_{k=c+1}^{i} s_k p_{i,c} \qquad \text{eqn 2}$$

where $H_c$ is the number of larvae hatched on day $c$ and $s_k$ is the proportion of the total larvae that survive from day $k-1$ to day $k$. We used a Bayesian model to estimate the parameters $\eta_c$ and $\theta_c$ for each cohort $c$. The likelihood function was informed by data on the daily pupal eclosion, $P_i$, cohort hatch sizes $H_c$ and daily larval survival estimates $s_k$ based on three counts per week (Appendix S3). The prior functions are uniform distributions (Appendix S2). A blockwise Metropolis–Hastings Markov chain Monte Carlo (MCMC) algorithm was used to estimate the

 

posterior distributions of means and standard deviations of the pupation probability distributions of larval cohorts (Appendix S2). Cohorts hatched in the same week were assumed to have the same pupation probability distribution, thus requiring the estimation of 38 parameters for the 19 weeks of hatching.

### *Estimating per-capita female fecundity*

We estimated the per-capita female fecundity, $\lambda_c$, defined here as the number of first-instar larvae hatched on day $c$ per adult female old enough to potentially contribute offspring to this cohort. This procedure required estimates of the time lag between oviposition and hatching of eggs, $h$, the daily rate of pupal and adult survival, $\mu$, the minimum time required for adult females to mate, blood-feed and become gravid following emergence, $T_G$, and the time required for pupal development $T_P$. We set $T_G = 4$ days, $T_P = 2$ days and $\mu = 0{\cdot}95$ based on past studies (McMeniman *et al.* 2009; Wong *et al.* 2011) and $h = 6$ days based on our semi-field cage data (Appendix S5). Then:

$$\lambda_c = \frac{H_c}{A^G_{c-h}} \qquad \text{eqn 3}$$

where $A^G_i$ is the number of adult females in the semi-field cage population on day $i$ who are at least $T_G$ days old, estimated by:

$$A^G_i = 0{\cdot}5 \sum_{k=0}^{i-T_P-T_G} P_k \mu^{i-k-1},\ i \geq T_P + T_G \qquad \text{eqn 4}$$

Our results are based on the per-capita female fecundity averaged over each week, $\bar{\lambda}_w = \sum_{c \in w} \lambda_c / n_w$, where $n_w$ is the number of days in week $w$ on which eggs were hatched.

Our estimates of per-capita female fecundity over the time period following *Wolbachia* introduction were adjusted to consider only the subpopulation of uninfected females who did not have an incompatible mating with an infected male. This methodology used data on the *Wolbachia* infection frequency in the samples of first-instar larvae and eclosed pupae and is detailed in Appendix S4. In summary, we made two adjustments to the fecundity estimates $\lambda_c$ (eqn 3). First, we excluded the estimated number of infected adult females old enough to be potentially gravid and their offspring. The accuracy of this approximation benefited from the fact that the introduced infected females produced almost all of the infected first-instar larvae. Secondly, we excluded the estimated number of uninfected adult females old enough to be potentially gravid who had incompatible matings with infected males. These estimates made three simplifying assumptions: (i) the sex ratio in the semi-field cage population is equal; (ii) maternal transmission of *Wolbachia* is perfect; and (iii) the population is panmictic (random mating).

### ASSESSING EFFECTS OF *WOLBACHIA* ON LARVAL FITNESS COMPONENTS

We assessed effects of *Wolbachia* infection on two of our three focal mosquito demographic variables: first instar-to-pupal survival and larval development time. Our data do not allow effects of *Wolbachia* on adult female fecundity to be assessed (see the Discussion). We used our model-predicted larval development time distributions together with the first-instar *Wolbachia* infection frequency estimate for each cohort to forecast the *Wolbachia* infection frequency in the eclosed pupae over time. This forecast represents the null hypothesis that *Wolbachia* infection does not affect the first instar-to-pupal larval survival or the larval development time. We note that $f_{L,c}$, the estimated *Wolbachia* infection frequency in each cohort of first-instar larvae $c$, follows a beta distribution Beta($h_{c,W} + 1$, $h_{c,U} + 1$) where $h_{c,W}$ and $h_{c,U}$ are the numbers of newly hatched infected and uninfected first-instar larvae observed in the sample from cohort $c$. Then, on the $j$th draw from the posterior distribution of $P_{i,c}$ (eqn 2), the expected number of *Wolbachia*-infected larvae in a cohort hatched on day $c$ that eclose as pupae on day $i$, $P^{(j)}_{i,c,W}$, is estimated by:

$$P^{(j)}_{i,c,W} = f^{(j)}_{L,c} P^{(j)}_{i,c} \qquad \text{eqn 5}$$

where $f^{(j)}_{L,c}$ is the $j$th draw from the beta distribution. We estimated the distribution of $P_{i,W}$, the number of *Wolbachia*-infected pupae that eclose on day $i$, using 100 000 draws from the posterior distribution.

### ASSESSING ADULT BODY SIZE VARIATION

Adult body size, measured by wing length, is an important *A. aegypti* fitness component that is also known to vary in response to changing larval density, with higher density larval conditions resulting in smaller adults (Barrera 1996; Walsh *et al.* 2013). We measured the wings of a subsample of the adults that emerged from the pupae sampled from our semi-field cage population in weeks 1–10 using standard procedures (Williams *et al.* 2013). Wings were not measured from samples taken in the time period following *Wolbachia* introduction (weeks 11–28) to avoid contamination prior to PCR analysis. Our subsample comprised wings from 200 individuals obtained by selecting 20 individuals (10 of each sex) at random from each week of samples. We compare the wing length distribution of our samples and that of samples obtained from a previous field survey of *A. aegypti* pupae from Cairns, north-eastern Australia (Williams *et al.* 2013).

## Results

### DENSITY-DEPENDENT POPULATION REGULATION IN *A. AEGYPTI*

The daily rate of pupal eclosion displayed short-period fluctuations (Fig. 1a; black line) of typically less than a week, with greater variability in the initial 2 months of the time period and rising to a peak at the end of the study. In contrast, the total abundance of larvae increased rapidly over the first 2 months due to high daily larval survival that was estimated to be above 0·85 for the entire study period (Fig. 1b and Appendix S3). Following the commencement of *Wolbachia* introductions, a second, higher peak in larval abundance occurred (Fig. 1b).

Our Bayesian model estimates of the average pupal eclosion for each day (eqn 2) provide a smooth fit to the daily pupal eclosion data (Fig. 1a; red line), which validates the corresponding model-predicted development time distributions of each larval cohort (Fig. 1c). Cohorts hatched in the first 2 weeks had a clear advantage, with fast development (in 1–2 weeks) (Fig. 1c) and relatively high first instar-to-pupae survival (Fig. 1d; black line).

 

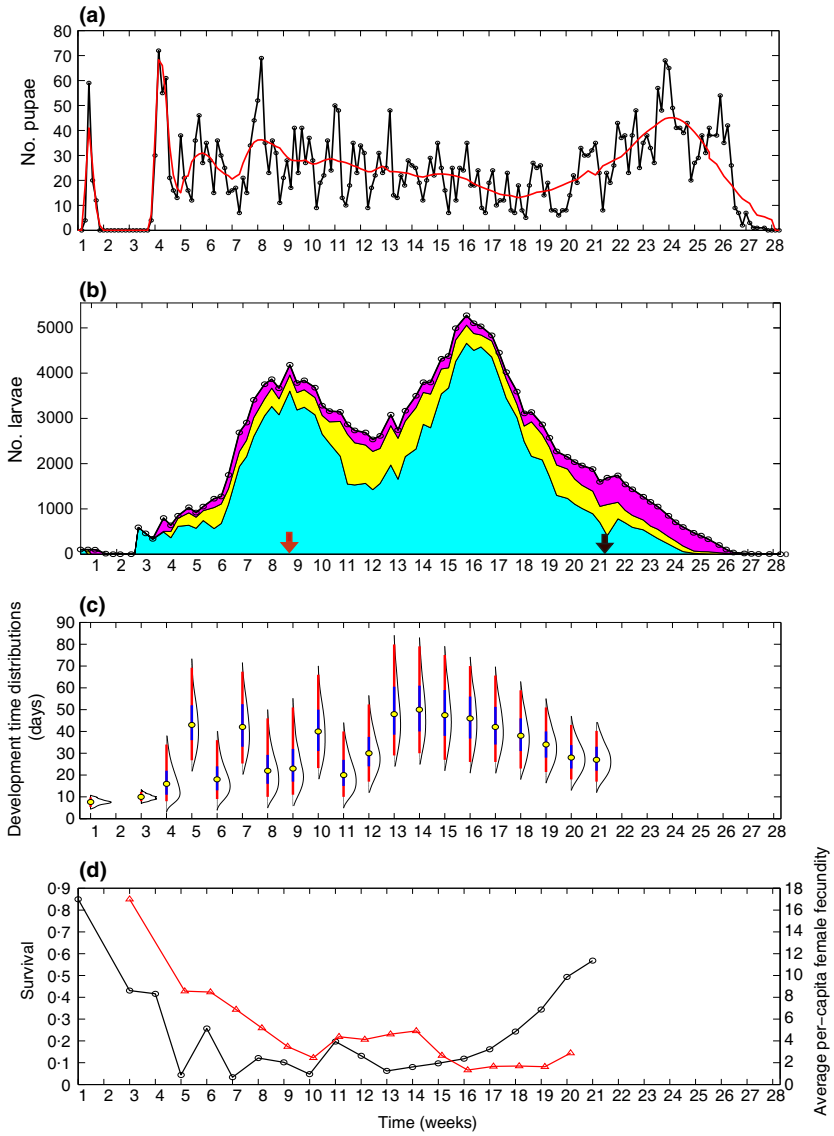


**Fig. 1.** (a) The observed (black lines and markers) and model-predicted (red line) number of pupae that eclose on each day; (b) the observed abundance of larvae over time. Black lines and markers show the total number of larvae and the shaded areas shows the proportion of larvae that are either first or second instars (blue), third instars (yellow) and fourth instars (pink), respectively. The red arrow indicates the day that introductions of *w*Mel-infected pupae were initiated and the black arrow indicates the day that both *w*Mel introductions and egg hatching were terminated; (c) the estimated development time distributions of larvae hatched in each week. Yellow markers show the median, blue lines show the 75% quantiles and red lines show the 95% quantiles. The black lines show the shape of the distributions; (d) the estimated first instar-to-pupal survival of cohorts hatched in each week (black lines and markers) and the estimated week average per-capita female fecundity (red lines and markers).

The estimated development times of subsequent cohorts are much longer (up to 80 days), with much greater variation in development times amongst individuals hatched in the same week. First instar-to-pupal survival declined rapidly over the first 4 weeks and remained low (~10%) for most of the study period before rising steadily in the final 4 weeks (Fig. 1d; black line). Both larval development time and first instar-to-pupal survival showed greater temporal variation in the first part of the period (weeks 1–12) compared to the latter part (weeks 13–21). This may be due to differences in the age-structure and growth rate of larval population between the first and latter parts of the study period. Our estimates of per-capita female fecundity also displayed a clear temporal pattern, and were highest at the start of the season and lowest at the end of the season (Fig. 1d; red line). Per-capita female fecundity was strongly correlated with the weekly rate of growth in total larval abundance ($r = 0{\cdot}84$, $N = 16$).

Having estimated these three demographic variables over time, we used generalized linear regression to assess their relationship to the larval density that individuals experienced during development. In the case of larval development time, we used the mean of the estimated development time distribution for each larval cohort as the independent variable (Fig. 1c). All three demographic variables showed a highly significant relationship to larval density: Fig. 2 shows the fitted models and details of the methodology and results are given in Appendix S5. For the first instar-to-pupal survival and the mean development time of larvae from cohorts hatched in a given week, the covariate was the estimated average larval density that the surviving pupae experienced over the course of their development (Appendix S5). For first instar-to-pupal survival, logistic regression gave a highly significant negative larval density coefficient ($P < 2 \times 10^{-16}$; McFadden $R^2 = 0{\cdot}68$; Fig. 2a). Mean larval development time was described by log–log regression with a highly significant positive coefficient ($P = 3{\cdot}3 \times 10^{-5}$; Adjusted $R^2 = 0{\cdot}61$; Fig. 2b). For the weekly average per-capita female fecundity, the covariate was the 3-week average larval density at

 

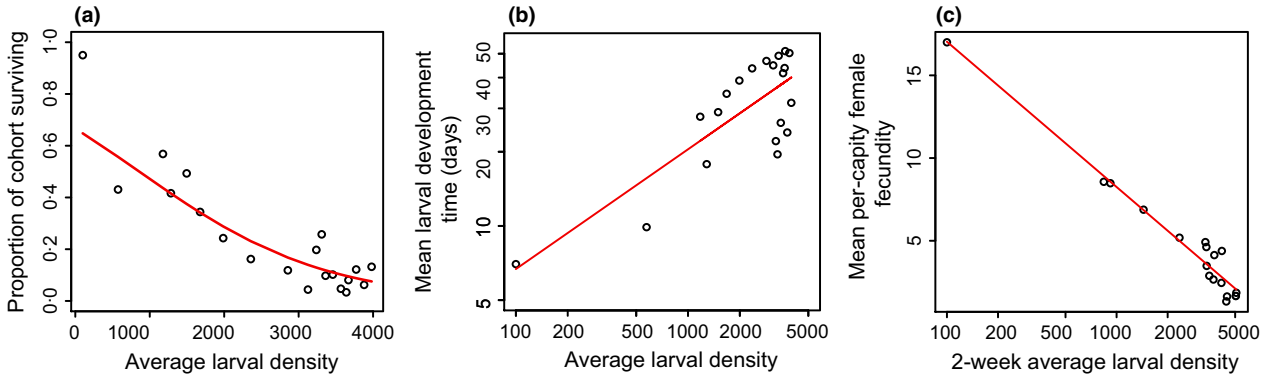


**Fig. 2.** The fitted generalized linear regression models of (a) first instar-to-pupal survival; (b) mean larval development time; (c) weekly mean per-capita female fecundity as functions of a covariate describing average total larval density. Red lines and black circles show the fitted models and data, respectively.

a lag of 3 days prior to the first day of each week (Appendix S5). A loglinear regression produced a highly significant negative coefficient ($P = 7{\cdot}3 \times 10^{-12}$; Adjusted $R^2 = 0{\cdot}96$; Fig. 2c).

Overall our *A. aegypti* population exhibited strong density-dependent population regulation, with higher larval densities being strongly associated with lower first instar-to-pupal survival, longer mean larval development times and lower per-capita female fecundity. This imposed considerable competitive pressure amongst individual larvae. The wide variation in development times amongst individuals of similar ages indicates high phenotypic variation in larval competitive ability (Fig. 1c).

### *WOLBACHIA* INFECTION DYNAMICS AND FITNESS EFFECTS

*Wolbachia* was first detected in the first-instar larvae 19 days after the introductions of infected pupae began (Fig. 3a). The observed infection frequencies in the first instar cohorts were below 0·5 for all but two cohorts that were hatched in weeks 15–16 (Fig. 3a). *Wolbachia* was first detected in the eclosed pupae exactly 5 weeks after its first detection in the first-instar larvae (Fig. 3b). This agrees well with our model prediction (Fig. 3b), indicating that the development times of these infected individuals do not differ significantly from those of other larvae in the same cohort. In the remaining weeks, the observed dynamics of *Wolbachia* in the eclosed pupae agree well with our model prediction with the exception of weeks 19 and 20. In these weeks, there is weak evidence that the *Wolbachia* is under-represented in the pupae compared to the model prediction. The rate of pupal eclosion is at the lowest value of the season in these weeks (Fig. 1a), and the low *Wolbachia* frequency may be due to a period of larval starvation (see the Discussion). For the other weeks, there is no credible effect of *Wolbachia* infection on the first instar-to-pupal survival or the development time of the eclosed pupae (Fig. 3b).

Despite this similarity between infected and uninfected larvae with regard to these fitness components, the results reveal powerful effects of density-dependent larval competition on *Wolbachia* invasion. The rate of *Wolbachia* spread is limited by the development times of the larval cohorts, and is slowed considerably by the protraction of

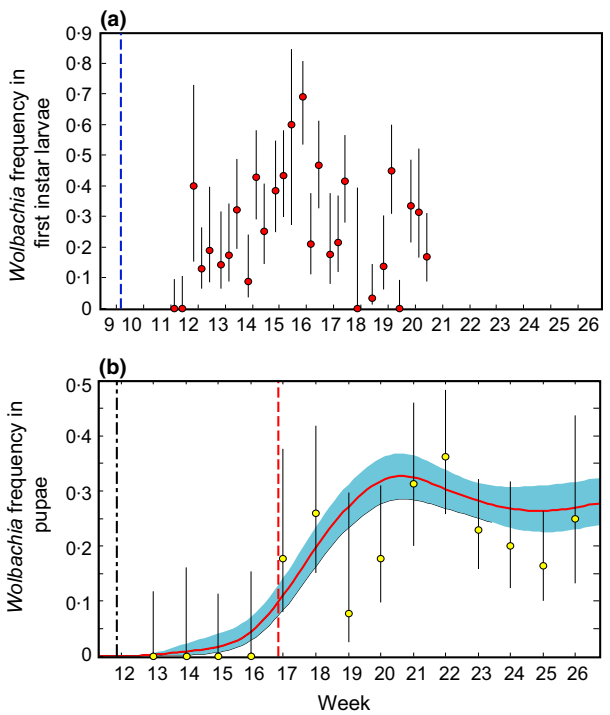


**Fig. 3.** (a) The observed *w*Mel frequency in the first-instar larvae. Red circles show the observed frequency based on samples of 30 larvae from each hatch and black lines show the Bayesian 95% credible interval. The blue dashed line shows the day that the introductions of *w*Mel-infected pupae commenced. (b) The observed *w*Mel frequency in the emerging pupae in weeks following *Wolbachia* introduction compared to the model prediction. Yellow circles show the observed frequency in pupae samples pooled across each week, and black lines show the 95% Bayesian credible interval. The red line shows the median infection frequency predicted by the model and the blue shaded area represents the predicted 95% Bayesian credible interval. The black dot-dashed line corresponds to the day that *w*Mel was first detected in the first-instar larvae and the red dashed line shows the day that *w*Mel was first detected in the eclosed pupae.

 

larval development times due to the high intrinsic level of larval density-dependent competition (Fig. 1c). This effect was strongly exacerbated by the increase in larval density following the immigration of infected mosquitoes (Fig. 1b). Thus, the first cohorts that contained *Wolbachia*-infected larvae experienced the longest development times (>5 weeks) observed over the entire study period (Figs 1b,c and 3). Further, the larval cohorts that experienced these increased density conditions had low survival, with the surviving adult females having low fecundity, relative to individuals that emerged at other times in the study period (Fig. 1d). Thus, the density-dependent response of the mosquito population demography to increasing larval density reduced the fitness of the entire population, and increased larval development times slowed the spread of *Wolbachia*.

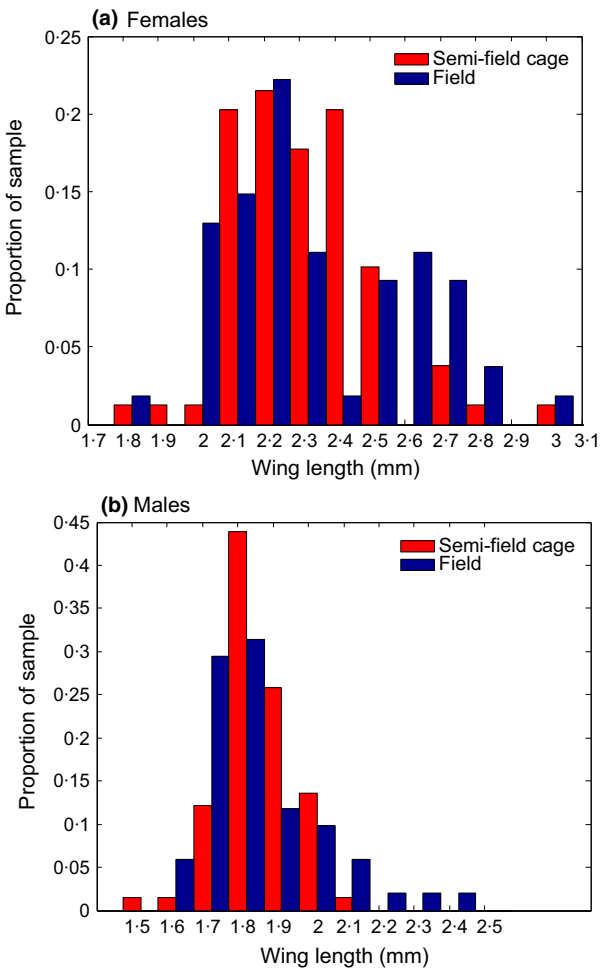


**Fig. 4.** Comparison of the wing length distributions of (a) female and (b) male adults from our semi-field cage population (red bars) and wild populations (blue bars). Wing length measurements from wild mosquitoes were obtained from a previous field survey (Williams *et al.* 2013); data from bucket container habitats only are shown (54 females and 51 males). Wing length measurements from our semi-field cage population were obtained from individuals sampled in the first 10 weeks of the study period (87 females and 84 males) (see Materials and methods).

### WING LENGTH COMPARISON

The distributions of male and female wing lengths obtained from a subsample of adults from our semi-field cage population (Fig. 4) were similar to those given by individuals sampled from wild *A. aegypti* larval container habitats in Cairns, north-eastern Australia (Williams *et al.* 2013).

## Discussion

We developed an empirical and mathematical model of *A. aegypti–Wolbachia* dynamics under competitive conditions of limited larval food resources. The rate of *Wolbachia* spread over time in our system was considerably slower than that expected based on the predictions of previous studies that do not consider larval resource limitation (see Appendix S6; Hoffmann *et al.* 2011; Walker *et al.* 2011). Our system showed highly variable and protracted larval development times, whereas previous studies of *Wolbachia* dynamics in *A. aegypti* assume a fixed larval development time of relatively short duration (Turelli 2010; Hancock, Sinkins & Godfray 2011; Hoffmann *et al.* 2011; Walker *et al.* 2011). We used our model's forecast of the *Wolbachia* frequency in the eclosed pupae to estimate the effects of *Wolbachia* on larval fitness components in the presence of intrinsic larval demographic heterogeneity. We showed that the slow development and low survival of the *Wolbachia*-infected larvae was not caused by fitness costs of *Wolbachia,* but was a result of the reduced fitness and slow dynamics of the whole mosquito population (Fig. 1c). These density-dependent effects prevented the *Wolbachia* from attaining a high frequency over the study period of 4–5 months (during which 55 overlapping generations were created).

The slowing of *Wolbachia* spread caused by density-dependent competition in the mosquito population reduces the speed at which *Wolbachia* invades *A. aegypti*. Slowing the rate of increase in *Wolbachia* frequency is highly influential because the fitness advantage conferred by *Wolbachia* to its host is frequency-dependent. In *A. aegypti* infected with *w*Mel, CI is nearly complete (Walker *et al.* 2011), and the expected reduction in the average fecundity of uninfected females is almost directly proportional to the *Wolbachia* frequency (Turelli 1994). Our study found that female fecundity varied by more than a factor of ten due to differing levels of larval density-dependent competition, and similarly strong variation in other mosquito fitness components. Therefore, while the *Wolbachia* frequency remains at low-to-moderate levels, larval density-dependent variation in fitness throughout the mosquito population can potentially influence *Wolbachia* dynamics more strongly than fitness heterogeneity caused by *Wolbachia*. Our results therefore emphasize that density-dependent heterogeneity in mosquito fitness components can have multiple interacting effects on *Wolbachia* invasion in *A. aegypti* populations and needs to be considered in models of *Wolbachia* dynamics (Turelli 1994; Hancock, Sinkins & Godfray 2011).

 

In designing strategies for *Wolbachia* release, our results indicate that releases that attain a high infection frequency in the mosquito population will be critical to increasing both the speed and likelihood of *Wolbachia* establishment. In addition to releasing large numbers of infected mosquitoes, manipulation of the availability of larval habitat and food resources in field populations could improve *Wolbachia's* invasion potential. If the offspring of the released mosquitoes experience low competition for food they are more likely to have fast development (i.e. short generation time) and high survival and fecundity relative to the wild-type population (Fig. 2). This may be achieved by suppressing uninfected mosquito populations prior to release and also potentially during the release programme (Hoffmann 2014).

Our evidence for larval density-dependent population growth in our study population is based on strong associations between larval density and three demographic variables (Fig. 2). However, other variables may also explain variation in these demographic rates over time. In particular, temperature is known to affect mosquito demographic rates (Mohammed & Chadee 2011). For our population, we found that water temperature in the larval habitat was significantly associated with mean larval development time and per-capita female fecundity but explained a much lower proportion of the variance than larval density (Appendix S7). Our inferences are necessarily based on a single study population, for which we provide a robust description of population dynamics over an ecologically relevant time period. Additional replicated experiments could be undertaken to directly compare the importance of larval density on *Wolbachia* invasion rates across time.

While we found no significant costs of *w*Mel Wolbachia on larval fitness components, further research is needed to assess the fitness effects of *Wolbachia* in the presence of density-dependent variation in mosquito fitness. Any effects of *w*Mel infection on larval survival and development time may not be have been detected given our sample sizes, particularly under the high larval density conditions experienced in our semi-field cage population. In weeks 19–20, our results suggest a possible reduction in the survival of infected larvae relative to uninfected larvae. During this time period, which follows the highest peak in total larval density, the rate of pupal eclosion is at the minimum value of the season (Fig. 1a,b). When food availability is extremely low and starvation occurs in the larval population, *Wolbachia* infection may reduce larval survival (Ross *et al.* 2014).

Moreover, our study did not estimate effects of *Wolbachia* on adult female fecundity, which is an important component in predicting *Wolbachia* invasion and spread (Turelli 2010). We could not estimate this effect because most of the *Wolbachia*-infected adults originated from a population external to our semi-field cage population. Therefore, we cannot reasonably assess effects of *Wolbachia* on per-capita female fecundity, because any differences could be attributed to differences in the larval environment experienced by infected and uninfected subpopulations.

While our semi-field cage system aims to simulate similar intrinsic and extrinsic processes to those occurring in natural *A. aegypti* populations, our system differs in some important ways from natural populations. Adult mosquito survival is expected to be relatively high in our semi-field cage compared to the higher risk conditions experienced by field populations (Muir & Kay 1998). Moreover, environmental conditions will be more variable in natural populations, with stronger variation in egg mortality, the rate of egg hatching, larval food availability, larval survival and other important demographic and environmental variables.

However, our model *A. aegypti* population also shares some important demographic properties in common with wild populations. Field populations of *A. aegypti* display a characteristic pyramidal juvenile age-structure where the numbers of eggs exceeds the numbers of pupae by 1–3 orders of magnitude (Southwood *et al.* 1972). This implies a typically low rate of larval survival that is consistent with the larval survival rates occurring in our study population. Further, the body size of wild-caught *A. aegypti* adults (as measured by their wing length) is typically smaller than that of well-nourished laboratory-reared individuals and varies over a wide range (Williams *et al.* 2013; Yeap *et al.* 2013). We found that the distributions of wing lengths in our semi-field cage population were similar to those in the field. This suggests that wild *A. aegypti* populations may experience density-dependent larval resource competition over a similar range to that experienced in our study population.

In conclusion, our results show how density-dependent fluctuation in multiple mosquito fitness components regulates population growth in our *A. aegypti* population. This heterogeneity in mosquito fitness greatly inhibited *Wolbachia* spread, demonstrating that density-dependent mosquito population dynamics have an important influence on *Wolbachia* invasion. Understanding natural mosquito population dynamics, and how they interact with *Wolbachia* spread, will be critical to predicting the dynamics of *Wolbachia* release strategies for dengue control.

## Acknowledgements

This research was supported by a Marie Curie International Outgoing Fellowship within the 7th European Community Framework Programme (Grant no. 326551-WOLBACHIA_MOD). We thank Jane Lloyd, Gavin Omodei, Chris Paton, Alex Roberts and Mick Townsend for their generous and continued help and support. We are also grateful to Nancy Endersby and Anjali Goundar for their generous assistance with PCR analysis.

## Data accessibility

Mosquito age-class abundances and *Wolbachia* infection frequencies: Figshare doi: 10.6084/m9.figshare.2067534 (Hancock *et al.* 2016).

## References

Altizer, S.M. & Augustine, D.J. (1997) Interactions between frequency-dependent and vertical transmission in host-parasite systems. *Proceedings of the Royal Society B-Biological Sciences*, **264**, 807–814.

 

Barrera, R. (1996) Competition and resistance to starvation in larvae of container-inhabiting *Aedes* mosquitoes. *Ecological Entomology*, **21**, 117–127.

Bhatt, S., Gething, P.W., Brady, O.J., Messina, J.P., Farlow, A.W., Moyes, C.L. *et al.* (2013) The global distribution and burden of dengue. *Nature*, **496**, 504–507.

Bian, G.W., Xu, Y., Lu, P., Xie, Y. & Xi, Z.Y. (2010) The endosymbiotic bacterium *Wolbachia* induces resistance to dengue virus in *Aedes aegypti*. *PLoS Pathogens*, **6**, e1000833.

Caspari, E. & Watson, G.S. (1959) On the evolutionary importance of cytoplasmic sterility in mosquitoes. *Evolution*, **13**, 568–570.

Darbro, J.M., Johnson, P.H., Thomas, M.B., Ritchie, S.A., Kay, B.H. & Ryan, P.A. (2012) Effects of *Beauveria bassiana* on survival, blood-feeding success, and fecundity of *Aedes aegypti* in laboratory and semi-field conditions. *American Journal of Tropical Medicine and Hygiene*, **86**, 656–664.

Dye, C. (1984) Models for the population dynamics of the yellow fever mosquito *Aedes aegypti*. *Journal of Animal Ecology*, **53**, 247–268.

Haine, E.R. (2008) Symbiont-mediated protection. *Proceedings of the Royal Society B-Biological Sciences*, **275**, 353–361.

Hancock, P.A., Sinkins, S.P. & Godfray, H.C.J. (2011) Population dynamic models of the spread of *Wolbachia*. *American Naturalist*, **177**, 323–333.

Hancock, P.A., Linley-White, V., Callahan, A.G., Godfray, H.C.J., Hoffmann, A.A. & Ritchie, S.A. (2016) Data from: density-dependent population dynamics in *Aedes aegypti* slow the spread of *w*Mel *Wolbachia*. *Figshare*, http://dx.doi.org/10.6084/m9.figshare.2067534

Hilgenboecker, K., Hammerstein, P., Schlattmann, P., Telschow, A. & Werren, J.H. (2008) How many species are infected with *Wolbachia*? A statistical analysis of current data. *Fems Microbiology Letters*, **281**, 215–220.

Hoffmann, A.A. (2014) Facilitating *Wolbachia* invasions. *Austral Entomology*, **53**, 125–132.

Hoffmann, A.A., Montgomery, B.L., Popovici, J., Iturbe-Ormaetxe, I., Johnson, P.H., Muzzi, F. *et al.* (2011) Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission. *Nature*, **476**, 454–457.

Laven, H. (1956) Cytoplasmic inheritance in *Culex*. *Nature*, **177**, 141–142.

Lee, S.F., White, V.L., Weeks, A.R., Hoffmann, A.A. & Endersby, N.M. (2012) High-throughput PCR assays to monitor *Wolbachia* infection in the dengue mosquito (*Aedes aegypti*) and *Drosophila simulans*. *Applied and Environmental Microbiology*, **78**, 4740–4743.

Legros, M., Lloyd, A.L., Huang, Y.X. & Gould, F. (2009) Density-dependent intraspecific competition in the larval stage of *Aedes aegypti* (Diptera: Culicidae): revisiting the current paradigm. *Journal of Medical Entomology*, **46**, 409–419.

Lipsitch, M., Siller, S. & Nowak, M.A. (1996) The evolution of virulence in pathogens with vertical and horizontal transmission. *Evolution*, **50**, 1729–1741.

McMeniman, C.J., Lane, R.V., Cass, B.N., Fong, A.W.C., Manpreet, S., Wang, Y. & O'Neill, S.L. (2009) Stable introduction of a life-shortening *Wolbachia* infection into the mosquito *Aedes aegypti*. *Science*, **323**, 141–144.

Mohammed, A. & Chadee, D.D. (2011) Effects of different temperature regimens on the development of *Aedes aegypti* (L.) (Diptera: Culicidae) mosquitoes. *Acta Tropica*, **119**, 38–43.

Moreira, L.A., Iturbe-Ormaetxe, I., Jeffery, J.A., Lu, G.J., Pyke, A.T., Hedges, L.M. *et al.* (2009) A *Wolbachia* symbiont in *Aedes aegypti* limits infection with Dengue, Chikungunya, and *Plasmodium*. *Cell*, **139**, 1268–1278.

Muir, L.E. & Kay, B.H. (1998) *Aedes aegypti* survival and dispersal estimated by mark-release-recapture in northern Australia. *American Journal of Tropical Medicine and Hygiene*, **58**, 277–282.

Phuc, H., Andreasen, M.H., Burton, R.S., Vass, C., Epton, M.J., Pape, G. *et al.* (2007) Late-acting dominant lethal genetic systems and mosquito control. *BMC Biology*, **5**, 11.

Reeson, A.F., Wilson, K., Cory, J.S., Hankard, P., Weeks, J.M., Goulson, D. & Hails, R.S. (2000) Effects of phenotypic plasticity on pathogen transmission in the field in a *Lepidoptera*-NPV system. *Oecologia*, **124**, 373–380.

Ross, P., Endersby, N., Yeap, H. & Hoffmann, A. (2014) Larval competition extends developmental time and decreases adult size of *w*MelPop *Wolbachia*-infected *Aedes aegypti*. *American Journal of Tropical Medicine and Hygiene*, **91**, 198–205.

Sibly, R.M., Barker, D., Denham, M.C., Hone, J. & Pagel, M. (2005) On the regulation of populations of mammals, birds, fish, and insects. *Science*, **309**, 607–610.

Simmons, C.P., Farrar, J.J., Nguyen, V.V.C. & Wills, B. (2012) Dengue. *New England Journal of Medicine*, **366**, 1423–1432.

Southwood, T.R.E., Murdie, G., Yasuno, M., Tonn, R.J. & Reader, P.M. (1972) Studies on the life budget of *Aedes aegypti* in Wat Samphaya, Bangkok, Thailand. *Bulletin of the World Health Organisation*, **46**, 211–226.

Turelli, M. (1994) Evolution of incompatibility inducing microbes and their hosts. *Evolution*, **48**, 1500–1513.

Turelli, M. (2010) Cytoplasmic incompatibility in populations with overlapping generations. *Evolution*, **64**, 232–241.

Walker, T., Johnson, P.H., Moreira, L.A., Iturbe-Ormaetxe, I., Frentiu, F.D., McMeniman, C.J. *et al.* (2011) The wMel *Wolbachia* strain blocks dengue and invades caged *Aedes aegypti* populations. *Nature*, **476**, 450–453.

Walsh, R.K., Facchinelli, L., Ramsey, J.M., Bond, J.G. & Gould, F. (2011) Assessing the impact of density dependence in field populations of *Aedes aegypti*. *Journal of Vector Ecology*, **36**, 300–307.

Walsh, R.K., Aguilar, C.L., Facchinelli, L., Valerio, L., Ramsey, J.M., Scott, T.W., Lloyd, A.L. & Gould, F. (2013) Regulation of *Aedes aegypti* population dynamics in field systems: quantifying direct and delayed density dependence. *American Journal of Tropical Medicine and Hygiene*, **89**, 68–77.

Washburn, J.O., Mercer, D.R. & Anderson, J.R. (1991) Regulatory role of parasites – impact on host population shifts with resource availability. *Science*, **253**, 185–188.

Williams, C.R., Johnson, P.H., Ball, T.S. & Ritchie, S.A. (2013) Productivity and population density estimates of the dengue vector mosquito *Aedes aegypti* (*Stegomyia aegypti*) in Australia. *Medical and Veterinary Entomology*, **27**, 313–322.

Wong, J., Astete, H., Morrison, A.C. & Scott, T.W. (2011) Sampling considerations for designing *Aedes aegypti* (Diptera: Culicidae) oviposition studies in Iquitos, Peru: substrate preference, diurnal periodicity, and gonotrophic cycle length. *Journal of Medical Entomology*, **48**, 45–52.

Xi, Z.Y., Khoo, C.C.H. & Dobson, S.L. (2005) *Wolbachia* establishment and invasion in an *Aedes aegypti* laboratory population. *Science*, **310**, 326–328.

Yeap, H.L., Endersby, N.M., Johnson, P.H., Ritchie, S.A. & Hoffmann, A.A. (2013) Body size and wing shape measurements as quality indicators of *Aedes aegypti* mosquitoes destined for field release. *American Journal of Tropical Medicine and Hygiene*, **89**, 78–92.

Zug, R. & Hammerstein, P. (2012) Still a host of hosts for *Wolbachia:* analysis of recent data suggests that 40% of terrestrial arthropod species are infected. *PLoS ONE*, **7**, e38544.

*Received 21 April 2015; accepted 1 February 2016*
*Handling Editor: Yann Clough*

## Supporting Information

Additional Supporting Information may be found in the online version of this article.

**Appendix S1.** Procedures and schedule for semi-field cage population maintenance.

**Appendix S2.** Bayesian MCMC estimation of larval emergence probability distributions.

**Appendix S3.** Estimating daily larval survival.

**Appendix S4.** Estimating per-capita female fecundity during *Wolbachia* immigration.

**Appendix S5.** Linear regression analysis of larval density-dependent demography.

**Appendix S6.** Comparing models with and without density dependence.

**Appendix S7.** Linear regression analysis of temperature-dependent demography.

 