Assessing temperature-dependent competition between two invasive mosquito species

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Abstract. Invasive mosquitoes are expanding their ranges into new geographic areas and interacting with resident mosquito species. Understanding how novel interactions can affect mosquito population dynamics is necessary to predict transmission risk at invasion fronts. Mosquito life-history traits are extremely sensitive to temperature, and this can lead to temperature-dependent competition between competing invasive mosquito species. We explored temperature-dependent competition between *Aedes aegypti* and *Anopheles stephensi*, two invasive mosquito species whose distributions overlap in India, the Middle East, and North Africa, where *An. stephensi* is currently expanding into the endemic range of *Ae. aegypti*. We followed mosquito cohorts raised at different intraspecific and interspecific densities across five temperatures (16–32°C) to measure traits relevant for population growth and to estimate species' per capita growth rates. We then used these growth rates to derive each species' competitive ability at each temperature. We find strong evidence for asymmetric competition at all temperatures, with *Ae. aegypti* emerging as the dominant competitor. This was primarily because of differences in larval survival and development times across all temperatures that resulted in a higher estimated intrinsic growth rate and competitive tolerance estimate for *Ae. aegypti* compared to *An. stephensi*. The spread of *An. stephensi* into the African continent could lead to urban transmission of malaria, an otherwise rural disease, increasing the human population at risk and complicating malaria elimination efforts. Competition has resulted in habitat segregation of other invasive mosquito species, and our results suggest that it may play a role in determining the distribution of *An. stephensi* across its invasive range.

Key words: Aedes aegypti; Anopheles stephensi; invasive species; malaria; mosquito ecology; temperature-dependent competition.

INTRODUCTION

Species invasions are an increasingly common phenomenon, driven by the expansion of global transport and commerce. Following an introduction, if and how far a species will spread in a novel environment depends on niche and fitness differences relative to the resident community, including competitive interactions (MacDougall et al. 2009). Competitive interactions between invasive and resident species often determine whether the two species can coexist across their distributions. However, competition pressure is not constant across space and can depend on the environmental context, particularly abiotic variables such as temperature (Chamberlain et al. 2014). Temperature-dependent competition has been observed in many systems, especially for temperature-sensitive organisms such as *Tribolium* beetles (Park 1954), *Daphnia* spp. (Fey and Cottingham 2011), and aphids (Grainger et al. 2018). Therefore, the strength and direction of competition may shift across a climate gradient, limiting an invasive species' spread in certain environmental contexts.

This kind of temperature-dependent competition is common amongst mosquito species, who experience interspecific interactions while in shared larval habitats. In fact, environmentally dependent competition has affected the invasion dynamics and co-occurrence patterns of multiple mosquito species (Livdahl and Willey 1991, Bevins 2008, Freed et al. 2014, Lounibos and Juliano 2018). This has been particularly apparent in recent decades, with multiple species invading into new

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regions, for example, *Aedes albopictus* in the United States and Europe (Medlock et al. 2012), *Culex coronator* in the United States (Wilke et al. 2020), and *Aedes japonicus* in Europe (Schaffner et al. 2009). *Aedes aegypti* is perhaps the most well-known invasive mosquito, having expanded from its endemic range in East Africa to a currently near-global distribution (Powell and Tabachnick 2013). In contrast, *Anopheles stephensi*, a newly invasive mosquito, is expanding from its historic range on the Indian sub-continent and the Arab peninsula, where it is the urban vector of malaria (Singh et al. 2017), to parts of Northeast Africa (Seyfarth et al. 2019, Surendran et al. 2019). These two species co-occur in the endemic range of *An. stephensi*, and *An. stephensi* is expanding into the endemic range of *Ae. aegypti*. Unlike other *Anopheles* species that only breed in natural water bodies, *An. stephensi* breeds in artificial containers in urban areas (Thomas et al. 2016). In these habitats, *An. stephensi* is often found cohabiting with other container species, including *Ae. aegypti* (Mariappan et al. 2015). Whether these species interact has not been studied.

Both species demonstrate sensitivity to temperature, but their specific life-history traits differ in their responses, with *An. stephensi* preferring cooler temperatures than *Ae. aegypti*, (Mordecai et al. 2013, 2017), and these differences may translate to temperature-dependent differences in competitive ability. Although both species experience a range of temperatures throughout their geographic distributions, the optimum temperature for *An. stephensi* is around 25°C (Mordecai et al. 2013, Sinka et al. 2020), compared to an optimum temperature of 29°C for *Ae. aegypti* (Mordecai et al. 2017). *An. stephensi* is expanding into warmer environments as it invades the endemic range of *Ae. aegypti* in northeast Africa, and the combination of competition and climate may present an obstacle to its spread. Additionally, *Ae. aegypti* is a primary vector for arboviruses such as yellow fever and dengue, and *An. stephensi* is a primary vector of urban malaria in its endemic range. Understanding how these species interact can aid in predicting how the local mosquito vector community, and therefore human disease risk, may be affected by the invasion of *An. stephensi*.

We tested for the existence of and measured the strength of temperature-dependent competition between *Ae. aegypti* and *An. stephensi* across a range of temperatures (16–32°C). Given mosquitoes' sensitivity to temperature and the species' thermal preferences, we expected that competition would be temperature-dependent, with *An. stephensi* having higher competitive ability at cooler temperatures than *Ae. aegypti*, which we expected to perform best at warmer temperatures. At each temperature, we followed cohorts of mosquitoes reared at different intra- and interspecific densities and measured life-history traits (e.g., larval survival, time to emergence, fecundity, adult longevity, and wing length) relevant to population dynamics. We calculated per

capita growth rates from these trait measurements and fit competition models to these data. From these models, we then calculated competition coefficients to estimate the relative competitive ability of each species at each temperature.

METHODS

Experiment

We used a response surface design across 15 density treatments (Appendix S1: Table S1) and five temperature treatments (16°, 20°, 24°, 28°, 32°C) to explore pairwise competition between *Ae. aegypti* and *An. stephensi*. Response surface designs vary the densities of competing species independently across a range of total densities, which allows for the fitting and parameterization of competition models (Inouye 2001). Densities were chosen so that the median total density corresponded to rearing conditions used for this strain during colony maintenance (200 larvae/1 L of water), resulting in a range of densities centered on conditions that both strains had been reared at since their colonies were initiated. The range of temperatures represented the full range at which each species can persist when reared individually, including temperatures at which both species are found in the field (Brady et al. 2014, Sinka et al. 2020). For example, in Delhi, where both species are found, temperatures of mosquito larval habitat range from 14.3° to 36.1°C, depending on the habitat type (Kumar et al. 2018). The strain of *Ae. aegypti* was an outbred field-derived population originating from Tapachula, Chiapas, Mexico, in 2016. The F5 generation was used in this experiment. The strain of *An. stephensi* (Liston, type-form) was sourced from a long-standing colony housed at Pennsylvania State University (State College, Pennsylvania, USA) that was originally obtained from the Walter Reed Army Institute of Research (Silver Spring, Maryland, USA). This commonly used strain is an urban form of *An. stephensi* referred to as the "type" or "typical" form and derives from a colony established from mosquito populations near Delhi, India in 1947 (Rutledge et al. 1970). This experimental design was replicated three times, without additional replication within each temporal replicate.

Larvae were hatched on experimental Day 0. On Day 1, first instar mosquito larvae were placed in quart-size mason jars with 250 mL of reverse-osmosis filtered water and 0.1 g cichlid pellet food (Hikari Cichlid Cod Fish pellets). Rearing jars were placed in incubators (Percival Scientific), following the intended temperature treatments with 85% ($\pm$5%) relative humidity (RH), and 12:12-h light:dark diurnal cycle. Temperature regimens were programmed to a mean given by the experimental treatment (16°, 20°, 24°, 28°C, 32 $\pm$ 0.5°C) and daily periodic fluctuation of 9°C, following the Parton-Logan equation (Parton and Logan 1981), which is

characterized by a sine wave during the daytime and exponential curve at night. Rearing jars were inspected daily for emerged mosquitoes and the numbers of males and females emerging on each day recorded. Following emergence, adults were pooled by day of emergence, temperature, species, and density treatment. Adults were kept in a 16-oz paper cup in a walk-in incubator (Percival Scientific) at 27°C (±0.5°C), 85% RH (±5%), and 12:12-h light:dark cycle and offered a 10% sucrose solution ad libitum.

Mosquitoes that emerged up to and including the day of peak emergence were allowed to mate 4–6 d before being offered a blood meal. Forty-eight hours prior to blood feeding, the sucrose was removed and replaced with deionized water, which was then removed 24 h later. Blood meals were administered via a water-jacketed membrane feeder at 38°C for 30 min. A maximum of 10 blood-fed females per treatment were sorted into individual oviposition containers and kept at 27°C (±0.5°C), 85% (±5%) RH, 12:12-h dark:light cycle. Oviposition containers consisted of a 50-mL centrifuge tube with a damp cotton ball and filter paper at the bottom to collect eggs. Centrifuge tubes were covered with a fine mesh to keep mosquitoes inside and allow for air circulation. During this time, females had access to a 10% sucrose solution ad libitum. Females were monitored daily for oviposition events. The date of the oviposition event was noted, and the number of eggs was counted the following day to allow for females who were monitored while ovipositing to finish laying eggs. After oviposition, the filter paper and cotton ball were removed and each female was monitored daily until death. Wing length was recorded for all female mosquitoes to estimate fecundity for those mosquitoes whose fecundity was not directly measured (Armbruster and Hutchinson 2002). All females' wings were mounted on a glass side to measure the wing length from the distal end of the alula to the apex of the wing using a dissecting scope and micrometer.

Life-history traits

We measured five traits relevant to population dynamics: larval survival, time to emergence, fecundity, adult longevity, and wing length (a standard proxy for fecundity; Armbruster and Hutchinson 2002). Larval survival was modeled as a binomial random variate for the number of female larvae surviving from the first-instar larval stage until adult emergence. Time to emergence was measured as the median time for female larvae to develop from first instar to an adult per jar, in days. The median was used because the distribution of emergence times within a jar was right-skewed. Fecundity was the number of eggs laid during the first oviposition event. Females that did not oviposit were assigned a fecundity of zero. Adult longevity was the number of days between female adult emergence and death. Wing length was the distance from the distal end

of the alula to the apex of the wing in millimeters. All traits were only measured for females, and the number of females per treatment at the start of the experiment was assumed to be 50% of the initial number of larvae within each jar.

We used generalized linear mixed models to test for the effect of temperature, *Ae. aegypti* density, *An. stephensi* density, and the interactions between temperature and species' densities on each life-history trait. We included replicate as a random intercept in all analyses. The models for larval survival were fit using a binomial distribution and a logit link (i.e., logistic regression). Binomial logistic regressions, weighted by the number of trials or individuals, are commonly used to model variables that represent proportions, such as percent survival. Because of the frequent occurrence of jars with no *An. stephensi* surviving, we used a hurdle model, which allows the degree of zero inflation to vary across observations dependent on other predictor variables (Brooks et al. 2017). In our model, the structural zero-inflation term was dependent on both species' densities. The time to emergence was modeled as the day that 50% of the females emerged and was fit with a generalized Poisson distribution and log link for both species. The generalized Poisson distribution is a mixture of Poisson distributions that is similar to a negative binomial distribution, but is more appropriate for right-skewed data due to its long tail (Joe and Zhu 2005). Fecundity was modeled with a negative binomial distribution and log link, including a term for zero-inflation to account for some females that laid no eggs. Adult longevity was modeled with a generalized Poisson distribution and log link. Wing length was modeled with a Gaussian distribution and identity link. Because of the low survival of *An. stephensi* larvae, sample size for this species was low, and models with interactions only converged when the response variable was the time to emergence or wing length. Therefore, the other three *An. stephensi* models included main effects only, as our data did not contain enough information to explain these interactions. Mosquitoes are small ectotherms, so their life-history traits exhibit a unimodal response to temperature (Mordecai et al. 2019), which we accounted for in our models by fitting temperature using a three-degree polynomial basis spline. All models were fit using the *glmmTMB* package (Brooks et al. 2017) and we assessed the residuals for divergence from normality using the *DHARMa* package (Hartig 2019) in R v. 3.6.4 (R Development Core Team 2018).

Calculating the population growth rate

We calculated the per capita growth rate for each treatment following Chmielewski et al. (2010), substituting our own empirically measured traits. We define the per capita growth rate r, as the change in the population-level abundance of female mosquitoes attributable to one female mosquito:

$$r = \frac{\ln R_0}{\tau}, \tag{1}$$

where R_0 is the change in population size (ΔN) in one generation and τ is the generation time, or mean time to maturity and reproduction in days. R_0 is the total population fecundity divided by the initial population size (N_0, assumed to be 50% of the initial larvae to only represent the females):

$$R_0 = \frac{\Sigma_x A_x F_x}{N_0}, \tag{2}$$

where F_x is the total lifetime reproduction of an individual emerging on day x (eggs) and A_x is the number of female individuals emerging on day x.

Lifetime fecundity F_x is calculated from measured values of gonotrophic cycle length in days (g), adult lifespan (l), and the number of eggs per gonotrophic cycle (f_x):

$$F_x = \frac{f_x g^{-1} l}{2}. \tag{3}$$

We define f_x as the number of eggs laid by the female in the oviposition chamber for each individual during the first gonotrophic cycle. When f_x was not directly measured for an individual, it was approximated by a species-specific linear regression relating wing length (w_x [mm]) to fecundity from experimental data from this experiment (Ae. aegypti: $f_x = -98.51 + 52.42 w_x$, Adj. $R^2 = 0.3624$, $n = 862$; An. stephensi: $f_x = -51.57 + 36.75 w_x$, Adj. $R^2 = 0.0815$, $n = 117$). Some females had no intact wings to measure, and these were assigned the mean fecundity value for that temperature and density treatment for that species in that replicate. Because only 50% of offspring are female, we divide this calculation of lifetime fecundity F_x by two to continue to include only females in our calculation of the growth rate.

Following Livdahl and Sugihara (1984), τ was weighted by the overall contribution to population fecundity:

$$\tau = \frac{\Sigma_x (x+g)(A_x F_x)}{\Sigma_x A_x F_x}, \tag{4}$$

where x is the day of emergence, g is the gonotrophic cycle length in days, and A_x and F_x are the number of females that emerged on day x and their predicted lifetime fecundity, respectively.

This results in a final equation for the per capita growth rate for each density × temperature treatment:

$$r' = \frac{\ln\left(\frac{1}{N_0}\Sigma_x A_x w_x g^{-1} l\right)}{\frac{\Sigma_x (x+g)(A_x w_x g^{-1} l)}{\Sigma_x A_x w_x g^{-1} l}}. \tag{5}$$

Similar to the other life-history traits, we tested for the effect of temperature, Ae. aegypti density, An. stephensi

density, and the interactions between temperature and species' densities on the population growth rate using a generalized linear mixed model, including replicate as a random intercept. To aid with model fit, we exponentiated r', transforming the metric into the finite rate of increase (λ), allowing us to fit the models using a Gaussian distribution and identity link. Because of the frequent occurrence of jars with $\lambda = 0$ for An. stephensi, we used a hurdle model with a structural zero-inflation term dependent on Ae. aegypti density in that model.

We conducted a sensitivity analysis to quantify the contribution of our four measured life-history traits (larval survival, larval development rate, adult fecundity, and adult longevity) to variation in per capita growth rates for each species. Following standard procedures for bootstrap uncertainty analyses, we sampled each parameter independently 5,000 times from our data set with replacement and assessed each parameter's contribution by estimating the partial rank correlation coefficient between the parameter and the calculated per capita growth rate. The sensitivity analyses were conducted using the *sensitivity* package in R v. 3.6.4 (R Development Core Team 2018).

Measuring the temperature-dependence of competition

As there is no consensus model for competitive interactions between mosquito species, we fit five theoretical discrete-time competition models that differ primarily in the shape of the response of the per capita growth rate to increasing species densities for each temperature treatment and mosquito species. We selected the best fit model using AIC (Appendix S1: Table S2). All models were within 2 AIC of each other, and we chose the model with the lowest AIC, which approximates a Lotka-Volterra competition model where growth rates decline linearly with increasing species densities:

$$e^{r_i} = \lambda_i - \alpha_{ii} N_i - \alpha_{ij} N_j, \tag{6}$$

where r_i is the per capita growth rate of species i (corresponding to r' from Eq. 5), λ_i is the intrinsic growth rate of species i, α_{ii} is the competition coefficient of intraspecific competition, α_{ij} is the competition coefficient of interspecific competition, and N_i and N_j are the starting population densities of species i and j corresponding to densities from our experimental treatments. We fit this model separately for each temperature level and species to explore how λ, α_{ii}, and α_{ij} changed as a function of the larval environment, and therefore if competitive interactions are temperature-dependent. We estimated the three parameters (λ_i, α_{ii}, and α_{ij}) by fitting a nonlinear least squares regression in R v. 3.6.3 (R Core Team 2018).

We used the estimated parameters from these equations to calculate each species' competitive ability (K_i) at each temperature following Hart et al. (2018):

$$K_i = \frac{\lambda_i - 1}{\sqrt{\alpha_{ii}\alpha_{ij}}}. \qquad (7)$$

Briefly, this definition of competitive ability is derived from the concept of mutual invasibility, which states that each species must be able to increase when rare while the other is at equilibrium in order for the species to coexist (Chesson 2000). Solving for the conditions that allow for mutual invasion results in a ratio representing the average fitness difference of the two species, from which we derive each species fitness, or competitive ability, K_i, with competitive ability increasing with increasing K. Importantly, this metric incorporates growth in the absence of competition ($\lambda_i - 1$) and the species ability to tolerate both intra- and interspecific competition ($\sqrt{\alpha_{ii}\alpha_{ij}}$). Because K incorporates a species' growth rate and tolerance for competition, it allows for the possibility of a species with a low overall growth rate, but which is very robust to competition, to be the dominant long-term competitor. Thus, a species that is tolerant of competition, even it grows slowly, can persist and grow to high overall abundance, increasing the overall amount of competitive pressure and ultimately outcompeting less tolerant species regardless of their growth rates. By choosing to use these parameters, rather than direct measurements of fecundity, this definition of competitive ability incorporates differences across all life-history stages to evaluate long-term competitive outcomes.

Results

Our factorial design included 15 species density combinations across five temperature treatments (75 treatments total) replicated three times. In one replicate, one jar (8 *Ae. aegypti*: 24 *An. stephensi* at 24°C) had only male *Ae. aegypti* emerge, and so this was dropped from all analyses, resulting in 179 jars across three replicates. After adult emergence, we followed a total of 1,242 *Ae. aegypti* and 134 *An. stephensi* females to estimate fecundity and adult longevity.

Life-history traits

Both species' larval survival rates exhibited a unimodal response to temperature, with survival highest at intermediate temperatures (Fig. 1A, D; Appendix S1: Tables S3, S4). This response was strongest for *Ae. aegypti* mosquitoes at high overall densities. In general, *Ae. aegypti* survival decreased with increasing intraspecific densities, although the shape of this relationship depended on temperature (Fig. 1B; Appendix S1: Table S3). There was no evidence for an effect of
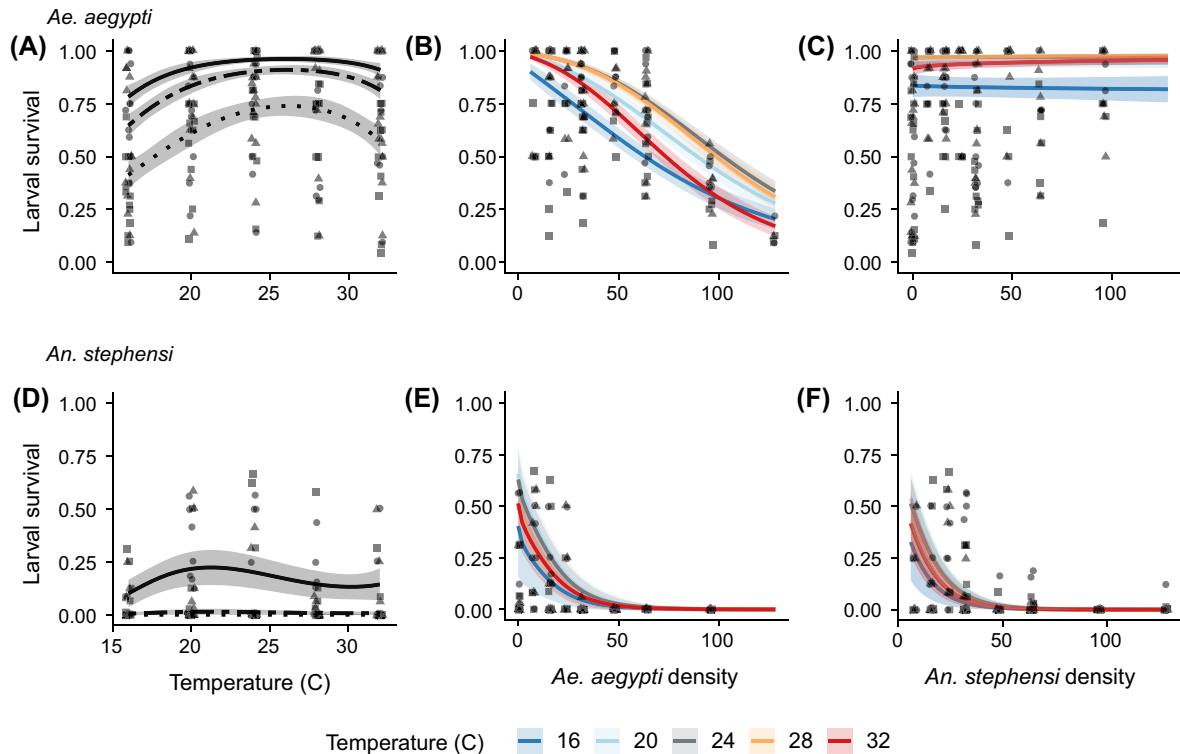

Fig. 1. Effect of temperature (A), (D); *Aedes aegypti* density (B), (E); and *Anopheles stephensi* density (C), (F) on each species' larval survival. Top row (A)–(C) is *Ae. aegypti* and bottom row (D)–(F) is *An. stephensi*. The points represent raw data, with each replicate denoted by a different symbol, and solid lines represent model fits with 95% confidence interval. In panels (A) and (D), three lines are shown for three unique species ratios, 16:16 (solid), 32:32 (dashed), 64:64 (dotted). In panels (B), (C) and (E), (F), solid lines represent the model fit with the other species density held constant at 16. Species densities are per 250 mL.

interspecific densities on *Ae. aegypti* larval survival (Fig. 1C; Appendix S1: Table S3). *Anopheles stephensi* survival was strongly negatively impacted by both interspecific and intraspecific densities and no *An. stephensi* survived above interspecific densities of 32 (Fig. 1E, F; Appendix S1: Table S4). Survival was lower for *An. stephensi* than *Ae. aegypti* with mean jar-level survival rates across all treatments and replicates of 0.075 ± 0.158 SD and 0.682 ± 0.285 SD, respectively.

Both species emerged more quickly with increasing temperatures and neither species' time to emergence was impacted by interspecific or intraspecific densities (Appendix S1: Fig. S1, Tables S3, S4). However, the time to emergence was generally longer for *An. stephensi* larvae than *Ae. aegypti* larvae at all treatment combinations (Appendix S1: Fig. S1).

We found no evidence for an effect of temperature on *Ae. aegypti* female fecundity (Appendix S1: Fig. S2A, Table S3). Higher intraspecific densities resulted in lower fecundity (Appendix S1: Fig. S2B, Table S3) regardless of temperature treatment, but interspecific density had no effect (Appendix S1: Fig. S2C, Table S3). We found no evidence for an effect of temperature, interspecific density, or intraspecific density on *An. stephensi* fecundity (Appendix S1: Fig. S2E, F, Table S4).

Aedes aegypti longevity was highest in females that were reared at intermediate temperatures (Appendix S1: Fig. S3A, Table S3). We failed to find evidence for an effect of intraspecific or interspecific densities on *Ae. aegypti* longevity (Appendix S1: Fig. S3B, C, Table S3). We found no evidence for a difference in *An. stephensi* longevity across any of the three treatments (Appendix S1: Fig. S3D, E, F, Table S4). Across all temperatures, *Ae. aegypti* females lived approximately twice as long as *An. stephensi* females, with mean adult lifespans of 32.1 ± 16.0 SD and 16.7 ± 9.80 SD days, respectively.

Increasing temperatures led to shorter wing lengths for both species (Appendix S1: Fig. S4 A, D, Tables S3, S4). *Aedes aegypti* wing lengths also decreased with increasing intraspecific densities (Appendix S1: Fig. S4B, Table S3), but were unaffected by interspecific densities (Appendix S1: Fig. S4C, Table S3). Neither species' density was found to influence *An. stephensi* wing lengths (Appendix S1: Fig. S4E, F, Table S4). Across all temperatures, *An. stephensi* were slightly larger than *Ae. aegypti*, with mean wing lengths of 2.89 ± 0.31 SD mm and 2.74 ± 0.31 SD mm, respectively.

There was no evidence of correlation between life-history traits for both species (Appendix S1: Fig. S6, S7), supporting our approach to analyze each individually. However, wing length was relatively more correlated with fecundity and time to emergence for both species than other pairwise correlations. This is to be expected, given that wing length is often used as a proxy for fecundity (Armbruster and Hutchinson 2002). In fact, we used wing length in this way to approximate the fecundity of female mosquitoes for which we did not directly observe an oviposition event.

Population growth rates

Temperature significantly influenced the population growth rate of *Ae. aegypti*, with the species exhibiting a unimodal relationship with temperature (Fig. 2A; Appendix S1: Table S3). There was no evidence for an effect of interspecific densities on *Ae. aegypti* growth rates; however, the growth rate did decrease with increasing conspecific densities (Fig. 2B, C; Appendix S1: Table S3). We found no support for an effect of temperature on *An. stephensi* growth rates (Fig. 2D, Appendix S1: Table S4). However, there was a strong negative relationship between both species' densities and *An. stephensi* growth rates, with positive growth rates ($\lambda > 1$) only at the lowest total densities (Fig. 2E, F, Appendix S1: Table S4). *Aedes aegypti* population growth rates were higher than *An. stephensi* population growth rates across all density and temperature treatments. The sensitivity analysis revealed that variation in calculated *Ae. aegypti* finite growth rates was explained the most by measured variation in the species' time to emergence, followed by larval survival rates and adult longevity (Appendix S1: Fig. S5). Variation in *An. stephensi* growth rates was explained primarily by variation in female fecundity, with the other four life-history traits having correlation coefficients overlapping or near zero (Appendix S1: Fig. S5).

Temperature-dependence of competition

The parameters from fitted competition models differed across temperature levels for both species, although there was higher uncertainty surrounding these estimates for *An. stephensi* than *Ae. aegypti* (Fig. 3). *Ae. aegypti* growth rates had a unimodal relationship with temperature, peaking at 28°C (Fig. 3A). The relationship between the intrinsic growth rate of *An. stephensi* and temperature had wide confidence intervals, although the trend suggests it may also exhibit a unimodal relationship (Fig. 3A). Both species' intraspecific competition coefficients (α_{ii}) tended to increase with temperature (Fig. 2B). The effect of *Ae. aegypti* density on *An. stephensi* population growth rates did not show a clear trend across temperatures, and the effect of *An. stephensi* on *Ae. aegypti* did not differ from zero at any temperature (Fig. 3C). Inserting these parameters into the general equation for competitive ability (Eq. 7) illustrates that *Ae. aegypti* is the dominant competitor at all temperatures tested (Fig. 2D).

DISCUSSION

Understanding how novel interactions may affect mosquito population dynamics is necessary for predicting disease risk at invasion fronts, such as that of *An. stephensi* (Seyfarth et al. 2019, Takken and Lindsay 2019). We found strong evidence for asymmetric competition between *Ae. aegypti* and *An. stephensi* across the
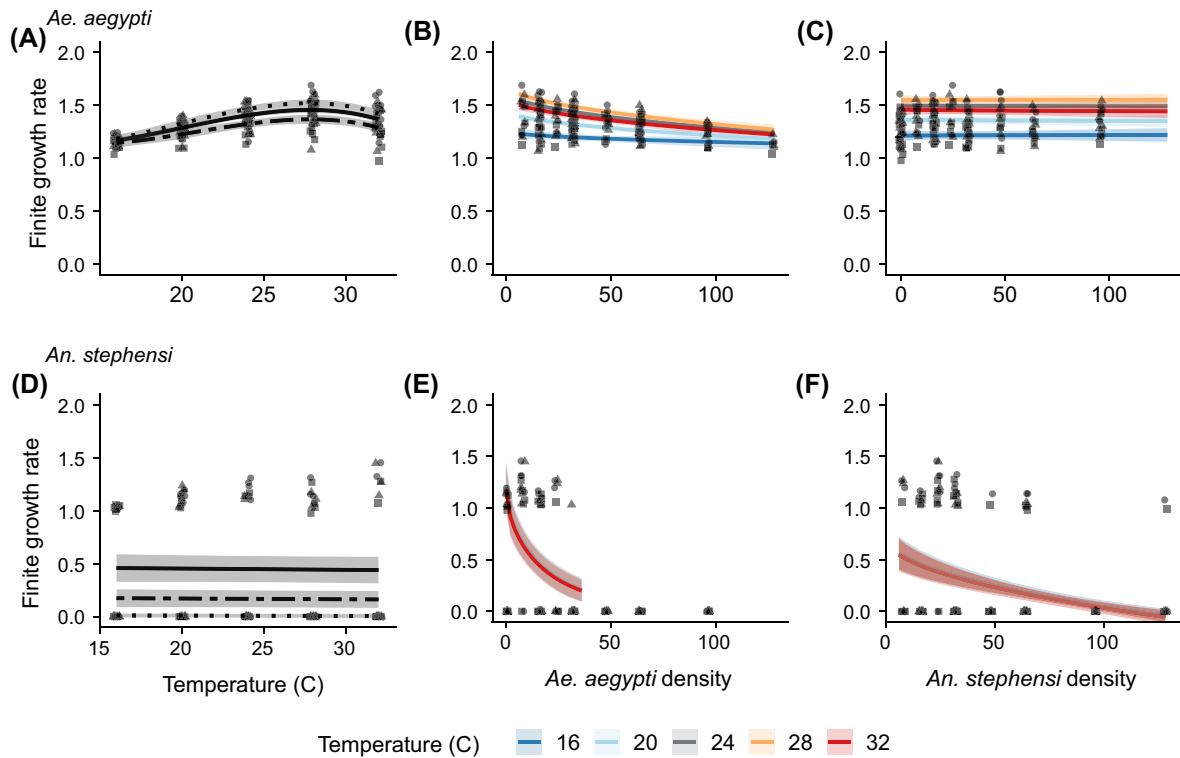
FIG. 2. Effect of temperature (A), (D); *Aedes aegypti* density (B), (E); and *Anopheles stephensi* density (C), (F) on each species' finite population growth rate. Top row (A)–(C) is *Ae. aegypti* and bottom row (D)–(F) is *An. stephensi*. The points represent raw data, with each replicate denoted by a different symbol, and solid lines represent model fits with 95% confidence interval. In panels (A) and (D), three lines are shown for three unique species ratios, 16:16 (solid), 32:32 (dashed), 64:64 (dotted). In panels (B), (C), (E), and (F), solid lines represent the model fit with the other species density held constant at 16. Species densities are per 250 mL.

full range of temperatures tested in this experiment, with *Ae. aegypti* consistently emerging as the dominant competitor. The intrinsic growth rate of *An. stephensi* was lower than that of *Ae. aegypti* at all temperatures, and *An. stephensi* was less tolerant of interspecific competition than *Ae. aegypti*. Given the global range of *Ae. aegypti*, this competitive interaction has the potential to reduce or slow the rate of spread of *An. stephensi* in its invasive range.

Life-history traits were influenced by both temperature and species densities. Metabolic theory predicts that colder temperatures result in longer development times for all ectotherms (Kingsolver and Huey 2008), implying that female mosquitoes from colder temperatures may be larger bodied and have higher fecundity rates. This has often been the case in mosquito systems (Armbruster and Hutchinson 2002; but see Reiskind and Zarrabi 2012). In our study, colder temperatures resulted in longer development times and wing lengths for both species, but the effect of temperature on fecundity was much weaker. The size–fecundity relationship in mosquitoes is typically weakest towards the thermal minima (Costanzo et al. 2018), with fecundity saturating with decreasing temperatures. In this study, measured fecundity at 16° and 20°C was less than predicted by a linear

size–fecundity relationship (Appendix S1: Fig. S3A), mirroring this breakdown of the relationship at cold extremes. Interestingly, we did not find evidence that species' densities influenced either species' time to emergence, in contrast with previous studies on larval competition and mosquito development times (Couret et al. 2014). However, *Ae. aegypti* wing length and fecundity did decrease with increasing conspecific densities. This is in agreement with other studies that found that limiting resources via competition leads to smaller-bodied mosquitoes (Alto et al. 2005, Juliano et al. 2014). Rather than delaying emergence to develop into larger-bodied mosquitoes given resource limitations, mosquitoes under higher competitive pressures in our study emerged at similar times, but with smaller bodies.

Our laboratory experiment suggests that long-term coexistence between the two species is unlikely; yet the two species co-occur at the landscape scale in the endemic range of *An. stephensi* and *An. stephensi* is currently expanding into the range of *Ae. aegypti*. Our study only considers interactions within one larval habitat and does not account for mechanisms that act at a larger scale that could explain the co-occurrence patterns found in the field. One such mechanism is the classical competition–dispersal trade-off: metacommunity level
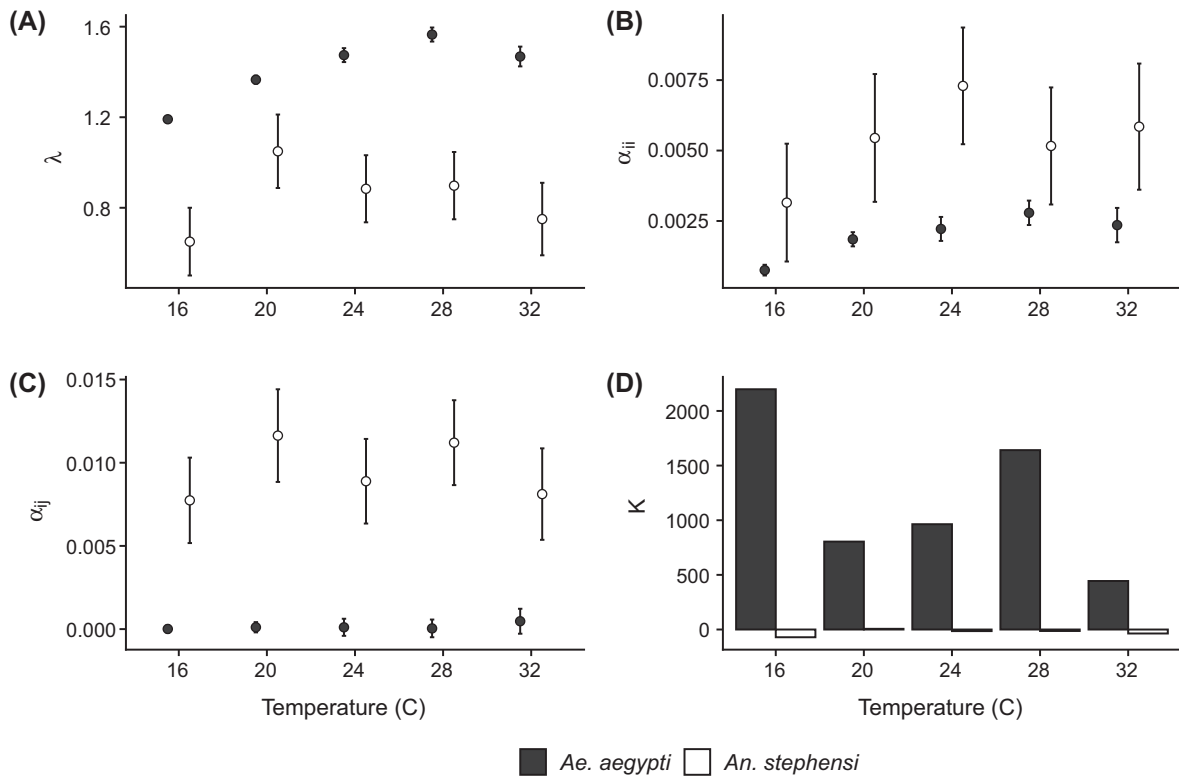
FIG. 3. *Aedes aegypti* outcompetes *Anopheles stephensi* at all temperatures. Parameter estimates from the competition model for *Ae. aegypti* (solid) and *An. stephensi* (open). Panels represent (A) intrinsic growth rates λ_i, (B) the intraspecific competition coefficient α_{ii}, (C) the interspecific competition coefficient α_{ij}, and (D) a comparison of species competitive abilities. Error bars represent standard error.

coexistence is possible if the inferior competitor's superior dispersal ability allows it to colonize new patches where the competitive pressure is lower (Hastings 1980). Indeed, *Ae. aegypti* flight range is estimated to be 83.4 ± 52.2 m, compared to a longer dispersal distance of 144.5 ± 53.0 m for *An. stephensi* (Verdonschot and Besse-Lototskaya 2014). Thus, although the species may not coexist within a single larval habitat, the wider dispersal range of *An. stephensi* may allow for landscape-scale co-occurrence at the level of the metacommunity. Spatial aggregation of the superior competitor has been shown to reduce the competitive impact of *Ae. albopictus* on *Ae. aegypti* at the landscape scale (Fader and Juliano 2013) and could potentially mediate competition between *Ae. aegypti* and *An. stephensi* as well. Pairing studies that explore landscape-scale patterns of co-occurrence in the field with semifield metacommunity experimental designs could inform how the patch-level dynamics measured in this experiment scale up.

Similarly, species-specific microhabitat preferences may reduce the frequency of habitat overlap, and thereby competition, during the larval stage. Although *An. stephensi* and *Ae. aegypti* are found together in small artificial containers, *An. stephensi* also oviposit in larger water bodies, such as overhead water tanks,

where *Ae. aegypti* is less common (Thomas et al. 2016). The *An. stephensi* preference for larger water bodies could result in lower species densities and therefore less exposure to competition during the larval stage. Indeed, *An. stephensi* are less tolerant of overcrowding than *Ae. aegypti* (Yadav et al. 2017), and a preference for larger water bodies may help the species avoid crowding in the larval environment. In addition, the foraging strategies of each species are adapted to their habitat preferences. *Anopheles stephensi* is a filter feeder that feeds primarily at the water surface, whereas *Ae. aegypti* is a shredder that feeds throughout the water column, but most often in the sediment layer (Merritt et al. 1992). This difference in strategies may reduce competition within a single habitat that contains multiple microhabitats, as well as lead to competition-reducing segregation across habitat types because of differing oviposition preferences (Reiskind et al. 2009, Rey and O'Connell 2014). Therefore, larger water bodies may serve as a refugia for *An. stephensi* from high competitive pressure by *Ae. aegypti* and allow for landscape-scale co-occurrence.

Finally, interpopulation variation in competitive phenotypes could limit the applicability of our experiment to species coexistence in the field. The two strains used

in this experiment have different domestication histories because of constraints on strain availability. The *Ae. aegypti* strain was recently derived from Mexico, and the *An. stephensi* strain was originally established from an Indian population several decades ago and since kept in laboratory conditions. A laboratory strain could exhibit a high tolerance for crowding (Kesavaraju et al. 2012), but may also have reduced overall fitness because of inbreeding (Koenraadt et al. 2010). Additionally, these strains are not sympatric in nature, having originated in Mexico and India, and this combination may not reflect the competitive interactions between *Ae. aegypti* and *An. stephensi* in North Africa, as has been shown regarding competition between *Ae. albopictus* and *Ae. aegypti* (Leisnham and Juliano 2010). Further, invasive *An. stephensi* populations may have an increased competitive ability compared to those in endemic areas (e.g., the evolution of increased competitive ability hypothesis; Strayer et al. 2006). If the invader genotype of *An. stephensi* is able to coexist with, or even outcompete, *Ae. aegypti*, then the result of strong asymmetric competition we found with our specific populations may not apply to competition in the region of North Africa where *An. stephensi* is currently invading. Further research that includes a variety of field-derived genotypes in competition experiments is needed to explore the importance of genotype × genotype interactions in this system.

Although we found the difference in competitive ability between the two species to be strong across all temperatures, this could be an artifact of our specific abiotic and biotic conditions, especially given the low survival rates of *An. stephensi* at higher species densities. However, another study of this same strain of *An. stephensi* in laboratory settings found similar larval survival of between 30% and 40% at low to high food availability (Moller-Jacobs et al. 2014), and *An. stephensi* is particularly sensitive to intraspecific competition, with survival rates decreasing precipitously with increasing larval densities (Reisen 1975, Yadav et al. 2017). This agrees with our findings that *An. stephensi* had mean survival rates of 34.5% at 24°C for combined species densities of 32 larvae/250 mL that decreased to 17.3% at 64 larvae/250 mL and 11.5% at 128 larvae/250 mL at 24°C. Further, larval survival did not explain more variation in *An. stephensi* population growth rates than the other life-history traits. *Anopheles stephensi* fitness did vary more than the fitness of *Ae. aegypti* for nearly all life-history traits, making it difficult to identify strong trends. This may be because of the low replication of our experiment, which was unable to account for this wide variability of one species. However, it may also be that this strain of *An. stephensi*, which has been reared in a controlled environment for hundreds of generations, is more sensitive to changes in environmental conditions than the more recently domesticated *Ae. aegypti* strain. This poor performance of laboratory-adapted species occurs in *Ae. aegypti* (Koenraadt et al. 2010, Ross et al.

2019), but has not been tested in *An. stephensi*. Rather than an artifact of the experiment, these findings provide further evidence of the sensitivity of *An. stephensi* to inter- and intraspecific density in the larval environment.

We only considered one environmental gradient in our study—temperature. Other environmental contexts, such as food type and availability (Yee et al. 2007, Murrell and Juliano 2008, Juliano 2010) or the presence of predators or parasites (Juliano 2009, Westby et al. 2019), are known to alter the outcome of competition between other container mosquitoes and could play a role in this system as well. Particularly, aquatic mosquito habitats in the field consist primarily of plant-detritus–based ecosystems that experience episodic resource pulses (Yee and Juliano 2012). In comparison, these laboratory strains are maintained with a regime of a single pulse of animal-based food, and this feeding regime was also used in this experiment. Resource quality and type alter the outcome of competition in other mosquito systems (Reiskind et al. 2012, Winters and Yee 2012, Allgood and Yee 2014), and, given the two species different foraging preferences, are likely to be important here as well. Understanding how additional environmental contexts alter interactions between *An. stephensi* and *Ae. aegypti* could help determine when these laboratory findings apply to patterns of coexistence in the field.

Although more work is needed to assess the applicability of our results to the field, our study does suggest that competition is an important factor to consider in the context of the expanding range of *An. stephensi*. Although *An. stephensi* is spreading into the endemic range of *Ae. aegypti* in northeast Africa (Surendran et al. 2019), it may not significantly impact the population abundance of existing *Ae. aegypti* populations given its low competitive ability relative to *Ae. aegypti*. Further, if *Ae. aegypti* is limiting the spread of *An. stephensi* into urban areas in the Middle East and North Africa, vector control efforts targeting *Ae. aegypti* could have unexpected consequences for *An. stephensi* dynamics via competitive release. This was observed when *Ae. albopictus* invaded urban centers in Manila, Philippines following a reduction of its competitor *Ae. aegypti* because of targeted insecticide spraying (Gilotra et al. 1967, Lounibos 2007). In areas with both mosquito vectors, vector control efforts should include a variety of approaches, rather than ones that target a single species, to avoid the competitive release of other mosquito vectors. For invasive species more broadly, biotic resistance is a known community assembly process that potentially limits the success of invasive species (Gallien and Carboni 2017). Our study suggests that species interactions are also important for population dynamics of *An. stephensi*, and that these interactions should be empirically tested and, when appropriate, incorporated into our predictions of mosquito species ranges and invasion dynamics.

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SUPPORTING INFORMATION

Additional supporting information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/eap.2334/full

DATA AVAILABILITY

All code and data (Evans 2021) needed to reproduce this analysis are stored in Figshare. https://dx.doi.org/10.6084/m9.figshare.12547187