Full title: Impacts of temperature on Zika virus transmission potential: combining empirical and mechanistic modeling approaches

Short title: Impacts of temperature on Zika virus transmission

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Abstract

Diseases like Zika, dengue, and chikungunya, which were once considered tropical and sub-tropical diseases, are now threatening temperate regions of the world due to climate change, globalization, and increasing urbanization. Temperature is a strong driver of vector-borne disease transmission, and characterizing the thermal range and optimum for transmission is essential to accurately predicting arbovirus emergence and spread. To advance our fundamental scientific understanding of the relationship between temperature and key pathogen traits for emerging arboviruses, we conducted a series of experiments to estimate the thermal performance of Zika virus (ZIKV) in field-derived *Aedes aegypti* across eight constant temperatures. We observed strong, unimodal effects of temperature on vector competence, extrinsic incubation period, and mosquito survival. We used thermal responses of these traits to update an existing temperature-dependent R_0 (the basic reproductive number) model, to infer how temperature impacts ZIKV transmission. We demonstrated that ZIKV transmission is optimized at a mean temperature of approximately 29°C, and has a thermal range of 22.7°C to 34.7°C. The predicted thermal minimum for Zika transmission is 5°C warmer than for dengue virus which suggests that current estimates on the global environmental suitability for Zika transmission are over-predicting its possible range. Accurately characterizing the unimodal effect of temperature on emerging arboviruses, like ZIKV, is critical for estimating the potential geographic and seasonal range for transmission, and accurately predicting where future climate change will increase, decrease, or have minimal impact on transmission.

Introduction

Mosquito-borne viruses are an emerging threat impacting human health and well-being. Epidemics of dengue, chikungunya, and Zika have spilled out of Africa to spread explosively throughout the world creating public health crises. Worldwide, an estimated 3.9 billion people living within 120 countries are at risk [1]. This pattern began with the growing distribution of dengue virus (DENV) over the past 30 years, which today infects 390 million people annually [2]. More recently, chikungunya and Zika viruses rapidly followed suit. Chikungunya virus (CHIKV) emerged in the Americas in 2013 and have caused 1.8 million suspected cases from 44 countries and territories to date [3]. In 2015-2016, Zika virus (ZIKV) spread throughout the Americas including the continental U.S., resulting in over 360,000 suspected cases, with likely many more undetected [3]. With the rise of neurological disorders and birth defects, such as Guillain-Barré and congenital Zika virus syndrome [4, 5], ZIKV became widely feared and was declared a "public health emergency of international concern" by the World Health Organization in 2016 [6]. In spite of growing research efforts to develop new therapeutics, vaccines, and innovative mosquito control technologies, mitigating arbovirus disease spread still depends on conventional mosquito control methods and public education.

The primary route of transmission is through the bite of infectious female *Aedes* mosquitoes. In much of the world, the invasive, widespread, and highly anthropophilic *Ae. aegypti* is the main vector responsible for the transmission of these viruses. Diseases like Zika, dengue, and chikungunya, which were once considered tropical and sub-tropical diseases, are now threatening temperate regions of the world due to climate change, globalization, and increasing urbanization [7]. The growing burden of these diseases and their potential to spread into new areas have incited a flurry of research focusing on the epidemiology, vector control, and

predictive models of how these viruses will spread seasonally, geographically, and with the effects of climate change.

There are several key gaps that potentially affect our ability to predict, and ultimately, mitigate the factors that influence transmission risk and arbovirus emergence globally. First, current models predicting mosquito distributions or virus transmission are often limited by a relatively poor understanding of the relationships among mosquito vectors, pathogens, and the environment. There is substantial evidence that temperature variability is a key driver of disease transmission across diverse vector-borne pathogen systems [2, 8-16]. Mosquitoes are small ectothermic animals and their physiology (e.g. immunity [17-19]), life history (e.g. development, reproduction, survival [14, 20]), and arbovirus fitness (e.g. extrinsic incubation period (EIP) and vector competence [9, 21-23]) exhibit unimodal responses to changes in temperature. Transmission depends in large part on the ability of mosquitoes to survive the EIP, become infectious, and bite new hosts, so differential (unimodal) impacts of temperature on survival, vector competence, and EIP have highly nonlinear effects on transmission. Warmer temperatures do not necessarily translate into more infectious mosquitoes [14, 21, 24]. Second, current models often ignore the low quality and quantity of existing data. Even in systems that are fairly well-studied (e.g. *Plasmodium falciparum* and DENV), key parameters are often estimated from only few studies. Finally, current transmission models often assume, with little justification, that the relationship between temperature and the EIP is monotonic [25], or that the relationships between temperature, EIP, and vector competence of less-studied arboviruses (e.g. CHIKV and ZIKV) are similar to DENV [14, 26, 27].

To advance our fundamental scientific understanding of the relationship between temperature and key pathogen traits for emerging arboviruses, we conducted a series of

experiments to estimate the thermal performance of ZIKV (vector competence, the extrinsic incubation rate, and the daily per capita mosquito mortality rate) in field-derived *Ae. aegypti* across eight different constant temperatures ranging from 16 - 38°C. We fit a series of nonlinear functions to estimate the thermal responses of the above traits. These thermal responses were incorporated into a temperature dependent basic reproductive number (R_0) model developed for *Ae. aegypti* and DENV [14] to infer how temperature variation will impact ZIKV transmission.

Methods

Virus culture

We used the ZIKV isolate MEX1-44 obtained from the University of Texas Medical Branch (UTMB) Arbovirus Reference Collection. The virus was isolated in January 2016 from a field-caught *Ae. aegypti* mosquito from Tapachula, Chiapas, Mexico. For all mosquito infections, we used pass ten stock virus that was passaged four times at the UTMB and additional six times at the University of Georgia. Four days after inoculation in Vero cells, we harvested the virus, centrifuged it at 2,500xg for 5 min, and stored it at -80°C. The virus tested negative for *Mycoplasma* contamination using MycoSensor PCR Assay Kit (Agilent) and was titrated using standard plaque assays on Vero cells [28]. Briefly, we infected the cells with six 10-fold serial dilutions for 1-2 hours. After incubation, we removed the inoculum and replaced it with 1.5% agarose DMEM (UltraPure LMP Agarose, Fisher Scientific). The cells were kept at 37°C, 5% CO_2 for four days when they were fixed with 4% formalin and stained with crystal violet. The titers were expressed in plaque forming units per milliliter (PFU/mL).

112 **Mosquito rearing**

113 Outbred *Ae. aegypti* mosquito colonies were generated from ovitrap collections in
114 Tapachula, Chiapas, Mexico, spring 2016. Mosquito eggs were hatched in ddH_2O under reduced
115 pressure in a vacuum desiccator and dispersed larvae in rearing trays. Each tray contained 200
116 larvae in 1L ddH_2O and 4 fish food pellets (Hikari Cichlid Gold Large Pellets). Adult mosquitoes
117 were kept in rearing cages and provided with 10% sucrose *ad libitum*. We maintained the
118 colonies on whole human blood (Interstate Blood Bank) and collected eggs on paper towels. The
119 first three generations (F1-F3) were used for building the colony and generating sufficient eggs
120 for downstream experiments, which were run with F4 mosquitoes. Larvae and adults were
121 maintained under standard, controlled insectary conditions at 27°C ± 0.5°C, 80% ± 10% relative
122 humidity, and a 12:12 light: dark diurnal cycle in a dedicated environmental walk-in room
123 (Percival Scientific).
124
125 **Experimental mosquito infections and forced salivations**
126 For each biological replicate, we separated 8,000 1 to 3-day-old females and transported
127 them to the ACL3 facility at the University of Georgia 48 hours prior to ZIKV infection.
128 Mosquitoes were kept in 64 oz. paper cups and provided with water, which was withdrawn 12
129 hours before feeding. We offered them either an infectious blood meal containing ZIKV at a
130 final concentration of 10^6 PFU/mL or an uninfected, control blood meal. The blood meal was
131 prepared by washing human blood three times in RPMI medium and the pelleted red blood cells
132 (50%) were resuspended in 33% DMEM, 20% FBS, 1% sucrose, and 5 mmol/L ATP. Lastly, for
133 the infectious blood meal, we mixed the blood mixture with ZIKV diluted in DMEM ($2*10^6$
134 PFU/mL) at a 1:1 ratio. Mosquitoes were blood-fed through a water-jacketed membrane feeder

for 30 min, after which we randomly distributed 2,000 ZIKV-exposed engorged mosquitoes and 2,000 unexposed blood-fed control mosquitoes into mesh-covered paper cups (250 mosquitoes per cup). We then placed two cups, one ZIKV-exposed and one control, at each temperature treatments (Percival Scientific): 16°C, 20°C, 24°C, 28°C, 32°C, 34°C, 36°C, and 38°C ± 0.5°C. Chambers were set to 80% ± 10% relative humidity, a 12:12 hours light: dark photoperiod, and mosquitoes were provided with 10% sucrose for the duration of the experiment. We monitored mosquito mortality every day by recording and removing dead mosquitoes.

Every three days, up to day 21, we force-salivated 20 ZIKV-exposed mosquitoes per treatment group. Twenty-three infected mosquitoes from each treatment groups were separated and provided only water 24 hours prior to forced salivation. The same number of mosquitoes were removed from the uninfected control group. To force-salivate mosquitoes, we immobilized mosquitoes by cold knock-down on ice and then by removing their legs and wings. We placed the proboscis of each mosquito into a pipet tip containing 35 µL FBS with 3 mmol/L ATP and red food dye, after which they were allowed to salivate for 30 min on a 35°C warming plate. After salivation, we collected mosquito saliva, put mosquito heads with previously collected legs, and bodies into separate tubes containing 700 µL of DMEM with 1x antibiotic/antimycotic. Each tissue was homogenized using 5 mm stainless steel beads in a QIAGEN TissueLyzer at 30 cycles/second for 30 seconds, and centrifuged at 17,000xg for 5 minutes at 4°C. To measure the proportion of mosquitoes that became infected, disseminated infection, and became infectious at each temperature, we tested for the presence/absence of the ZIKV in mosquito bodies, legs and heads, and saliva, respectively. All the samples were tested using plaque assays on Vero cells as described above. We performed two full biological replicates of this experiment (S1 Fig).

158 **Statistical analysis**

159 The effects of temperature was assessed on four different metrics of ZIKV infection. We
160 used the numbers of mosquitoes becoming infected (ZIKV positive bodies), disseminated (ZIKV
161 positive legs / heads), and infectious (ZIKV positive saliva) out of total numbers of mosquitoes
162 exposed to assess the effect of temperature on the likelihood of ZIKV infection, dissemination,
163 and infectiousness at the population level. We also used the numbers of mosquitoes that became
164 infectious out of those successfully infected as a measure of ZIKV dissemination efficiency. For
165 each response variable, we used generalized linear mixed models (IBM® SPSS® Statistics
166 1.0.0.407), normal distribution and identity link function, to estimate the effects of temperature
167 (16°C, 20°C, 24°C, 28°C, 32°C, 34°C, 36°C, 38°C), days post infection (dpi 3, 6, 9, 12, 15, 18,
168 21), and the interaction between temperature and dpi (fixed factors). Mosquito batch nested
169 within experimental replicate was included in all models as a random factor. We determined the
170 best model fit and distributions based on Akaike Information Criterion (AIC), the dispersion
171 parameter, and by plotting model residuals. Sequential Bonferroni tests were used to assess the
172 significance of pairwise comparisons within a significant main effect or interaction, and *p*-values
173 greater than 0.05 were considered non-significant. Finally, to estimate the effects of temperature,
174 ZIKV exposure and the interaction between temperature and ZIKV exposure on the daily
175 probability of mosquito survival, we used the same framework in a Cox proportional hazards
176 model (SAS® Studio, 3.6 Basic Edition) with temperature, infection status (ZIKV-exposed or
177 control) and the interaction as fixed factors, with mosquito batch nested within experimental
178 replicate as a random factor.

179

180 **Mechanistic R_0 model**

Temperature affects a variety of mosquito and virus traits that drive transmission, including mosquito demography that affects population size—egg-to-adult development rate (*MDR*), survival probability (p_{EA}), and fecundity (*EFD*; eggs per female per day)—as well as biting rate (a), adult mosquito mortality rate (μ), extrinsic incubation rate (*EIR*), and vector competence (bc; equal to the proportion of exposed mosquitoes that become infected times the proportion of infected mosquitoes that become infectious, with virus in their saliva). In previous work, we assembled trait thermal response estimates from laboratory experiments that manipulated temperature and measured each of these traits for *Ae. aegypti* and DENV, and synthesized them into an estimate for the thermal response of R_0, the expected number of new cases generated by a single infectious person or mosquito introduced into a fully susceptible population throughout the period within which the person or mosquito is infectious [14]:

$$R_0(T) = \sqrt{\frac{a(T)^2\, bc(T)\, exp\left(-\mu(T)/EIR(T)\right)\, EFD(T)\, p_{EA}(T)\, MDR(T)}{r\, \mu(T)^3}}$$

where r is the human recovery rate, T is environmental temperature, and T attached to a parameter indicates that the parameter is dependent on temperature. Here, we update three of these thermal response functions—average adult mosquito lifespan ($lf=1/\mu$), extrinsic incubation rate (*EIR*), and vector competence (bc) —using the new experimental data from *Ae. aegypti* mosquitoes exposed to ZIKV-infected blood meals across a range of constant temperatures.

Experimental data on lifespan, vector competence, and extrinsic incubation rates were used across temperatures to estimate trait thermal response functions for calculating $R_0(T)$. Because we destructively sampled mosquitoes to assess infection status and did not follow all

mosquito cohorts to the end of their lifespan, we used Gompertz survival curves to estimate average lifespan. First, Kaplan-Meier daily probabilities of survival for each experimental replicate, infection status, and temperature were estimated. Then, we used the 'nls' function in R [29] to fit a Gompertz function to the daily survival probabilities for each infection status by trial and temperature combination. To estimate the average female lifespan of exposed and control mosquitoes for each temperature treatment and experimental replicate, we calculated the area under the curve by integrating the associated Gompertz function. Vector competence for each temperature was estimated from the average proportion of mosquitoes observed to become infectious at each temperature. For estimating the ZIKV extrinsic incubation rate (*EIR*) at each temperature, we calculated the time required for half of the average proportion of the population to become infectious (and defined this as the average extrinsic incubation period, *EIP*), then inverted this time interval to estimate a daily rate of ZIKV development for each temperature (*1/EIP*).

Using these data, we fit thermal response functions for lifespan, EIR, and vector competence as either symmetric (Quadratic: $-c(T-T_0)(T-T_m)$) or asymmetric (Briere: $cT(T-T_0)(T_m-T)^{(1/2)}$) functions, where T is experimental temperature, T_0 is the minimum temperature, T_m is the maximum temperature, c is a rate constant, and both functions are truncated at zero for negative values [14, 30]. As in previous work [14], we fit the thermal response functions using Bayesian inference with uninformative priors, which are restricted to biologically reasonable ranges: $T_0 \sim$ Uniform (0, 24), $T_m \sim$ Uniform (25, 45), $c \sim$ Gamma (1, 10) for Briere and $c \sim$ Gamma (1, 1) for Quadratic [14]. In the model, we assume that the sampling process is a normal distribution centered on the thermal response function calculated at the experimental temperature, with precision τ (where $\tau=1/\sigma$) assigned the prior: Gamma ~ (0.0001, 0.0001). We

fit the models using JAGS [31] and R [29] and the R package 'rjags' [32], by running five Markov Chain Monte Carlo simulations for a 5,000-step burn-in followed by 5,000 additional steps, then thinning the posterior samples by saving every fifth sample [14, 30].

The three new thermal response functions (*lf*, *EIR*, and *bc*) were combined with the remaining previously-fitted thermal response functions [14] to calculate $R_0(T)$ for ZIKV. To do so, we propagated the posterior distribution of each parameter thermal response through the $R_0(T)$ function to calculate a posterior distribution on $R_0(T)$.

Results

We force-salivated a total of 1,865 mosquitoes for ZIKV infection and monitored the mortality of 8,000 mosquitoes in both the ZIKV-exposed and uninfected control treatment groups across two biological replicates and eight mean constant temperatures. We found significant effects of temperature, days post-infection (dpi), and the interaction between temperature and dpi on the number of mosquitoes that became infected (ZIKV-positive bodies), that disseminated infection (ZIKV-positive legs and heads), and that became infectious (ZIKV-positive saliva). We also found significant effects of temperature, dpi, and their interaction on the overall transmission efficiency of ZIKV. Finally, these effects translated into significant effects of temperature on R_0, or predicted risk of transmission for ZIKV.

The effect of temperature on ZIKV infection and infection dynamics

We observed strong, unimodal effects of temperature on the number of mosquitoes infected, with disseminated infections, and that became infectious (Table 1, Fig 1). While all three response variables dropped at both cool and warm temperatures, the extent of the decrease

was more pronounced as the virus spread through the mosquito (Fig 1), suggesting these traits exhibit different thermal sensitivities. For example, the likelihood of becoming infected was the most amenable to temperature variation, with few mosquitoes infected at 16°C (6%), maximized from 24°C-34°C (75% - 89%), and again decreased at 38°C (7%). The likelihood of viral dissemination was more constrained, with numbers of mosquitoes with disseminated infections minimized at 16-20^{o}C (4% - 3%), maximized at 28-34^{o}C (65% - 77%), and again minimized at 38^{o}C (5%). Finally, the likelihood of mosquitoes becoming infectious was the most sensitive to temperature, with the numbers of infectious mosquitoes minimized from 16-24^{o}C (0%-4%), maximized between 28-34^{o}C (23%-19%), and again minimized from 36-38^{o}C (5%-0.4%)

Table 1. The effects of temperature, day, and their interaction on the numbers (out to total mosquitoes exposed) of mosquitoes infected, and with disseminated infections, infectious, as well as a measure of overall dissemination efficiency (of those infected, the number of mosquitoes that became infectious).

response variables	temperature			day			temperature x day		
	F	d.f.	*p*-value	F	d.f.	*p*-value	F	d.f.	*p*-value
number infected	86.159	7	**<0.0001**	1.349	6	0.251	8.374	42	**<0.0001**
number disseminated	139.085	7	**<0.0001**	14.742	6	**<0.0001**	12.477	42	**<0.0001**
number infectious	34.012	7	**<0.0001**	8.876	6	**<0.0001**	4.846	42	**<0.0001**
dissemination efficiency	15.308	7	**<0.0001**	7.699	6	**<0.0001**	4.431	42	**<0.0001**

Results from generalized linear mixed effects models examining the effects of temperature, day, and the interaction on the numbers of mosquitoes infected, with disseminated infections, infectiousness, and a measure of dissemination efficiency.

Fig 1. Temperature effect on the proportion of mosquitoes infected, with disseminated infections, and infectious. The effect of eight different constant temperatures (16°C, 20°C,

24°C, 28°C, 32°C, 34°C, 36°C, 38°C) on the proportion of mosquitoes infected (ZIKV positive bodies compared to total number of exposed), with disseminated infections (ZIKV positive heads compared to total number exposed), and infectious (ZIKV positive saliva compared to total number exposed). Results with no common letters were significantly different ($p \leq 0.05$).

We also observed that temperature had a significant effect on the rate that virus disseminated through the mosquito and could be detected in saliva (Table 1, Fig 2). In general, across most temperature treatments (with the exception of 36°C and 38°C), we observed increases in the numbers of mosquitoes with ZIKV positive bodies, legs and heads, and saliva with time (Fig 2). In contrast, we see declines in the numbers of mosquitoes infected, with disseminated infections, and that were infectious over time at the warmest temperatures, due to the higher mosquito mortality when housed at 36°C and 38°C. As the temperature increased, the time at which ZIKV was detected in the samples decreased (Fig 2 and Table 2), suggesting ZIKV infections and dissemination becomes more efficient with warming temperatures.

Fig 2. Days post-infection and the proportion of mosquitoes infected, with disseminated infections, and infectious. The relationship between days post-infection (3, 6, 9, 12, 15, 18, 21) and the proportion of mosquitoes infected (A, ZIKV positive bodies), with disseminated infections (B, ZIKV positive legs and heads), and infectious (C, ZIKV positive saliva) out of the total mosquitoes exposed to ZIKV at eight different constant temperatures (16°C, 20°C, 24°C, 28°C, 32°C, 34°C, 36°C, 38°C).

Table 2. The time (days post infection, dpi) required for first detection and to reach the plateau of numbers of mosquitoes infected, with disseminated infections, and infectious.

temperature	16°C	20°C	24°C	28°C	32°C	34°C	36°C	38°C
time (dpi) to first detection of:								
infection	12	6	3	3	3	3	3	3
dissemination	12	15	6	3	3	3	3	3
infectiousness		21	15	9	6	6	6	3
time (dpi) to reach plateau of:								
infection	18	12	6	3	9	3	3	3
dissemination	18	21	15	15	9	6	6	3
infectiousness		21	18	15	12	9	6	3

The effects of temperature on ZIKV dissemination efficiency

We observed significant effects of temperature, dpi, and the interaction on the dissemination efficiency of ZIKV, measured as the number of mosquitoes that were successfully infected (ZIKV-positive bodies) that in turn went on to become infectious (ZIKV-positive saliva). ZIKV dissemination efficiency was maximized between 28 – 34°C, suggesting that ZIKV escape from the midgut and salivary gland invasion was most efficient at these temperatures (Fig 3A). In contrast, ZIKV dissemination efficiency was minimized at both cooler (16 - 20°C) and warmer temperatures (38°C). Interestingly, cooler temperatures had a more dramatic effect on ZIKV dissemination efficiency relative to warmer temperatures. For example, although 60% of exposed mosquitoes became successfully infected at 20°C, we had very low salivary gland invasion, with only one mosquito across both trials becoming infectious. In contrast, at warm temperatures infection and dissemination efficiencies were very robust (Fig S2), but the mortality associated with the warm temperatures resulted in low numbers of mosquitoes that were capable of being infectious. Finally, of those successfully infected, we observed successful salivary gland invasion to occur earlier in the infection process as temperatures warmed (Fig 3B).

Fig 3. Temperature effect on the dissemination efficiency

(A) The effect of eight different constant temperatures (16°C, 20°C, 24°C, 28°C, 32°C, 34°C, 36°C, 38°C) and (B) days post-infection (3, 6, 9, 12, 15, 18, 21) on the dissemination efficiency (proportion of ZIKV positive saliva relative to positive bodies). Results with no common letters were significantly different ($p \leq 0.05$).

The effect of temperature on mosquito survival

We observed significant effects of temperature and an interaction between temperature and ZIKV exposure on the daily probability of mosquito survival (Fig 4, Table 3). Overall, the daily probability of mosquito survival was highest for mosquitoes housed at 24°C and 28°C relative to cooler (16 - 20°C) and warmer (32 - 38°C) temperatures. Mosquito survival was lowest at the warmest temperature of 38°C, with no mosquitoes surviving past 3 dpi. ZIKV-exposed mosquitoes experienced a higher daily probability of survival at 24°C, 28°C, and 32°C relative to unexposed, control mosquitoes with greater than 90% survival at the optimal temperatures.

Fig 4. Temperature effects on the daily probability of mosquito survival. Kaplan-Meier estimates of daily probability of mosquito survival for unexposed (A) and ZIKV exposed (B) field-derived *Ae. aegypti* mosquitoes across eight different constant temperatures (16°C, 20°C, 24°C, 28°C, 32°C, 34°C, 36°C, 38°C).

Table 3. The effects of temperature, ZIKV infection, and potential interaction on the daily probability of mosquito survival.

effect	Chi-Square	d.f.	*p*-value
temperature	1138.226	7	**<0.0001**
infection	0.227	1	0.6338
temperature x infection	25.871	7	**0.0005**

Results from Cox mixed-effects model examining the effects of temperature (16°C, 20°C, 24°C, 28°C, 32°C, 34°C, 36°C, 38°C), infection status (exposed or not exposed) and the interaction on the daily probability of mosquito survival.

The effect of temperature on ZIKV transmission risk

Trait thermal responses for lifespan, vector competence, and extrinsic incubation rate were all unimodal (Fig 5). Mosquito lifespan and vector competence thermal responses were symmetrical, peaking at 24.2°C (95% CI: 21.9 – 25.9°C) and 30.6°C (95% CI: 29.6 – 31.4°C), respectively, while the extrinsic incubation rate thermal response was asymmetrical with a peak at 36.4°C (95% CI: 33.6 – 39.1°C). Applying these new trait thermal responses to the $R_0(T)$ model [14], we found that $R_0(T)$ peaked at 28.9°C (95% CI: 28.1 – 29.5°C), with lower and upper limits of 22.7°C (95% CI: 21.0 – 23.9°C) and 34.7°C (95% CI: 34.1 – 35.8°C), respectively (Fig 6).

Fig 5. Effect of temperature and estimated vector competence, extrinsic incubation rate and mosquito lifespan. Trait thermal responses, fit from laboratory experimental data. Vector competence (left), is the maximum proportion of virus-exposed mosquitoes with virus in their saliva, across temperatures and replicates. Extrinsic incubation rate (middle) is the inverse of the

time required to reach half of the maximum proportion infectious (days^{-1}) for each temperature and replicate. Lifespan is the average lifespan of mosquitoes in each temperature and replicate (days), shown in filled (virus-exposed) and open (sham-inoculated) points. Solid lines represent posterior means; dashed lines represent 95% credible intervals.

Fig 6. Effect of temperature on R_0. Effect of temperature on R_0 (top) and histograms showing the posterior distribution of the temperature minimum (bottom left), optimum (bottom center), and maximum (bottom right) for R_0. Solid line is the mean and dashed lines are the 95% credible intervals.

While there is some evidence that mosquito longevity varies for virus-exposed versus control mosquitoes, where unexposed mosquitoes had shorter lifespans at near-optimal temperatures (24°C and 28°C; Fig 5), we did not include this difference in the R_0 model for two reasons. First, with limited data to parameterize the low temperature range for survival, we are unable to characterize the differences in the lower end of the thermal response functions in detail. Second, the standard R_0 model does not incorporate differences in survival for infected versus uninfected mosquitoes because it assumes that the pathogen is rare and that all mosquitoes are uninfected. For this reason, we fit a single thermal response function for lifespan to the full dataset and used it in the R_0 model.

Discussion

The dynamics and distribution of vector-borne diseases depend on the interplay between the pathogen, the mosquito, and the environment [33]. Temperature is a strong driver of vector-borne disease transmission, and characterizing the thermal range and optimum for transmission is essential for accurately predicting how arbovirus emergence and transmission will be affected by seasonality, geography, climate and land use change. Yet current models of recently emerging arboviruses (e.g. CHIKV [26, 34] and ZIKV [10, 27, 35, 36]) are constrained by a lack of data on the thermal sensitivity of key pathogen traits. In this study, we experimentally estimated the relationship between temperature and measures of ZIKV vector competence, extrinsic incubation rate, and mosquito mortality. By incorporating these temperature-trait relationships into an existing mechanistic model, we demonstrate that like malaria [21, 30] and dengue virus [14], ZIKV transmission also has a strong unimodal relationship with temperature.

The effect of temperature on ZIKV transmission is shaped by the complex interaction of individual trait responses of the mosquito and the pathogen with temperature. As studies have demonstrated in other arbovirus systems, temperature significantly affects vector competence [14, 22, 23, 37-42]. We show that temperature has a unimodal relationship with vector competence with an estimated optimum at 30.6°C and an estimated thermal minimum and maximum of 22.9°C and 38.4°C, respectively (based on posterior median estimates for T_0 and T_m). ZIKV infection was limited by different mechanisms at the thermal minimum and maximum. Cool temperatures limited midgut escape and dissemination resulting in a lower proportion of the mosquito population that was infectious. This could be due to temperature effects on mosquito physiology [43], immunity [18, 44-47], and viral binding to specific receptors in the midgut, secondary tissues, and salivary glands [48]. Warmer temperatures, on

392 the other hand, were very permissive for ZIKV infection, resulting in 95% and 100% infection
393 among surviving mosquitoes at 36°C and 38°C, respectively (S2 Fig). However, high mosquito
394 mortality resulted in an overall low proportion of the mosquito population becoming infected and
395 infectious. A similar nonlinear effect of cool and warm temperatures on vector competence was
396 observed with *Ae. albopictus* infected with DENV2 [42]. In contrast, Adelman et al. [19]
397 demonstrated that cooler temperatures resulted in increased susceptibility to chikungunya and
398 yellow fever virus due to impairment of the RNAi pathway. However, we only exposed adult
399 mosquitoes to varying mean temperatures, while Adelman et al. [19] looked at the carry-over
400 effects of larval rearing temperature. Both larval and adult temperature variation will likely be
401 important in the field in determining temperature effects on mosquito and pathogen traits
402 comprising arbovirus transmission.

403 We also observed an asymmetrical unimodal relationship between temperature and the
404 extrinsic incubation rate of ZIKV, with the extrinsic incubation rate optimized at 36.4°C and
405 minimized at 19.7°C and 42.5°C (based on posterior median estimates for T_0 and T_m). The effects
406 of temperature on the extrinsic incubation periods of arboviruses and other mosquito pathogens
407 have been extensively studied (dengue virus [42, 49, 50], yellow fever virus [23], West Nile
408 virus [22], chikungunya virus [51], and malaria [52, 53]). Consistent with previous studies, we
409 show that the extrinsic incubation rate of ZIKV increased with warming temperatures, with no
410 infectious mosquitoes observed at 16°C after 21 days post infection and the first infectious
411 mosquito detected at day 3 post infection at 38°C. The extrinsic incubation rate was ultimately
412 constrained at the warmer temperatures due to high mosquito mortality. This is not surprising as
413 metabolic reaction rates tend to increase exponentially to an optimal temperature, then decline
414 rapidly due to protein degradation and other processes [54-56].

The optimal temperature for mosquito fitness and viral dissemination need not be equivalent, and the impacts of temperature on mosquito mortality relative to the extrinsic incubation rate of arboviruses can have important implications for the total proportion of the mosquito population that is alive and infectious [52, 57]. In our study, mosquito lifespan was optimized at 24.2°C and minimized at 11.7°C and 37.2°C, respectively (based on posterior median estimates for T_0 and T_m). The non-linear relationship between metrics of mosquito mortality or lifespan and temperature has also been demonstrated for *Ae. aegypti* [14], *Ae. albopictus* [14, 20] and various *Anopheles* spp. [21, 53]. Despite the fact that the extrinsic incubation period was optimized at a warm temperature (36.4°C), the optimal temperature for overall ZIKV transmission (R_0) was predicted to be cooler (28.9°C) because mosquitoes have a significantly shortened lifespan above 32°C. In contrast, even though mosquitoes are predicted to have relatively longer lifespans at cooler temperatures, the time required for mosquitoes to become infectious (>21 days at 16°C and 18 days at 20°C) may be longer than most mosquitoes experience in the field. As a result, large vector populations may not be sufficient for transmitting the virus if viral replication is inhibited or if the lifespan of the mosquito is shorter than the extrinsic incubation period [58]. One surprising result was that mosquitoes exposed to ZIKV were predicted to live significantly longer at temperatures that optimized mosquito survival as compared to unexposed mosquitoes (37 vs. 87 days at 24°C; 45 vs. 54 days at 28°C). Additionally, the temperature that optimizes mosquito lifespan might also vary between ZIKV exposed mosquitoes (24°C) and their uninfected counterparts (28°C). If similar trends hold for other arbovirus systems, current modeling efforts may be underestimating virus transmission potential under certain environmental scenarios. If a survival benefit of virus exposure regularly occurs at optimal temperatures across arbovirus systems, estimating mosquito mortality in the

438 field for mosquitoes of different infection statuses and the physiological underpinnings of this
439 response are important areas for future research.

440 After incorporating the relationships between temperature and vector competence, the
441 extrinsic incubation rate, and mosquito lifespan into a mechanistic model, we demonstrated that
442 ZIKV transmission is optimized at a mean temperature of approximately 29°C, and has a thermal
443 range of 22.7°C to 34.7°C. Because this relationship is nonlinear and unimodal, we can expect as
444 temperatures move toward the thermal optimum due to future climate change or increasing
445 urbanization [59], environmental suitability for ZIKV transmission should increase, potentially
446 resulting in expansion of ZIKV further north and into longer seasons. There is evidence that this
447 is already occurring with warming at high elevations in the Ethiopian and Columbian highlands
448 leading to increased incidence of malaria [16]. In contrast, in areas that are already permissive
449 and near the thermal optimum for ZIKV transmission, future warming and urbanization may lead
450 to decreases in overall environmental suitability [24]. Accurately estimating the optimal
451 temperature for transmission is thus paramount for predicting where climate warming will
452 expand, contract, or shift transmission potential.

453 By using a mechanistic model originally parameterized for DENV, we also explored a
454 common assumption made by multiple models that DENV transmission has a similar
455 relationship with temperature as ZIKV [10, 14, 27, 35, 36]. While the temperature optimum and
456 maximum for R_0 changed very little from our previous DENV R_0 model, the temperature
457 minimum for transmission increased by nearly five degrees in the updated model (S3 Fig). This
458 is mainly due to a higher thermal minimum for both vector competence and the extrinsic
459 incubation rate for ZIKV as compared to DENV (S3 Fig [14]). The reason for this difference
460 could be because DENV has a different thermal niche than ZIKV, or alternatively, our field

461 derived *Ae. aegypti* could have a different thermal niche then the *Ae. aegypti* populations
462 assessed in Mordecai et al. [14]. There is evidence in a range of invertebrate-pathogen systems
463 (spanning fruit flies, *Daphnia*, pea aphids, and mosquitoes [41, 60-63]) that the effects of
464 environmental variation on disease transmission are often modified by the genetic background of
465 the host and infecting pathogen [64-66]. Thus, more work is required to validate the
466 generalizability of these models, and the mechanisms underpinning temperature effects on
467 mosquito–virus interactions and temperature–transmission relationships. However, if the higher
468 thermal minimum for ZIKV is universal, it would suggest that mechanistic and statistical
469 modeling efforts to map the global environmental suitability for ZIKV transmission currently
470 and with future climate change are potentially over-predicting its possible range [10, 14, 36].
471 Finally, although we estimated the effects of mean constant temperatures on mosquito
472 and pathogen traits that integrate to shape ZIKV transmission, mosquitoes and their pathogens
473 live in a variable world where temperatures fluctuate daily and seasonally. The values for these
474 traits and transmission potential have been shown to differ in fluctuating environments relative to
475 constant temperature environments [24, 67-71]. While characterizing trait responses to mean
476 constant temperatures and incorporating these relationships into models of disease transmission
477 is tractable, more effort is needed in validating computational approaches to infer transmission in
478 a fluctuating environment (i.e. rate summation [14, 72]).
479 Accurately and precisely predicting arbovirus transmission will likely be influenced by
480 variation in other sources of abiotic (e.g. relative humidity, rainfall), biotic (e.g. availability and
481 quality of oviposition and resting habitats), and socioeconomic factors that influence human
482 exposure to biting mosquitoes. This is a fundamental first step for empirically defining and
483 validating current models on the environmental suitability for ZIKV transmission, in which

484 temperature will be a strong driver. Understanding the unimodal effect of temperature on
485 emerging arboviruses, like ZIKV, will contribute to accurate predictions about how climate
486 change and shifts in mosquito relevant microclimate with land use change could impact the risk
487 of arbovirus emergence and transmission. R_0 models have been used as a tool to guide vector-
488 borne disease interventions, and is a comprehensive metric of pathogen fitness. We anticipate as
489 with other vector-borne diseases [14, 21, 30] that environmental suitability for ZIKV
490 transmission could expand northwards with future warming, but will be more constrained at low
491 temperatures than DENV. We also predict areas that are already at or near the thermal optimum
492 of 29°C to experience a decrease in environmental suitability for ZIKV transmission [21, 24].
493 Further, land use change that modifies the microclimates mosquitoes experience could have
494 immediate impacts on ZIKV transmission [59], which might explain the explosive spread of
495 ZIKV in urban centers throughout the Americas.
496

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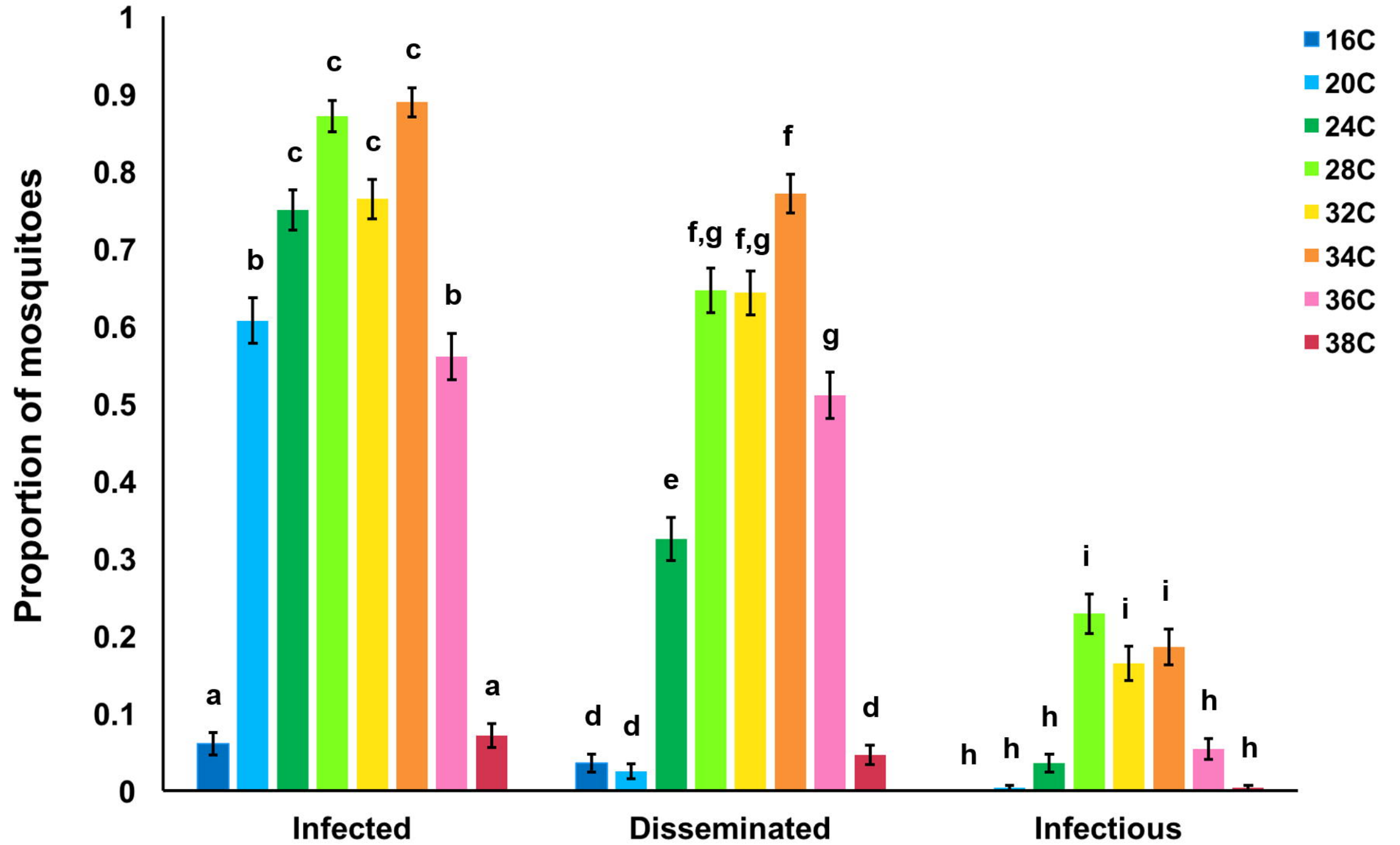
S1 Fig. Experimental design

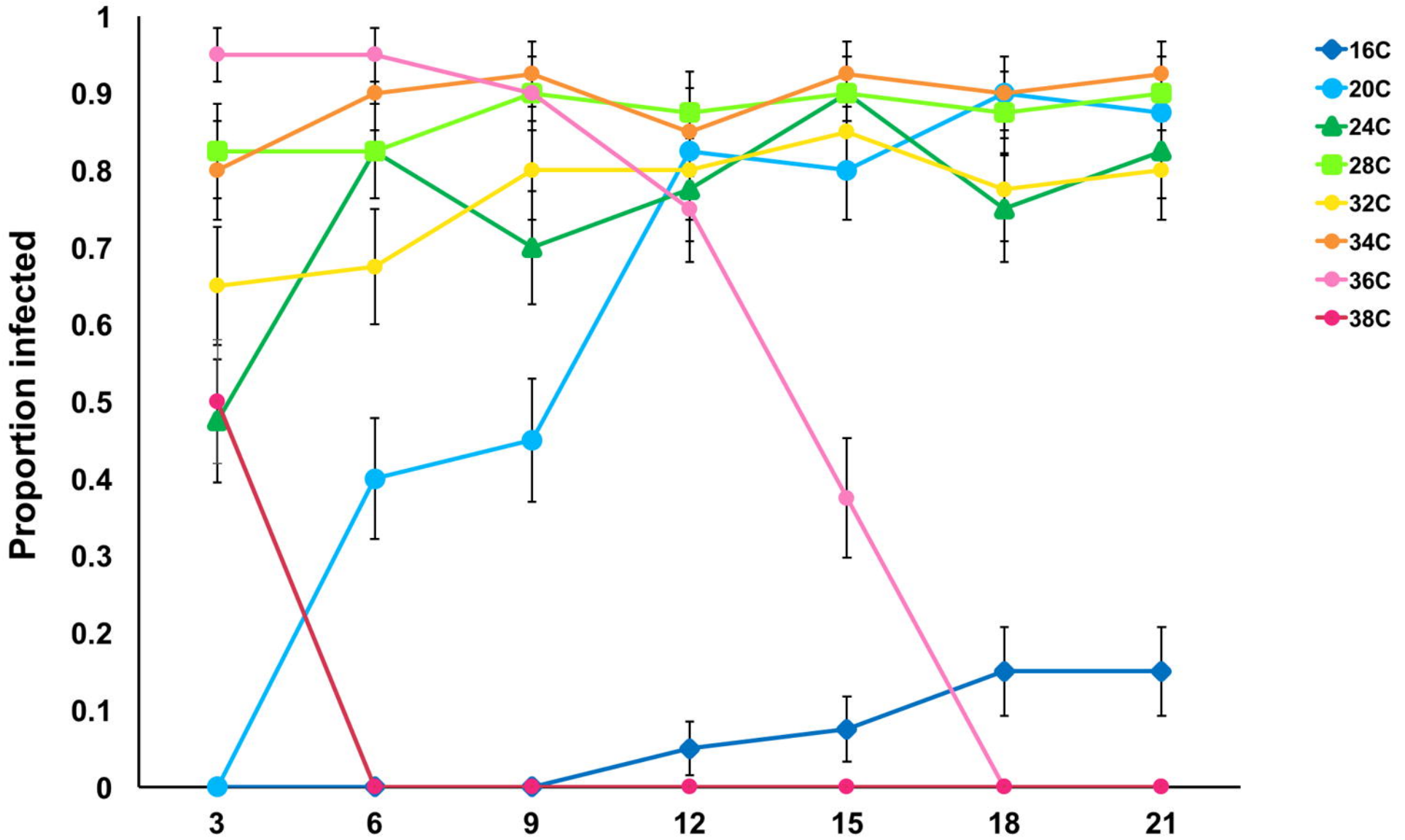
In each biological replicate, eight thousand female *Aedes aegypti* mosquitoes were offered either an infectious blood meal containing ZIKV at the final concentration of 10^6 PFU/mL or an uninfected, control blood meal. Two thousand ZIKV-exposed and two thousand control engorged mosquitoes were randomly distributed into mesh-covered paper cups (250 per cup) and put at one of eight temperature treatments 16°C, 20°C, 24°C, 28°C, 32°C, 34°C, 36°C, and 38°C. Every three days, up to day twenty-one, twenty ZIKV exposed mosquitoes per treatment group were force-salivated. After salivation, mosquito saliva, heads, legs, and bodies were collected into separate tubes. Each tissue was tested for the presence/absence of the ZIKV using plaque assays on Vero cells. Two full biological replicates were performed.

S2 Fig. Infection, dissemination and infectiousness among alive mosquitoes. The effect of eight different constant temperatures (16°C, 20°C, 24°C, 28°C, 32°C, 34°C, 36°C, 38°C) on the proportion of mosquitoes infected (ZIKV positive bodies compared to total number of processed mosquitoes), with disseminated infections (ZIKV positive heads compared to total number of processed mosquitoes), and infectious (ZIKV positive saliva compared to total number of processed mosquitoes).

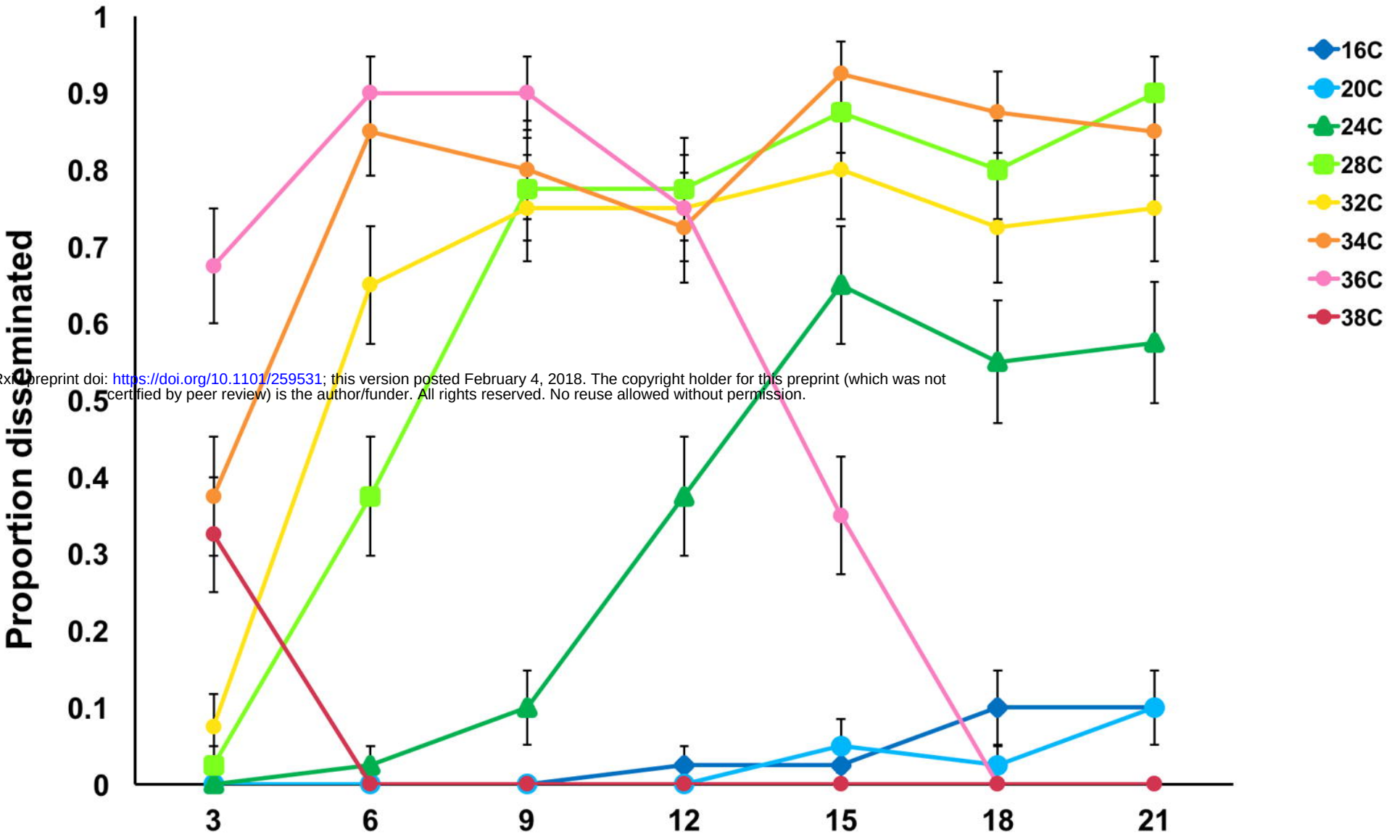
S3 Fig. Comparison of $R_0(T)$ for Zika virus with the previous estimate of $R_0(T)$ for dengue virus. Comparison of the new estimate of $R_0(T)$ for Zika virus (ZIKV; dark blue) with the previous estimate of $R_0(T)$ for dengue virus (DENV; light blue) (Mordecai et al. 2017). Top shows $R_0(T)$ means (solid lines) and 95% credible intervals (dashed lines) while bottom panels show the trait thermal response means for vector competence (bottom left), extrinsic incubation rate (bottom center), and lifespan (bottom right) for the new experimental data presented here (dark blue) and the previously published data (light blue) (Mordecai et al. 2017).


Proportion of mosquitoes
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
16C
20C
24C
28C
32C
34C
36C
38C
a
b
c
c
c
c
b
a
d
d
e
f,g
f,g
f
g
d
h
h
h
i
i
i
h
h
Infected
Disseminated
Infectious

A



B



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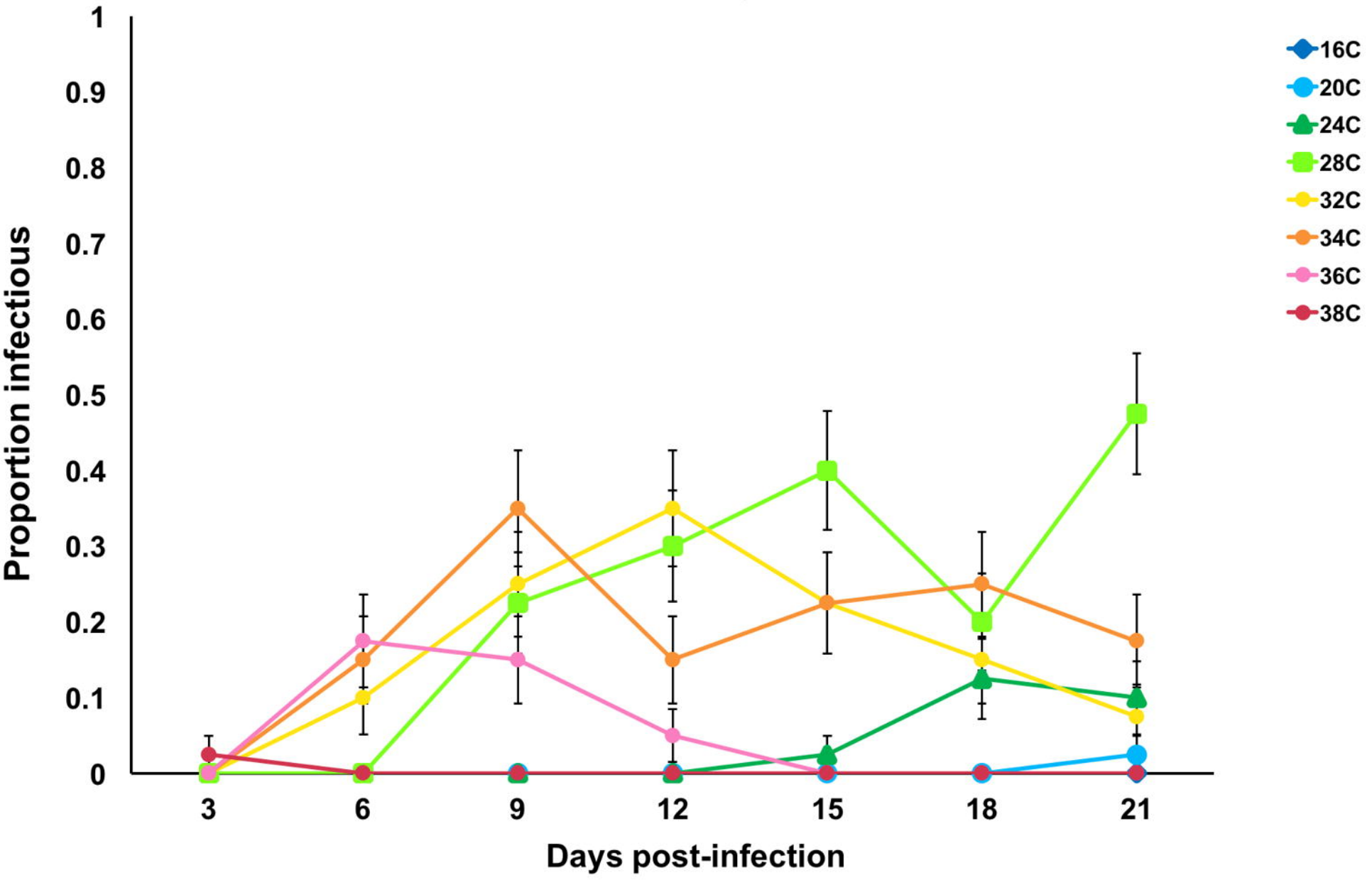
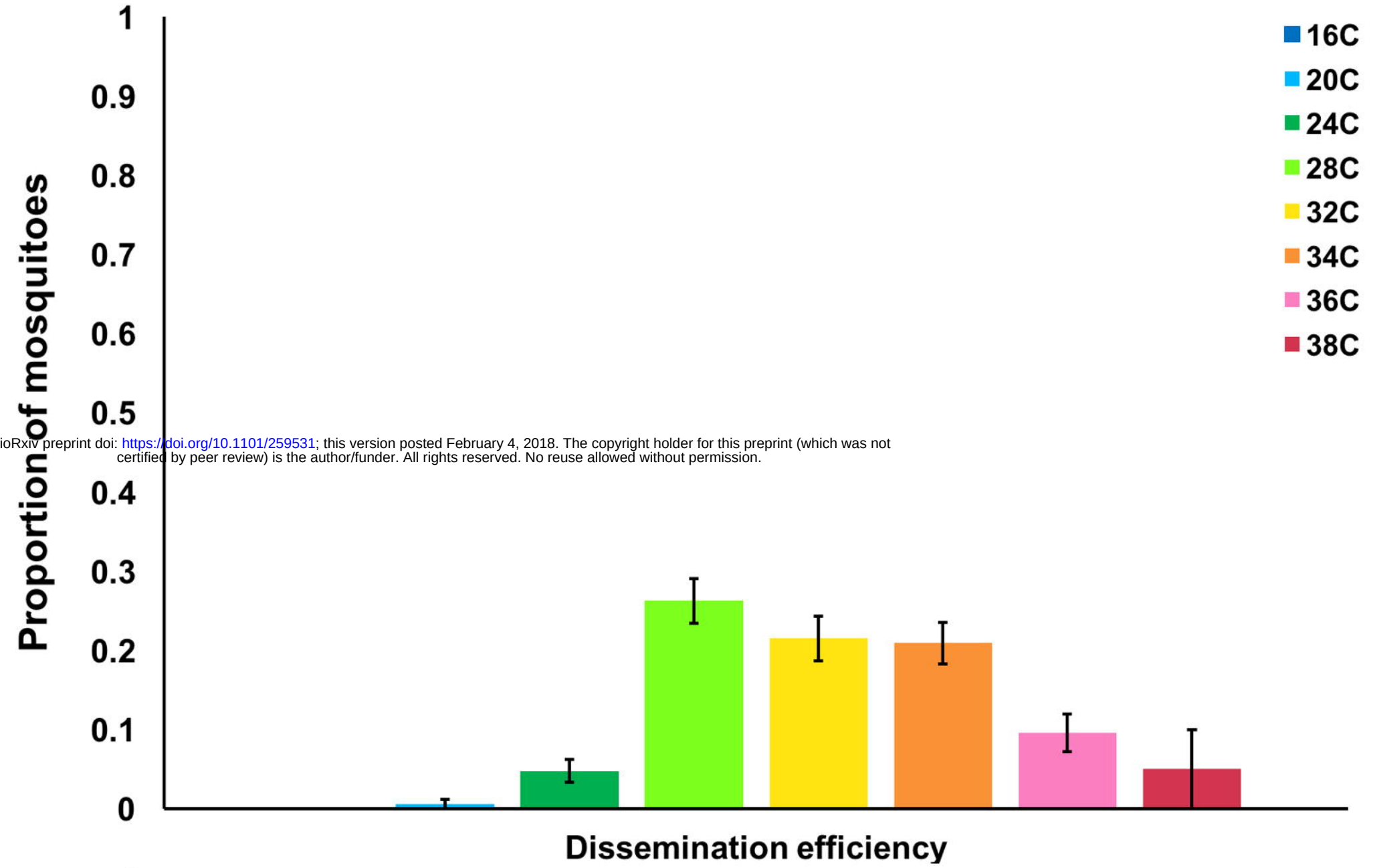


A



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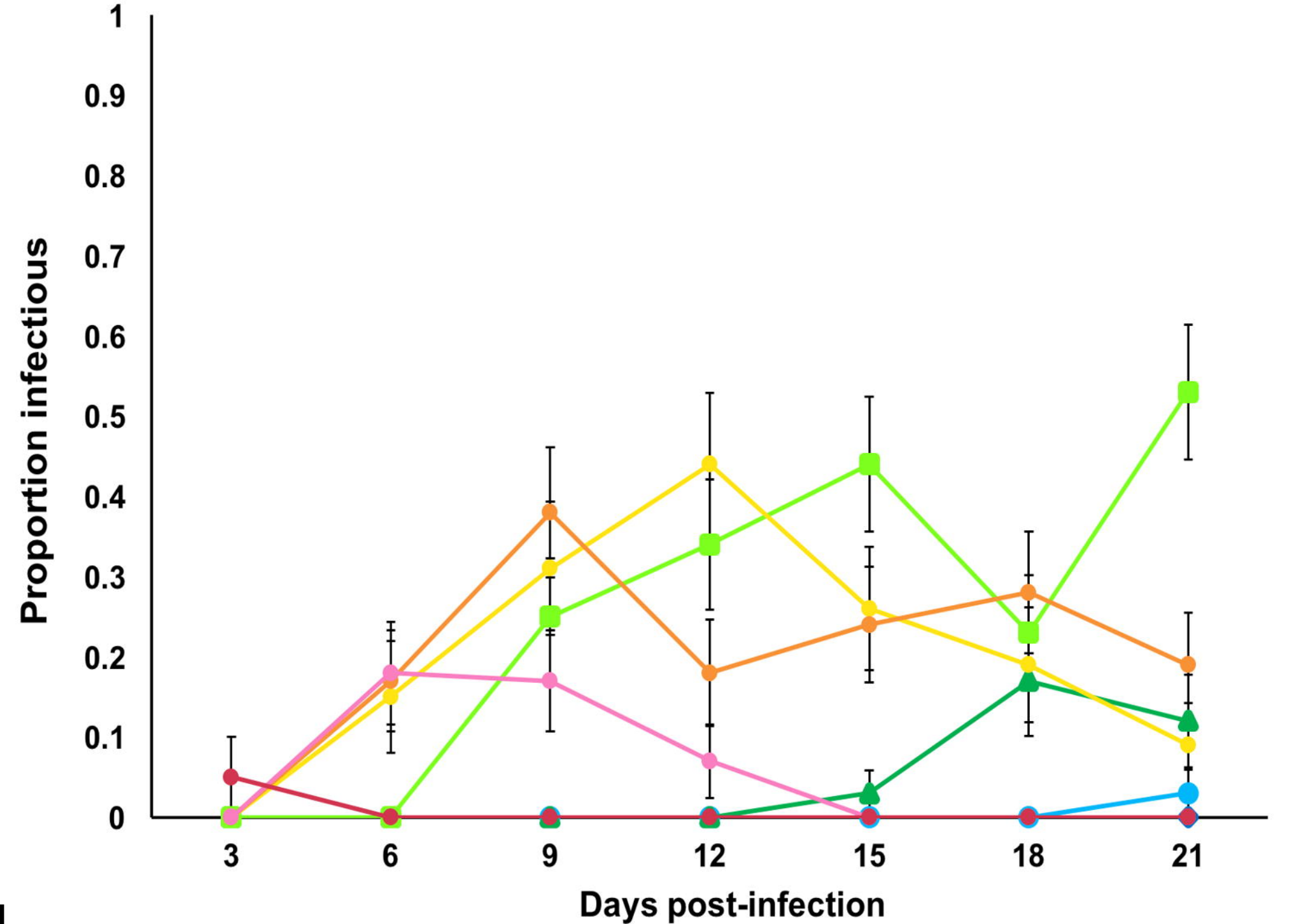
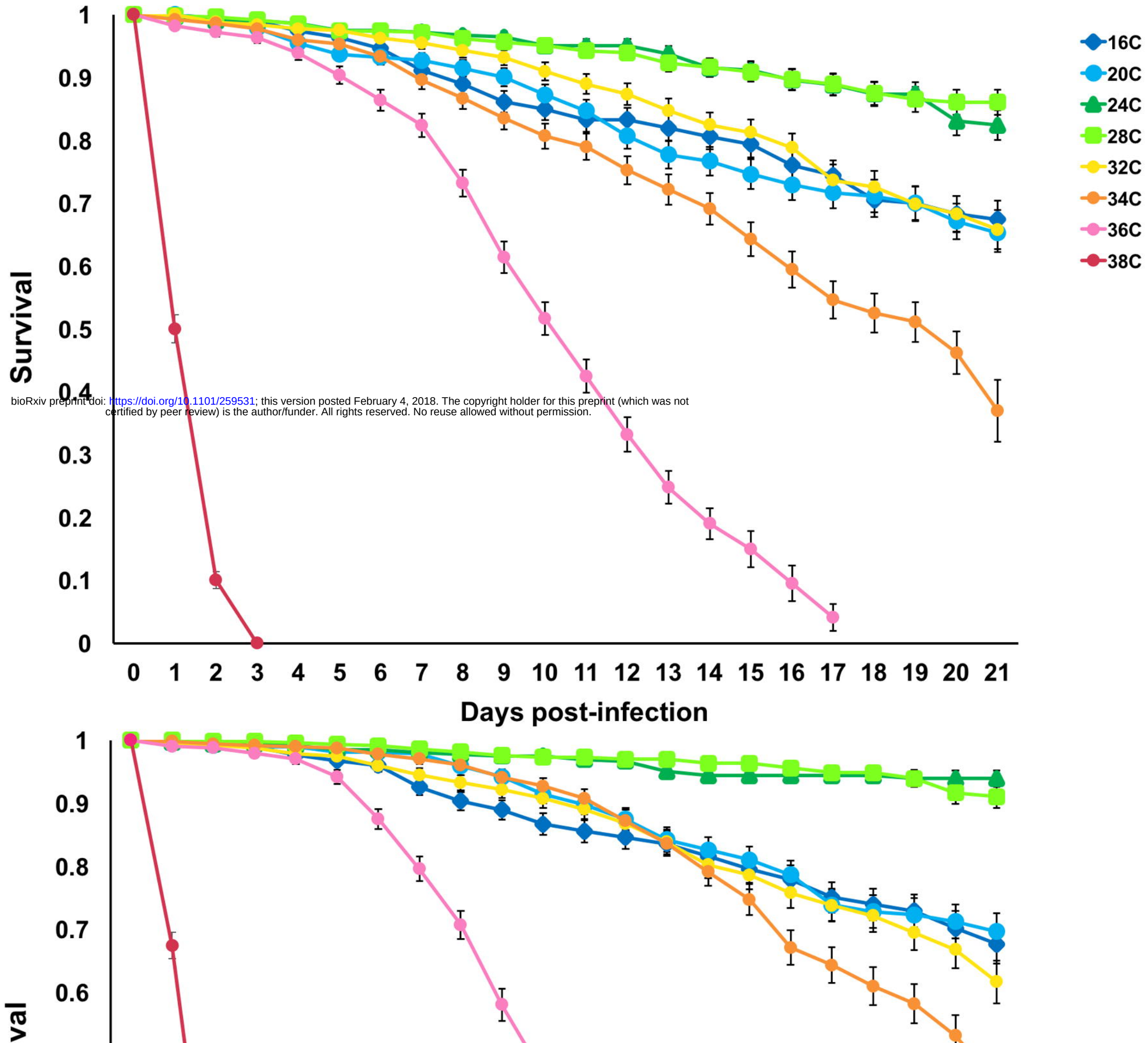


A



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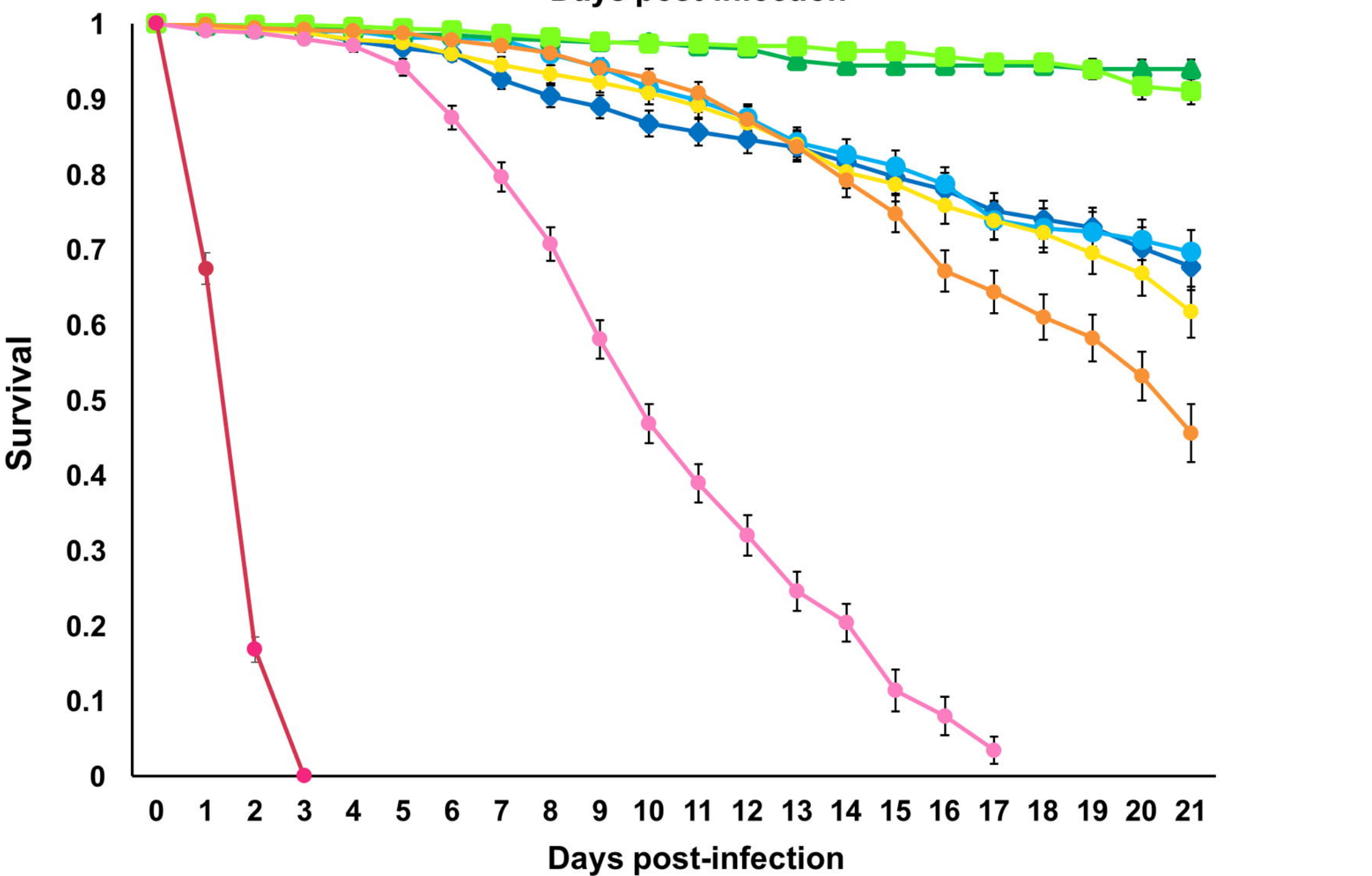
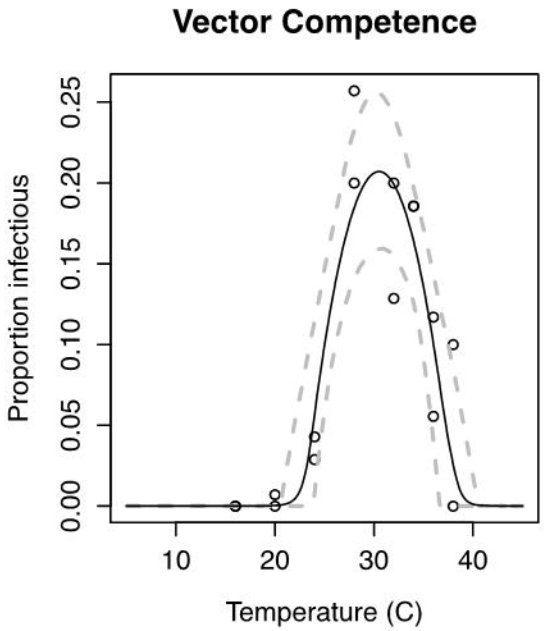
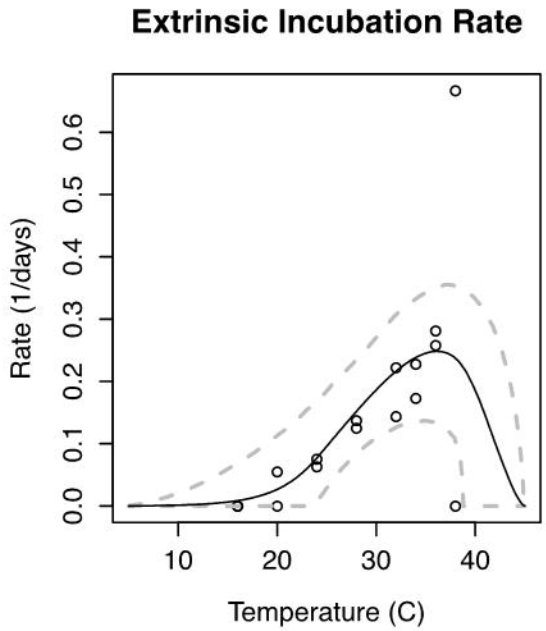
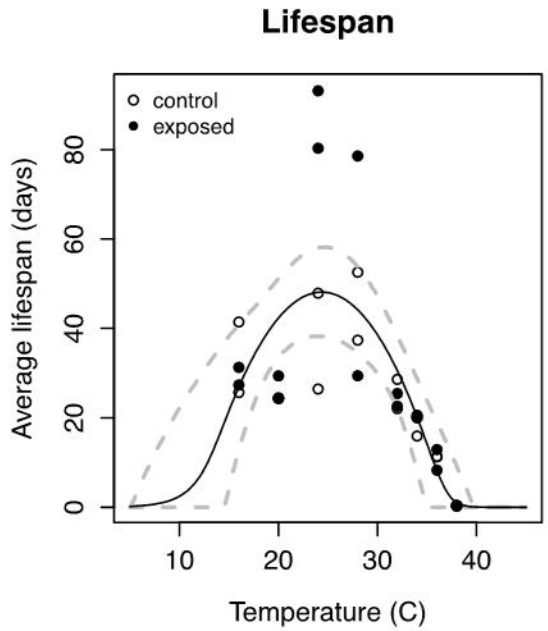
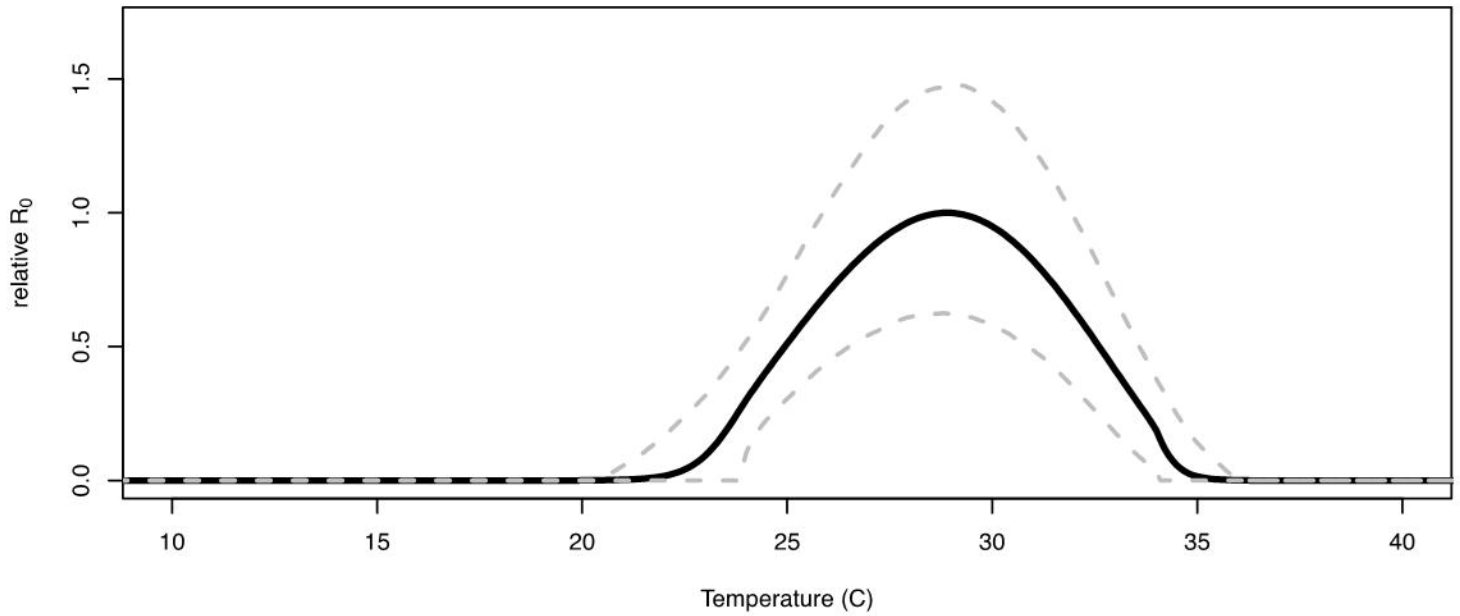



Vector Competence
Proportion infectious
0.00
0.05
0.10
0.15
0.20
0.25
10
20
30
40
Temperature (C)

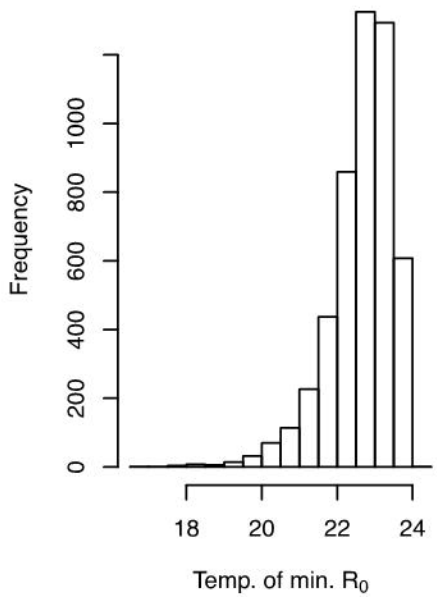

Extrinsic Incubation Rate
Rate (1/days)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
10
20
30
40
Temperature (C)

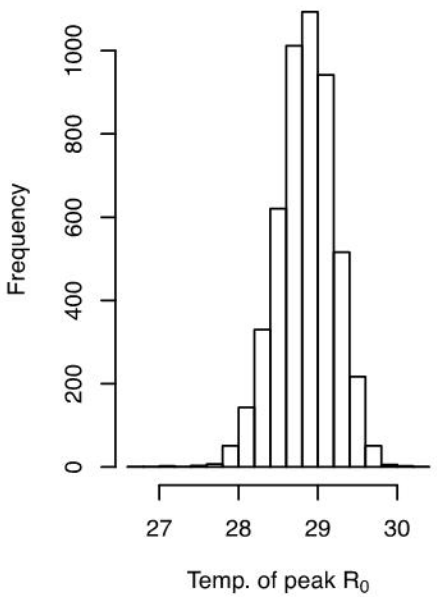

Lifespan
control
exposed
Average lifespan (days)
0
20
40
60
80
10
20
30
40
Temperature (C)

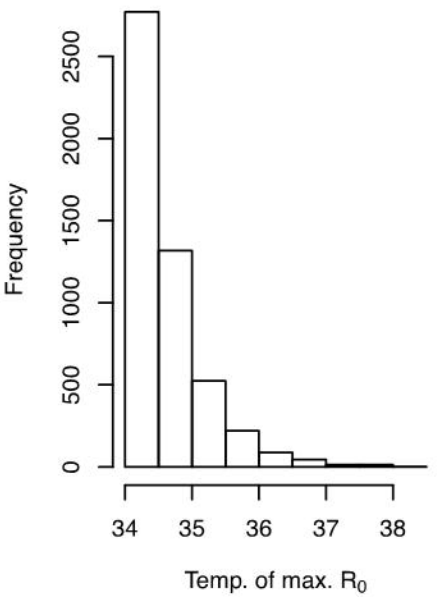
A


relative R_0
0.0
0.5
1.0
1.5
10
15
20
25
30
35
40
Temperature (C)

B


Frequency
0
200
400
600
800
1000
18
20
22
24
Temp. of min. R_0


Frequency
0
200
400
600
800
1000
27
28
29
30
Temp. of peak R_0


Frequency
0
500
1000
1500
2000
2500
34
35
36
37
38
Temp. of max. R_0