Temperature explains broad patterns of Ross River virus transmission across Australia

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ABSTRACT

Temperature impacts the physiology of ectotherms, including vectors that transmit disease. While thermal biology predicts nonlinear effects of temperature on vector and pathogen traits that drive disease transmission, the empirical relationship between temperature and transmission remains unknown for most vector-borne pathogens. We built a mechanistic model to estimate the thermal response of Ross River virus, an important mosquito-borne pathogen of humans in Australia, the Pacific Islands, and potentially emerging worldwide. Transmission peaks at moderate temperatures (26.4°C) and declines to zero at low (17.0°C) and high (31.5°C) temperatures. The model predicted broad patterns of disease across Australia. First, transmission is year-round endemic in the tropics and sub-tropics but seasonal in temperate zones. Second, nationwide human cases peak seasonally as predicted from population-weighted seasonal temperatures. These results illustrate the importance of nonlinear, mechanistic models for inferring the role of temperature in disease dynamics and predicting responses to climate change.
INTRODUCTION

Temperature impacts mosquito-borne disease transmission via effects on the physiology of mosquitoes and pathogens (Shocket et al. n.d.). Transmission requires that mosquitoes be abundant, bite a host and ingest an infectious bloodmeal, survive long enough for pathogen development and within-host migration (the extrinsic incubation period), and bite additional hosts (Shocket et al. n.d.). All of these processes depend on temperature. Support for temperature effects on mosquito-borne disease arises from mechanistic models based on thermal biology (Mordecai et al. 2013, 2017; Liu-Helmersson et al. 2014; Wesolowski et al. 2015; Paull et al. 2017) and statistical models that analyze variation in climate and disease over time and space (Werner et al. 2012; Stewart Ibarra & Lowe 2013; Siraj et al. 2015; Paull et al. 2017). However, important knowledge gaps remain. First, variation in how transmission responds to temperature across mosquito-borne diseases, and via what mechanisms, remains uncertain. Accordingly, the types of data needed to characterize thermal responses of additional or emerging diseases are unclear. Second, the impacts of temperature on transmission can appear idiosyncratic across locations and studies (Hu et al. 2004; Gatton et al. 2005; Jacups et al. 2008a; Bi et al. 2009; Werner et al. 2012; Koolhof et al. 2017), and inferring causality from field observations and statistical approaches alone remains challenging. Thermal biology may provide an explanation for this variation or a causal link. Filling these gaps is necessary to predict geographic, seasonal, and interannual variation in transmission of mosquito-borne pathogens, especially as the climate changes. Here, we address these gaps by building a model for temperature-dependent transmission of Ross River virus.

Ross River virus (RRV) is a mosquito-transmitted alphavirus that causes the most common mosquito-borne disease in Australia (1,500–9,500 human cases per year; Koolhof &
Carver 2017). RRV is also endemic in Pacific Island nations, following a 1979-80 epidemic that infected over 500,000 people (Klapsing et al. 2005; Aubry et al. 2015; Lau et al. 2017). RRV infection causes acute fever, rash, and joint pain, which can become chronic and cause disability (Harley et al. 2001). In some cases temperature predicts RRV cases (Gatton et al. 2005; Bi et al. 2009; Werner et al. 2012), while other cases it does not (Hu et al. 2004; Gatton et al. 2005).

Understanding RRV transmission ecology is critical because the virus is a likely candidate for emergence worldwide (Flies et al. 2018). A mechanistic model for temperature-dependent RRV transmission could help explain these disparate results and predict potential expansion.

Mechanistic models synthesize how environmental factors like temperature influence the host and parasite traits that drive transmission. For ectotherms, these traits depend nonlinearly on environmental temperature. Specifically, trait thermal responses are usually unimodal: they peak at an intermediate temperature optimum and decline towards zero at lower and upper thermal limits, all of which can vary across traits (Angilletta 2009; Dell et al. 2011; Mordecai et al. 2013, 2017). Mechanistic models combine the multiple, nonlinear thermal responses that shape transmission (Rogers & Randolph 2006; Mordecai et al. 2013). One commonly-used measure of disease spread is $R_0$, the basic reproductive number (expected number of secondary cases from a single case in a fully susceptible population). For mosquito-borne disease, $R_0$ is a nonlinear function of mosquito density, biting rate, vector competence (infectiousness given pathogen exposure), and adult survival; pathogen extrinsic incubation period; and human recovery rate (Dietz 1993). To understand the overall impact of temperature on transmission, we can incorporate empirically-estimated trait thermal responses into an $R_0$ model. Incorporating the full suite of nonlinear trait responses often produces predictions that are drastically different than models that assume linear or monotonic thermal responses or omit important temperature-
dependent processes (Mordecai et al. 2013, 2017). Previous mechanistic models have predicted different optimal temperatures for disease spread across pathogens and vector species: 25 °C for *falciparum* malaria and West Nile virus (Mordecai et al. 2013; Paull et al. 2017), and 29°C and 26 °C for dengue, chikungunya, and Zika viruses in *Ae. aegypti* and in *Ae. albopictus*, respectively (Liu-Helmersson et al. 2014; Wesolowski et al. 2015; Mordecai et al. 2017).

In this paper, we build the first mechanistic model for temperature-dependent transmission of RRV and ask whether temperature explains seasonal and geographic patterns of disease spread. We use data from laboratory experiments with the two most important and well-studied vector species (*Culex annulirostris* and *Aedes vigilax*) to parameterize the model with unimodal thermal responses. We then use sensitivity and uncertainty analyses to determine which traits drive the relationship between temperature and transmission potential and identify key data gaps. Finally, we illustrate how temperature currently shapes patterns of disease across Australia. The model correctly predicts that RRV disease should be year-round endemic in tropical, northern Australia and seasonally epidemic in temperate, southern Australia. It also accurately predicts the seasonality of human cases nationally. Thus, the temperature-dependent model for RRV transmission provides a mechanistic link between geographic and seasonal changes in temperature and broad-scale patterns of disease.

**MATERIAL AND METHODS**

*Natural History of RRV*

The natural history of RRV is complex: transmission occurs across a range of climates (tropical, subtropical, and temperate) and habitats (urban and rural, coastal and inland) and via many vector and vertebrate reservoir species (Claflin & Webb 2015; Stephenson et al. 2018).
The virus has been isolated from over 40 mosquito species in nature, and 10 species transmit it in laboratory studies (Harley et al. 2001; Russell 2002). However, four key species are responsible for most transmission to humans (Culex annulirostris, Aedes [Ochlerotatus] vigilax, Ae. [O.] notoscriptus, and Ae. [O.] camptorhynchus), with two additional species also implicated in human outbreaks (Ae. [Stegomyia] polynesiensis and Ae. [O.] normanensis).

The vectors differ in climate and habitat niches, leading to geographic variation in associations with outbreaks. We assembled and mapped records of RRV outbreaks in humans attributed to specific species (Fig. 1, Table S1 in Appendix S1 in Supporting Information; Rosen et al. 1981; Campbell et al. 1989; Russell et al. 1991; Lindsay et al. 1992, 1993a, b, 1996, 2007; McManus et al. 1992; Merianos et al. 1992; Whelan et al. 1992, 1995, 1997; McDonnell et al. 1994; Russell 1994, 2002; Dhileepan 1996; Ritchie et al. 1997; Brokenshire et al. 2000; Ryan et al. 2000; Harley et al. 2000, 2001; Frances et al. 2004; Kelly-Hope et al. 2004b; Biggs & Mottram 2008; Schmaedick et al. 2008; Jacups et al. 2008b; Lau et al. 2017). Ae. vigilax and Ae. notoscriptus contribute to transmission more in tropical and subtropical zones, Ae. camptorhynchus in temperate zones, and Cx. annulirostris throughout all climatic zones.

Freshwater-breeding Cx. annulirostris contributes to transmission in both inland and coastal areas, while saltmarsh mosquitoes Ae. vigilax and Ae. camptorhynchus contribute in coastal areas (Russell 2002) and inland areas affected by salinization from agriculture (Biggs & Mottram 2008; Carver et al. 2009). Peri-domestic, container-breeding Ae. notoscriptus contributes in urban areas (Russell 2002; Faull & Williams 2016). The vectors also differ in seasonality: Ae. camptorhynchus populations peak earlier and in cooler temperatures than Ae. vigilax, leading to seasonal succession where they overlap (Lindsay et al. 1992; Russell 1998). This latitudinal and
temporal variation suggests that vector species may have different thermal optima and/or niche breadths. If so, temperature may impact disease transmission differently for each species.

**Temperature-Dependent \( R_0 \) Models**

\( R_0 \) depends on a suite of vector, pathogen, and human traits, including vector density. Vector density in turn depends on reproduction, development rate, and survival at egg, larval, pupal, and adult stages, all of which are sensitive to temperature. However, mosquito density also depends on aquatic breeding habitat and other factors (Barton *et al.* 2004; Kokkinn *et al.* 2009; Jacups *et al.* 2015). For this reason, we developed two models of \( R_0 \): (eq. 1) the ‘Constant \( M \) Model’ assumes that mosquito density \( (M) \) does not depend on temperature (equation from Dietz 1993); (eq. 2) the ‘Temperature-Dependent \( M \) Model’ assumes that temperature drives mosquito density and includes vector life history trait thermal responses (Parham & Michael 2010; Mordecai *et al.* 2013, 2017; Johnson *et al.* 2015). The relative influence of temperature versus habitat availability and other drivers varies across settings, and these models capture two extremes. Because habitat availability is context-dependent and species-specific, and because we focus on temperature, we do not model vector habitat here.

**Constant \( M \) Model:**

\[
R_0(T) = \left( \frac{a(T)bc(T)e^{-\frac{\mu(T)}{PDR(T)}}}{N\mu(T)} \right)^{1/2}
\]

(eq. 1)

**Temperature-dependent \( M \) Model:**

\[
R_0(T) = \left( \frac{a(T)^2bc(T)e^{-\frac{\mu(T)}{PDR(T)}}p_{EA}MDR(T)}{N\mu(T)^3} \right)^{1/2}
\]

(eq. 2)

In both equations, \( (T) \) indicates a parameter depends on temperature, \( a \) is mosquito biting rate, \( bc \) is vector competence (proportion of mosquitoes that become infectious given exposure), \( \mu \) is adult mosquito mortality rate (adult lifespan, \( l_f = 1/\mu \)), \( PDR \) is parasite development rate...
(PDR = 1/EIP, the extrinsic incubation period), \( N \) is human density, and \( r \) is the rate at which humans recover from infection and become immune (all rates are measured in days\(^{-1} \)). The latter two terms do not depend on temperature. In the temperature-dependent \( M \) model, mosquito density \( (M) \) depends on fecundity \( (EFD, \) eggs per female per day), proportion surviving from egg-to-adult \( (p_{EA}) \), and mosquito development rate \( (MDR) \), divided by the square of adult mortality rate \( (\mu; \) Parham & Michael 2010). We calculated \( p_{EA} \) as the product of the proportion of egg rafts that hatch \( (pRH) \), the number of larvae per raft that hatch \( (nLR, \) scaled by the maximum at any temperature to calculate proportional egg survival within-rafts), and the proportion of larvae surviving to adulthood \( (p_{LA}) \). We initially compare results for both models, then focus on the temperature-dependent \( M \) model.

Since \( R_0 \) also depends on other factors, we scaled the model output between zero and one (‘relative \( R_0 \)’). Relative \( R_0 \) describes the temperature suitability for transmission, which combines with other factors like breeding habitat availability, vector control, humidity, human and reservoir host density, host immune status, and mosquito exposure to determine disease incidence. In this approach, only the relative response of each trait to temperature matters, which is desirable since traits can differ substantially based on other factors and in the laboratory versus the field (particularly mosquito survival: MacDonald 1952; Clements & Paterson 1981). With relative \( R_0 \) we cannot use the typical threshold for sustained disease transmission \( (R_0 > 1) \).

However, relative \( R_0 \) preserves the temperature-dependence of \( R_0 \), including key temperature values where transmission is possible \( (R_0 > 0; \) a conservative threshold where transmission is not excluded by temperature) and where \( R_0 \) is maximized.

We fit functions describing the thermal response of each trait in the \( R_0 \) models using previously published data (Table S2; (McDonald et al. 1980; Mottram et al. 1986; Russell 1986;
We exhaustively searched the literature for laboratory studies with trait measurements at three or more constant temperatures for the major vector species in Australia (Cx. annulirostris, Ae. vigilax, Ae. camptorhynchus, Ae. notoscriptus, and Ae. normanensis). Data were sparse: no species had data for all traits needed to parameterize the \( R_0 \) models (Fig. 1). Therefore, we combined mosquito life history traits from Cx. annulirostris (\( a, \mu, EFD, pRH, nLR, pLA, MDR \)) and pathogen infection traits measured in Ae. vigilax (\( bc, PDR \)) to build composite \( R_0 \) models. We also fit traits for other mosquito and virus species: MDR and \( pEA \) from Ae. camptorhynchus and Ae. notoscriptus, and PDR and \( bc \) from Murray Valley encephalitis virus in Cx. annulirostris (Appendix S1). We use sensitivity analyses to evaluate the potential impact of this vector species mismatch.

We fit unimodal thermal responses for traits using Bayesian inference with the ‘r2jags’ package (Plummer 2003; Su & Yajima 2009) in R (R Core Team 2017). Traits with asymmetrical thermal responses were fit as Brière functions of temperature: 

\[
qT(T - T_0)(T_{\text{max}} - T)^{1/2}
\]

(Brière et al. 1999). Traits with symmetrical thermal responses were fit as quadratic functions of temperature: 

\[
-q(T - T_0)(T - T_{\text{max}}).
\]

In both functions of temperature \( T \), \( T_0 \) and \( T_{\text{m}} \) are the critical thermal minimum and maximum, respectively, and \( q \) is a rate parameter. For priors we used gamma distributions where the hyperparameters were derived from thermal responses fit to data from other mosquito species with similar ecology (\( Ae. aegypti, Ae. albopictus, Ae. krombeini, \) and \( Ae. triseriatus, \) and \( Anopheles pseudopunctipennis; \) Table S4), allowing us to more accurately represent their fit and uncertainty. Our data did not include declining trait values at high temperatures for biting rate (\( a \)) and parasite development rate (\( PDR \)). Nonetheless, data from other mosquito species (Mordecai et al. 2013, 2017) and principles of thermal biology (Angilletta 2009; Dell et al. 2011) imply that these traits must decline at very high temperatures.
Thus, for those traits we included an artificial data point where the trait value approached zero at a very high temperature (40°C), allowing us to fit the Brière function. We used strongly informative priors to limit the effect of these traits on the upper thermal limit of \( R_0 \) (by constraining them to decline near 40°C). For comparison, we also fit all thermal responses with uniform priors; these results illustrate how our choices of priors impacted the results (Appendix S1).

Sensitivity and uncertainty analyses

We conducted sensitivity and uncertainty analyses of the temperature-dependent \( M \) model (eq. 2) to understand how trait thermal responses shape the thermal response of \( R_0 \). We examined the sensitivity of \( R_0 \) in two ways. First, we evaluated the impact of each trait by setting it constant while allowing all other traits to vary with temperature. Second, we calculated the partial derivative of \( R_0 \) with respect to each trait across temperature (i.e., \( \partial R_0 / \partial X \cdot \partial X / \partial T \) for trait \( X \) and temperature \( T \); Appendix S1). We also calculated the effect of each trait on uncertainty in \( R_0(T) \)—the proportion of total uncertainty due to each trait across temperature—to understand what data would most improve the model. To do so, we first propagated posterior samples from all trait thermal response distributions through to \( R_0(T) \) and calculated the width of the 95% highest posterior density interval (HPD interval; a type of credible interval) of this distribution at each temperature: the ‘full \( R_0(T) \) uncertainty’. Next, we sampled each trait from its posterior distribution while setting all other trait thermal responses to their posterior medians, and calculated the posterior distribution of \( R_0(T) \) and the width of its 95% HPD interval across temperature: the ‘single-trait \( R_0(T) \) uncertainty’. Finally, we divided each single-trait \( R_0(T) \) uncertainty by the full \( R_0(T) \) uncertainty.
Field Observations: Seasonality of Temperature-Dependent $R_0$ Across Australia

We used the temperature-dependent $M$ model (eq. 2) to predict the seasonality of $R_0(T)$ across Australia. We took monthly mean temperatures from WorldClim for seven cities spanning a latitudinal and temperature gradient (from tropical North to temperate South: Darwin, Cairns, Brisbane, Perth, Sydney, Melbourne, and Hobart) and calculated the posterior median $R_0(T)$ for each month at each location. We also compared the seasonality of a population-weighted $R_0(T)$ with the seasonality of nationally aggregated RRV cases. We used 2016 census estimates for the fifteen most populous urban areas, which together contain 76.6% of Australia’s population (Australian Bureau of Statistics 2017). We calculated relative $R_0(T)$ for each location and weighted by population to estimate a country-wide average. We compared this country-scale estimate of relative $R_0(T)$ with data on mean monthly human cases of RRV nationwide from 1992-2013 obtained from the National Notifiable Diseases Surveillance System.

We expected a substantial time lag between temperature suitability and reported human cases as mosquito populations increase, bite humans and reservoir hosts, acquire RRV, become infectious, and bite subsequent hosts, which then undergo an incubation period before (potentially) becoming symptomatic, seeking treatment, and reporting cases. Empirical work on dengue vectors in Ecuador identified a six-week time lag between temperature and mosquito oviposition (Stewart Ibarra et al. 2013). Subsequent mosquito development and virus incubation periods in mosquitoes and humans likely add another two- to four-week lag before symptomatic cases appear, resulting in an 8-10 week lag between temperature and observed cases (Hu et al. 2006; Jacups et al. 2008b; Mordecai et al. 2017). With monthly case data, we hypothesize a two-month time lag between $R_0(T)$ and RRV disease cases.
Mapping Temperature-Dependent $R_0$ Across Australia

To illustrate temperature suitability for RRV transmission across Australia, we mapped the number of months for which relative $R_0(T) > 0$ and $> 0.5$ for the posterior median, 2.5%, and 97.5% credibility bounds (Fig. S7) based on the temperature-dependent $M$ model (eq. 2). We calculated relative $R_0(T)$ at 0.2°C increments and projected it onto the landscape for monthly mean temperatures from WorldClim data at a 5-minute resolution (approximately 10km$^2$ at the equator). Climate data layers were extracted for the geographic area, defined using the Global Administrative Boundaries Databases (GADM 2012). We performed all map calculations and manipulations in R using packages ‘raster’ (Hijmans 2016), ‘maptools’ (Bivand & Lewin-Koh 2017), and ‘Rgdal’ (Bivand et al. 2017), and rendered GeoTiffs in ArcGIS 10.3.1 (ESRI 2015).

RESULTS

All traits varied with temperature (Fig. 2), with thermal optima ranging from 23.4°C for adult lifespan ($l_f$) to 33.0°C for parasite development rate ($PDR$). Unimodal thermal responses were supported for most traits, though declines at high temperatures were not directly observed for biting rate ($a$) and parasite development rate ($PDR$). Instead, data from other mosquito species and ectotherm physiology theory imply that these traits must decline at very high temperatures (~40°C).

The optimal temperature for transmission (relative $R_0$) peaked near 26.4°C regardless of whether mosquito density depended on temperature, because optimal transmission aligned with optimal mosquito density (Fig. 3; temperature-dependent $M$ model: 26.4°C, constant $M$ model: 26.6°C, mosquito density [$M$]: 26.2°C). By contrast, the range of temperatures that were suitable...
for transmission is much narrower when mosquito density depends on temperature because

temperature-dependent mosquito density constrains transmission at the thermal limits (Fig. 3; temperature-dependent $M$ model: $17.0 - 31.5^\circ C$, constant $M$ model: $12.9 - 33.7^\circ C$). Fecundity ($EFD$) and adult lifespan ($lf$) constrain $R_0$ at the upper thermal limit, while fecundity ($EFD$), larval survival ($pLA$), egg survival (raft viability [$pRH$] and survival within rafts [$nLR$]), and adult lifespan ($lf$) constrain $R_0$ at the lower thermal limit (Fig. S7). All of these traits (except adult lifespan) only occur in, and adult lifespan is quantitatively more important in, the temperature-dependent $M$ model. Correspondingly, uncertainty in these traits generated the most uncertainty in $R_0$ at the respective thermal limits (Fig. S7C). The intermediate optimal temperature for $R_0$ was most sensitive to adult mosquito lifespan (Fig. S7). Near the optimum, most uncertainty in $R_0$ was due to uncertainty in adult lifespan, egg raft viability, and fecundity. Substituting larval traits from alternative vectors or pathogen infection traits for Murray Valley Encephalitis virus did not substantially alter the $R_0$ thermal response, since $Cx.\ annulirostris$ life history traits strongly constrained transmission (Fig. S5).

Temperature suitability for transmission varies seasonally across Australia. In subtropical and temperate locations (Brisbane and further south), low temperatures force $R_0$ to zero for part of the year (Figs. 4A, 5). Monthly mean temperatures in these areas fall along the increasing portion of the $R_0$ curve for the entire year, so thermal suitability for RRV transmission increases with temperature in these areas. By contrast, in tropical, northern Australia (Darwin and Cairns), the temperature remains suitable throughout the year (Figs. 4, 5). Darwin is the only major city where mean temperatures are high enough to depress transmission. Most Australians live in southern, temperate areas of the country, making country-scale transmission seasonal; as hypothesized, human cases peak two months after population-weighted $R_0(T)$ (Fig. 6).
DISCUSSION

In a warming world, it is critical to understand effects of temperature on transmission of mosquito-borne disease. Characterizing nonlinear thermal responses by identifying transmission optima and limits is useful for predicting geographic, seasonal, and interannual variation in disease. Thermal responses can vary substantially among diseases and vector species (Mordecai et al. 2013, 2017), yet we lack mechanistic models based on empirical, unimodal thermal responses for many diseases and vectors. Here, we parameterized a temperature-dependent model for transmission of Ross River virus (Fig. 2) with data from two important vector species (Cx. annulirostris and Ae. vigilax; Fig. 1). The optimal temperature for disease transmission is moderate (26-27°C; Fig. 3), and largely determined by the thermal response of adult mosquito lifespan (Fig. S7). Both low and high temperatures limit transmission due to low mosquito fecundity and survival at all life stages (Fig. S7). Temperature explains the geography of year-round endemic versus seasonally epidemic disease (Figs. 4, 5) and accurately predicts the seasonality of human cases at the national scale (Fig. 6). Thus, the empirically-parameterized model for RRV transmission provides a mechanistic link between geographic and seasonal changes in temperature and broad-scale patterns of disease.

While the thermal response of RRV transmission generally matched those of other mosquito-borne pathogens, there were some key differences. The moderate optimal temperature for RRV (26-27°C) fit within the range of thermal optima for other diseases: malaria transmission by Anopheles spp. at 25°C, and dengue and other viruses by Ae. aegypti and Ae. albopictus at 29°C and 26°C, respectively (Fig. S6; Mordecai et al. 2013, 2017; Johnson et al. 2015). For all of these diseases, the specific optimal temperature for transmission was largely
determined by the thermal response of adult lifespan (Mordecai et al. 2013, 2017; Johnson et al. 2015). However, the traits that limited RRV transmission at high and low temperatures differed from other systems. The lower thermal limit for RRV was most constrained by fecundity and survival at all stages while the upper thermal limit was most constrained by fecundity and adult lifespan. By contrast, the thermal limits for malaria transmission were determined by parasite development rate at cool temperatures and egg-to-adult survival at high temperatures (Mordecai et al. 2013). As with previous models, the upper and lower thermal limits of RRV transmission are much more uncertain than the optimum (Fig. 3; Johnson et al. 2015; Mordecai et al. 2017), because trait responses are inherently harder to measure near their thermal limits where survival is low and development is slow or incomplete. Our results support a general pattern of intermediate thermal optima for transmission where the well-resolved optimal temperature is driven by adult mosquito lifespan, but upper and lower thermal limits are more uncertain and may be determined by unique traits for different vectors and pathogens.

We were able to fit thermal responses for all of the traits needed to calculate $R_0$, but the data were limited in two keys ways. First, two of the traits (fecundity and adult lifespan) had data from only three temperatures. We used priors derived from data from other mosquito species to minimize over-fitting and better represent the true fit and uncertainty in these trait responses (Fig. 2, versus uniform priors in Fig. S1). However, data from more temperatures would increase our confidence in the fitted thermal responses. Second, no RRV vector species had data for all traits (Fig. 1), so we combined mosquito traits from $Cx.\ annulirostris$ and pathogen infection traits in $Ae.\ vigilax$—two broadly-distributed vector species (Fig. 1)—to build composite $R_0$ models. Geographic and seasonal variation in vector populations suggests that $Ae.\ camptorhynchus$ and $Ae.\ vigilax$ have different thermal niches (cooler and warmer, respectively)
and *Cx. annulirostris* has a broader thermal niche (Fig. 1; Russell 1998). Temperature-dependent trait data for more species are needed to test the hypothesis that these niches are reflected in the species’ thermal responses. If true, predictions based on the model here may not be accurate for transmission by *Ae. camptorhynchus* and *Ae. vigilax*, since the model results are largely determined by *Cx. annulirostris* trait responses. That hypothesis could also explain why RRV persists over a wide climatic and latitudinal gradient. Thermal response experiments with other important RRV vectors are a critical area for future research.

The temperature-dependent $R_0$ model provides a mechanistic explanation for independently-observed patterns of RRV transmission across Australia. As predicted (Figs. 4, 5), RRV is endemic in tropical Australia and epidemic in subtropical and temperate Australia (Weinstein 1997). The model also accurately predicts the seasonality of cases at the national scale (Fig. 6), reproducing the *a priori* predicted lag of 8-10 weeks for temperature to affect reported human cases (Hu *et al.* 2006; Jacups *et al.* 2008b; Stewart Ibarra *et al.* 2013; Mordecai *et al.* 2017). Further, as temperatures increase from spring into summer, RRV transmission by *Cx. annulirostris* in inland areas often moves south along the latitudinal gradient (Russell 1998), matching the model prediction (Fig. 5). Although temperature is often invoked as a potential driver for such patterns, it is difficult to establish causality from statistical inference alone, particularly if temperature and disease both exhibit strong seasonality and therefore could both be responding to another latent driver (Sugihara *et al.* 2012). Thus, the mechanistic model is a critical piece of evidence linking temperature to patterns of disease.

In addition to explaining broad-scale patterns of RRV disease, the unimodal thermal model explains previously contradictory local-scale results. Specifically, statistical evidence for temperature impacts on local time series of RRV cases is mixed. RRV incidence is often—but
not always—positively associated with warmer temperatures (Tong & Hu 2001; Tong et al. 2002, 2004, Hu et al. 2004, 2010; Jacups et al. 2008b; Williams et al. 2009; Werner et al. 2012; Koolhof et al. 2017). However, variation in temperature impacts across local time series is expected from the intermediate thermal optimum for transmission, since the optimum is near observed mean temperatures in tropical and sub-tropical climates. The strongest statistical signal of temperature on disease is expected to occur in temperate regions where mean temperature varies along the rapidly rising portion of the $R_0$ curve (~20-26°C). If mean temperatures vary both above and below the optimum (as in Darwin), important effects of temperature may be masked in time series models that fit linear responses. Additionally, if temperatures are always relatively suitable (as in tropical climates) or unsuitable (as in very cool temperate climates), variation in disease may be due primarily to other factors. A nonlinear mechanistic model is critical for estimating impacts of temperature on transmission because the effect of increasing temperature by a few degrees can have a positive, negligible, or negative impact on $R_0$ along different parts of the thermal response curve. Although field-based evidence for unimodal thermal responses in vector-borne disease is rare (but see Mordecai et al. 2013; Peña-García et al. 2017), there is some evidence for high temperatures constraining RRV transmission and vector populations: outbreaks were less likely with more days above 35ºC in one area in Queensland (Gatton et al. 2005) and populations of *Cx. annulirostris* peaked at 25ºC and declined above 32ºC in Victoria (Dhileepan 1996). Future statistical analyses of RRV cases may benefit from using a nonlinear estimate of temperature-dependent $R_0$ as a predictor instead of raw temperature (Fig. 5B versus 5A).

Aquatic breeding habitat availability is also an important driver of mosquito abundance and mosquito-borne disease. Local rainfall and river flow have been linked to the abundance of
RRV vector species (Tall et al. 2014) and RRV disease cases (Tong & Hu 2001, 2002b; Hu et al. 2004; Kelly-Hope et al. 2004a; Tong et al. 2004; Gatton et al. 2005; Jacups et al. 2008b; Bi et al. 2009; Williams et al. 2009; Werner et al. 2012), as have unusually high tides in coastal areas with saltmarsh mosquitoes (Tong & Hu 2002a; Tong et al. 2004; Jacups et al. 2008b). Overlying models of species-specific breeding habitat with temperature-dependent models would better resolve the geographic and seasonal distribution of RRV transmission. $R_0$ peaked at similar temperatures whether or not we assumed that mosquito abundance was temperature-dependent (eq. 1 versus eq. 2); however, the range of suitable temperatures was much wider for the model that assumed a temperature-independent mosquito population (Fig. 3). Since breeding habitat availability can only impact vector populations when temperatures do not exclude them, it is critical to consider thermal constraints on mosquito abundance even when breeding habitat is considered a stronger driver. Nonetheless, many mechanistic models of temperature-dependent transmission of vector-borne disease do not include thermal effects on vector density (e.g., Paull et al. 2017). Our results demonstrate that the decision to include—or exclude—these relationships can have a critical impact on the model results, especially near thermal limits. Several important gaps remain in our understanding of RRV thermal ecology. First, we need better trait thermal response data, for more vector species. Second, the $R_0$ model needs to be more rigorously validated using time series of human cases to determine the importance of temperature at finer spatiotemporal scales. These analyses should incorporate daily and seasonal temperature variation (Paaijmans et al. 2010) and integrate species-specific drivers of breeding habitat availability, like seasonal rainfall and tidal patterns. Finally, translating environmental suitability for mosquito transmission into human cases also depends on past and current disease dynamics in reservoir host and human populations, and their impacts on population immunity.
For instance, in Western Australia when recruitment of susceptible juvenile kangaroos is low due to low rainfall in the preceding winter, heavy summer rains may fail to initiate RRV epidemics (Mackenzie et al. 2000). By contrast, large outbreaks occur in southeastern Australia when high rainfall follows a dry year, presumably from increased transmission within previously unexposed reservoir populations (Woodruff et al. 2002). Building vector species-specific $R_0$ models and integrating thermal ecology with other drivers are important next steps for accurately forecasting variation in RRV transmission.

Extending the results of the temperature-dependent model for RRV transmission to future climate regimes highlights the importance of characterizing nonlinear thermal responses. Climate warming is likely to increase the geographic and seasonal range of transmission potential in temperate, southern Australia where the majority of Australians live. However, climate change is likely to decrease transmission potential in tropical cities like Darwin, where moderate warming (~3°C) would push temperatures above the upper thermal limit for transmission for most of the year (Fig. 5). However, the extent of climate-driven declines in transmission will depend on how much Cx. annulirostris and Ae. vigilax can adapt to extend their upper thermal limits and whether warmer-adapted mosquito species (e.g., Ae. aegypti and potentially Ae. polynesiensis) can invade and sustain RRV transmission cycles. Thus, while the response of RRV transmission by current vector species to climate change is predictable based on vector and parasite trait thermal responses, future RRV disease dynamics will also depend on vector adaptation, potential vector species invasions, and climate change impacts on sea level and precipitation that drive vector habitat availability.

ACKNOWLEDGEMENTS
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REFERENCES


Austr. J. Entomol., 46, 60–64.


SUPPORTING INFORMATION

Additional Supporting Information may be downloaded via the online version of this article at Wiley Online Library (www.ecologyletters.com)
FIGURE 1: Vector species implicated in RRV outbreaks. Map of Australia and Pacific Islands showing outbreaks of RRV disease in which specific mosquito species were identified as vectors based on the collection of field specimens. Only the six most important species are included. Grid indicates thermal response data availability for each trait in the $R_0$ model for the five Australian species. Data sources are listed in Table S1.

FIGURE 2: Thermal responses of Cx. annulirostris and Ross River virus (in Ae. vigilax) traits that drive transmission. Functions were fit using Bayesian inference with priors fit using data from other mosquito species and viruses. For (E) fecundity and (C) adult lifespan, points show data means and error bars indicate standard error. Black solid lines are posterior distribution means; dashed red lines are 95% credible intervals.

FIGURE 3: Thermal response of relative $R_0$. (A) Posterior means of relative $R_0$ across temperature. Constant $M$ model (eq. 1) assumes mosquito density does not depend on temperature (light blue), while temperature-dependent $M$ model (eq. 2) accounts for temperature-dependence of mosquito life history traits (dark blue). Predicted mosquito density ($M$) is also shown for comparison (red). The y-axis illustrates relative $R_0$ (or $M$) across temperatures rather than absolute values, which would require information about additional drivers and vary across transmission settings. Histograms of (B) the critical thermal minimum, (C) thermal optimum, and (D) critical thermal maximum temperatures for both $R_0$ models (constant $M$: light blue; temperature-dependent $M$: dark blue).
FIGURE 4: Transmission potential for Ross River virus based on monthly mean temperatures. Color indicates the number of months of where (A) relative $R_0>0$ and (B) relative $R_0>0.5$. Model predictions are based on the median posterior probability for relative $R_0$. Points indicate selected cities (from Fig. 5), scaled by the percent of the total Australian population residing in each city.

FIGURE 5: Average seasonality of temperature and predicted temperature-dependent relative $R_0$ in Australian cities. The selected cities span a latitude and temperature gradient (Darwin = dark red, Cairns = red, Brisbane = dark orange, Perth = light orange, Sydney = aqua, Melbourne = blue, Hobart = dark blue). The sequence of months begins in July and ends in June (both during austral winter). (A) Mean monthly temperatures. Gray shaded areas show temperature at outer 95% CI (light gray), median (medium gray), and inner 95% CI (dark gray) for thresholds where $R_0>0$. Dashed gray line shows median temperature for $R_0$ optimum. (B) Relative $R_0$ as a function of temperature in each city.

Figure 6: Seasonality of temperature-dependent relative $R_0$ (line) and RRV infections. Human cases (bars) are aggregated nationwide from 1992-2013. Temperature-dependent $R_0$ (line) is weighted by population, calculated from the 15 largest cities in Australia (76.6% of the total population). The sequence of months begins in July and ends in June (during austral winter). Cases peak two months after $R_0(T)$, the a priori expected lag between temperature and reported cases.
Vector Species Implicated in Outbreaks:

- **Saltwater spp.**
  - *Aedes vigilax*
  - *Aedes camptorhynchus*
  - *Culex annulirostris*

- **Freshwater spp.**
  - *Aedes notoscriptus*
  - *Aedes normanensis*
  - *Aedes polynesiensis*

### Table: Vector / Trait

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<th>a</th>
<th>EFD</th>
<th>MDR</th>
<th>$p_{EA}$</th>
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<th>bc</th>
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FIGURE 2

A. Biting Rate (a)

B. Vector Competence (bc)

C. Adult Lifespan ($\mu^{-1}$)

D. Parasite Development Rate ($PDR$)

E. Fecundity ($EFD$)

F. Egg Raft Viability ($pRH$)

G. No. Viable Eggs per Raft ($nLR$)

H. Larval–to–Adult Survival ($pLA$)

I. Mosquito Development Rate ($MDR$)

Temperature ($°C$)
FIGURE 3

A: Relative R0 or M

- Temp.-Dep. M R0
- Constant M R0
- Mosquito Density (M)

B: Temperature of min R0 (°C)

C: Temperature of peak R0 (°C)

D: Temperature of max R0 (°C)
FIGURE 4

[Map of Australia with months and percentage of total population indicated]

- Months: 1 to 12
- Percentage of Total Population:
  - 20-25%
  - 15-20%
  - 10-15%
  - 5-10%
  - 1-5%
  - 0-1%
FIGURE 5

A: Monthly mean temperature (°C) for different cities. The shaded area represents the range of temperature variation.

B: Relative $R_0(T)$ as a function of the month of the year, beginning in July (winter). The graph shows the peak values during the winter months for each city.
Mean monthly RRV cases

Month of year – beginning in July (winter)

Relative $R_0(T)$