The use of maps and models to evaluate surveillance policies for dengue

A thesis submitted for the degree of *Doctor of Philosophy*

Oliver Jerome Brady

St Anne's College, University of Oxford

Hilary 2015

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Abstract

Dengue is a mosquito-borne disease that has gone from isolated sylvatic spill-overs to a pathogen of global public health importance in less than a century. Surveillance and control efforts have continually lagged behind contemporary needs and this thesis investigates the pressing need to reassess the current geographic limits and case burden of dengue including how these are measured.

First, the global distribution of reported dengue infections was mapped. In many areas dengue reporting is sparse as it can be asymptomatic or clinically similar to other diseases. The maps identified surveillance gaps, particularly in Africa and the Middle East, where targeted efforts are needed to reliably measure the contemporary global extent of dengue transmission.

Second, the global case burden of dengue was estimated by pairing risk maps with longitudinal cohort study data. This gave the first cartographic estimate of dengue cases and at 390 million per year (95% CI: 284-528) was over three times the World Health Organization official estimate. Reconciling these estimates with reported case data allowed an assessment of existing national surveillance strategies.

Third, existing methods for outbreak identification were compared. High burden dengue outbreaks are preventable if timely and appropriate measures are applied. This analysis suggested, however, that commonly used outbreak definitions are not consistent, which could have an adverse effect on outbreak response. An alternative framework for outbreak identification based on healthcare capacities is thus outlined.

Fourth, an analysis was undertaken that quantified how mosquito vectors place geographic and seasonal limits on dengue transmission. This involved updating estimates of mosquito longevity which were integrated with temperature-dependent dynamic transmission models to produce global maps. These suggested that while temperature places strong limits on the distribution of dengue in some settings, mosquito ecology may be more important in others.

Finally, these findings are discussed in the context of global dengue surveillance gaps, with recommendations of how these can be addressed at a time when providing an evidence base for deployment of new control strategies will be critical.

Statement of Contribution and Associated Publications

Each chapter of this thesis is comprised of a peer-reviewed, in press, or published manuscript. These pieces of work have been a collaborative effort including members from the Spatial Ecology and Epidemiology Group (SEEG) here in Oxford and individuals from various other institutions. In this section I outline my specific contributions and how they complement the separate contributions of my co-authors.

Chapter 2: Refining the known global distribution of dengue.

I was responsible for all aspects of this work. I conceived the study design (with PWG and SIH), collected the data, analysed the data (with JPM and SB) and wrote the first draft of the manuscript. The final manuscript text was edited and approved by all authors.

Brady, O. J., Gething, P. W., Bhatt, S., Messina, J. P., Brownstein, J. S., Hoen, A. G., Moyes, C. L., Farlow, A. W., Scott, T. W. and Hay, S. I. (2012). Refining the global spatial limits of dengue virus transmission by evidence-based consensus. *PLoS Neglected Tropical Diseases*. **6**(8): e1760.

Chapter 3: Estimating the global burden of dengue.

This chapter details a significant component part of a larger study to estimate the global case burden of dengue. My particular contribution focussed on gathering and standardising dengue incidence data to convert map-based estimates of environmental suitability for transmission into case numbers. While SB was chiefly responsible for the project and implemented all modelling analyses, the data for the burden model and the interpretation of the resulting numbers were solely my responsibility. This chapter is composed of two sections of the Supplementary Information to the published

manuscript which were written by myself and refined with the help of all co-authors. I also made significant contributions to all other areas of the project.

Bhatt, S., Gething, P. W., Brady, O. J., Messina, J. P., Farlow, A. W., Moyes, C. L., Drake, J. M., Brownstein, J. S., Hoen, A. G., Sankoh, O., Myers, M. F., George, D. B., Jaenisch, T., Wint, G. R. W., Simmons, C. P., Scott, T. W., Farrar, J. J. and Hay, S. I. (2013). The global distribution and burden of dengue. *Nature*. **496**(7446): 504.

Chapter 4: Assessing the applicability of dengue outbreak response guidelines.

I was responsible for all aspects of this work. I designed the experiment (with DLS, TWS and SIH), assembled the data, implemented all modelling analyses and wrote the first draft of the manuscript. The final manuscript text was edited and approved by all authors. The manuscript has recently been resubmitted to *Epidemics* following favourable peer-review.

Brady, O. J., Smith, D. L., Scott, T. W. and Hay, S. I. (2014). Dengue disease outbreak definitions are implicitly variable. *Epidemics*.(submitted).

Chapter 5: Modelling adult survival of the mosquito vectors of dengue.

I was responsible for all aspects of this work. I conceived and designed the experiments (with MAJ and PWG), assembled the data (with additional data provided by CAG, HD, MGG, PL, RM and LMS), designed and implemented the modelling analysis and wrote the first draft of the manuscript. The final manuscript text was edited and approved by all authors.

Brady, O. J., Johansson, M. A., Guerra, C. A., Bhatt, S., Golding, N., Pigott, D. M., Delatte, H., Grech, M.G., Leisnham, P.T., Maciel-de-Freitas, R., Styer, L.M., Smith, D. L., Scott, T. W., Gething, P. W. and Hay, S. I. (2013). Modelling adult *Aedes aegypti* and *Aedes albopictus* survival at different temperatures in laboratory and field settings. *Parasites and Vectors*. **6**: 351.

Chapter 6: Evaluating the potential for dengue transmission in temperate regions.

I was responsible for all aspects of this work. I conceived and designed the experiments (with DLS, PWG and SIH), assembled the data (with additional data provided by MUGK), designed and implemented the modelling approach (with support from NG) and wrote the first draft of the manuscript. The final manuscript text was edited and approved by all authors.

Brady, O. J., Golding, N., Pigott, D. M., Kraemer, M. U. G., Messina, J. P., Reiner, R. C., Scott, T. W., Smith, D. L., Gething, P. W. and Hay, S. I. (2014). Global temperature constraints on *Aedes aegypti* and *Ae. albopictus* persistence and competence for dengue virus transmission. *Parasites and Vectors*. **7**: 338.

* * *

The databases, methods and modelled outputs of these chapters have gone on to be used in a number of other projects which I have contributed to, but was not chiefly responsible for. These are referred to in the discussion of each chapter and are summarised below.

Related manuscripts for chapter 2:

Feldstein, L. R., Brownstein, J. S., Brady, O. J., Hay, S. I. and Johansson, M. A. (2015). The dengue microcosm: what islands tell us about the global ecology of dengue viruses. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. (in press).

Pigott, D. M., Bhatt, S., Golding, N., Duda, K. A., Battle, K. E., Brady, O. J., Messina, J. P., Balard, Y., Bastien, P., Pratlong, F., Brownstein, J.S., Freifield, C.C., Mekaru, S.R., Gething, P.W., George, D.B., Myers, M.F., Reithinger, R. and Hay, S.I. (2014). Global distribution maps of the leishmaniases. *eLife*. **3**: e02851.

Messina, J. P., Brady, O. J., Pigott, D. M., Brownstein, J. S., Hoen, A. G. and Hay, S. I. (2014). A global compendium of human dengue virus occurrence. *Scientific Data*. **1**: 140004.

Jaenisch, T., Junghanss, T., Wills, B., Brady, O. J., Eckerle, I., Farlow, A., Hay, S. I., McCall, P. J., Messina, J. P., Ofula, V., Sall, A. A., Sakuntabhai, A., Velayudhan, R., Wint, G. R. W., Zeller, H., Margolis, H. S. and Sankoh, O. (2014). Dengue Expansion in

Africa—Not Recognized or Not Happening? *Emerging Infectious Diseases*. **20**: e140487.

Related manuscripts for chapter 3:

Messina, J. P., Brady, O. J., Pigott, D. M., Golding, N., Kraemer, M. U. G., Scott, T. W., Wint, G. R. W., Smith, D. L. and Hay, S. I. (2015). The many projected futures of dengue. *Nature Reviews and Microbiology*. (in press).

Messina, J. P., Brady, O. J., Scott, T. W., Zou, C., Pigott, D. M., Duda, K. A., Bhatt, S., Katzelnick, L., Howes, R. E. and Battle, K. E. (2014). Global spread of dengue virus types: mapping the 70 year history. *Trends in Microbiology*. **22**(3): 138-146.

Brady, O. J., Messina, J. P., Scott, T. W. and Hay, S. I. (2014). Mapping the Epidemiology of Dengue. *Dengue and Dengue Hemorrhagic Fever*. Gubler, D.J., Ooi, E.E., Vasudevan, S.G. and Farrar, J., New York, CABI International.

Related manuscripts for chapter 4:

Hay, S. I., Brady, O. J., Messina, J. P. and Farrar, J. (2013). Football fever could be a dose of dengue. *Nature*. **503**: 439.

Related manuscripts for chapter 5:

Guerra, C. A., Reiner Jr, R. C., Perkins, A. T., Lindsay, S. W., Midega, J., Brady, O. J., Barker, C. M., Reisen, W. K., Harrington, L. C., Takken, W., Kitron, U., Lloyd, A. L., Hay, S. I., Scott, T. W. and Smith, D. L. (2014). A global assembly of adult female mosquito mark-release-recapture data to inform the control of mosquito-borne pathogens. *Parasites and Vectors*. **7**(276): 10.1186.

Related manuscripts for chapter 6:

Kraemer, M. U. G., Sinka, M. E., Mylne, A., Duda, K. A., Shearer, F. M., Barker, C. M., Moore, C. G., Carvalho, R. G., Coelho, G. E., Elyazar, I. R. F., Teng, H. J., Brady, O. J., Messina, J. P., Pigott, D. M., Smith, D. L., Scott, T. W., Wint, G. R. W., Golding, N. and Hay, S. I. (2015). The global distribution of the arbovirus vectors *Aedes aegypti* and *Aedes albopictus.* (in prep).

Brady, O. J., Godfray, H. C. J., Tatem, A. J., Gething, P. W., Cohen, J. M., McKenzie, F. E., Perkins, T. A., Reiner Jr, R. C., Tusting, L., Scott, T. W., Lindsay, S. W., Hay, S. I. and Smith, D. L. (2014). Adult vector control, mosquito ecology, and malaria transmission. *International Health*. (in press).

Brady, O. J., Godfray, H. C. J., Tatem, A. J., Gething, P. W., Cohen, J. M., McKenzie, F. E., Perkins, T. A., Reiner Jr, R. C., Tusting, L., Scott, T. W., Lindsay, S. W., Hay, S. I. and Smith, D. L. (2014). Vectorial capacity and vector control: reconsidering sensitivity to parameters. (in prep).

* * *

Finally, I have been involved in the following publications that are not directly related to my thesis, but were conducted in parallel to the aforementioned work:

Pigott, D. M., Golding, N., Mylne, A., Huang, Z., Henry, A. J., Weiss, D. J., Brady, O. J., Kraemer, M. U. G., Smith, D. L., Moyes, C. L., Bhatt, S., Gething, P. W., Horby, P. W., Bogoch, I. I., Brownstein, J. S., Mekaru, S. R., Tatem, A. J., Khan, K. and Hay, S. I. (2014). Mapping the zoonotic niche of Ebola virus disease in Africa. *eLife*. **3**: e04395.

Brady, O. J., Hay, S. I. and Horby, P. W. (2014). Scale up supply of experimental Ebola drugs. *Nature*. **512**(7514): 233.

Mylne, A.*, Brady, O. J.*, Huang, Z., Pigott, D. M., Golding, N., Kraemer, M. U. G. and Hay, S. I. (2014). A comprehensive database of the geographic spread of past human Ebola outbreaks. *Scientific Data*. **1**(140042).

Pigott, D. M, Golding, N., Mylne, A., Huang, Z., Weiss, D.J., Brady, O.J., Kraemer, M.U.G., Hay, S.I. (2015). Mapping the zoonotic niche of Marburg virus disease in Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. (in press).

* indicates joint first authorship

* * *

As his supervisor, I certify that the statements of contribution listed here are a fair representation of Oliver Brady's work:

Prof. Simon Hay

Date:

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Abbreviations

Admin0	National administrative level
Admin1	First administrative level
Admin2	Second administrative level
AFI	Acute febrile illness
Africa+	Africa, Saudi Arabia, Yemen and the Indian Ocean islands
AIC	Akaike information criterion
CDC	Centers for Disease Control and Prevention
CFR	Case fatality rate
CI	Credible interval
DDT	Dichloro-diphenyl-trichloroethane
DENV	Dengue virus
DF	Dengue fever
DHF	Dengue hemorrhagic fever
DSS	Dengue shock syndrome
DVI	Dengue Vaccine Initiative
DW^-	Dengue without warning signs
DW^+	Dengue with warning signs
EIP	Extrinsic incubation period (of the virus in a vector)
ELISA	Enzyme-linked immunosorbent assay
EMRO	Eastern Mediterranean Region Office
FGC	First gonotrophic cycle
FOI	Force of infection
GAM	Generalized additive model
GBD	Global Burden of Disease
GIDEON	Global Infectious Diseases and Epidemiology Online Network

GRUMP	Global Rural Urban Mapping Project
HE	Healthcare expenditure
HI	Hemagglutination inhibition
HMIS	Health management information system
I:A	Inapparent to apparent infection ratio
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IQR	Interquartile range
INDEPTH	International Network for the Demographic Evaluation of Populations and Their Health
MCMC	Markov chain monte carlo
MoH	Ministry of health
MRR	Mark-release-recapture
n	Number
PAHO	Pan American Health Organization
PCR	Polymerase chain reaction
PRNT	Plaque reduction neutralization test
ProMED	Program for Monitoring Emerging Diseases
RL	Relative likelihood
SD	Severe dengue
SEA	Southeast Asia
SEARO	South-East Asia Region Office
SEEG	Spatial Ecology and Epidemiology Group
WHO	World Health Organization
WPRO	Western Pacific Region Office

Chapter 1 - Introduction

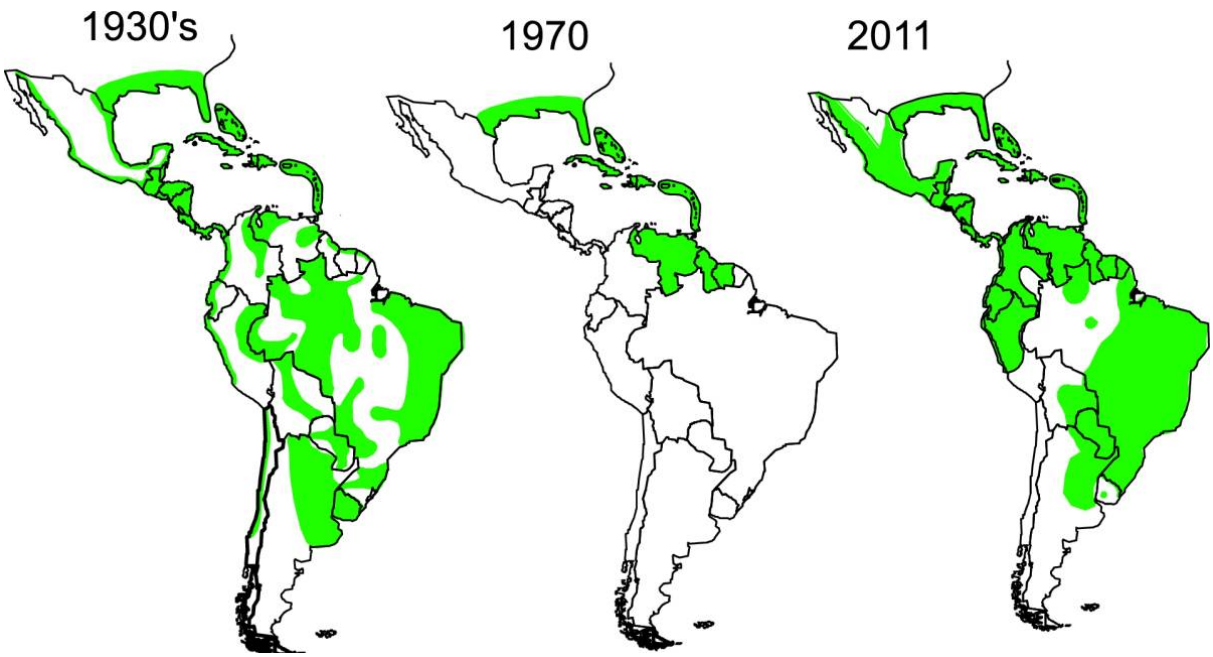
1.1. The Emergence of Dengue as a Global Public Health Priority

Dengue is a disease of the 21st century. Against a recent background of global recession of many major vector borne diseases, dengue bucks the trend. Its proliferation in urban environments along with vectors that can be easily spread by human trade and transport mean that the upward curve of socio-economic development is only making a more favourable world for dengue virus (DENV) transmission.

The first one to four thousand years of DENV transmission in humans (up to circa 1800) was marked by brief sylvatic spill-overs creating self-limiting outbreaks with limited pathogenicity *(Gubler, 2014; Holmes and Twiddy, 2003)*. The widespread disruption of infrastructure during the Second World War and the rapid socioeconomic changes in South East Asian cities that followed provided the first opportunities for frequent and sustained human DENV transmission. For the first time more than one of the four DENV serotypes could co-circulate as the highly anthopophillic *Aedes aegypti* mosquito spread throughout the dense, highly populous areas that were to become Asia's first megacities *(Halstead, 1980; Hammon et al., 1960)*. Secondary infection with a heterologous DENV serotype typically causes more severe clinical manifestations and this early virus co-circulation lead to the first outbreaks of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) that cemented dengue as a disease of public health concern *(Halstead, 1966; Midgley et al., 2011)*.

Throughout the decades spanning 1950-1980 the widespread availability of contact pesticides and a number of other public health tools brought the discussion of eradication of several infectious diseases back to the forefront *(Cockburn, 1963;*

Heimann, 1968). While not specifically targeted, these wide-scale vector control programs had a profound effect on the global epidemiology of dengue, none more so than the *Ae. aegypti* eradication program in the Americas. In an effort to combat urban yellow fever the Pan American Health Organization (PAHO) implemented a vertically structured, internationally coordinated control program. At its core was a heavy focus on targeted household surveys for *Ae. aegypti* larval breeding sites and widespread application of dichloro-diphenyl-trichloroethane (DDT) *(Gubler, 2005; Soper, 1965)*. Over the program's twenty year history, huge gains were made with elimination in over 20 countries (Fig. 1.1.) and the program likely delayed the establishment of dengue in the Americas by 25 years *(Gubler, 2014)*. In the 1970s, the eventual breakdown of coordinated political will, fading control efforts in the remaining foci and the ill-footed strategic switch to cheaper to implement ultra-low-volume spraying to kill adult mosquitoes led to the catastrophic collapse of the program *(Gubler, 2005; Schliessman and Calheiros, 1974)*. In just two decades the species re-established much of its pre-control range *(Gubler, 2005)* (Fig. 1.1.), a distribution which it retains and expands upon up to the current day *(Díaz-Nieto et al., 2013)*.



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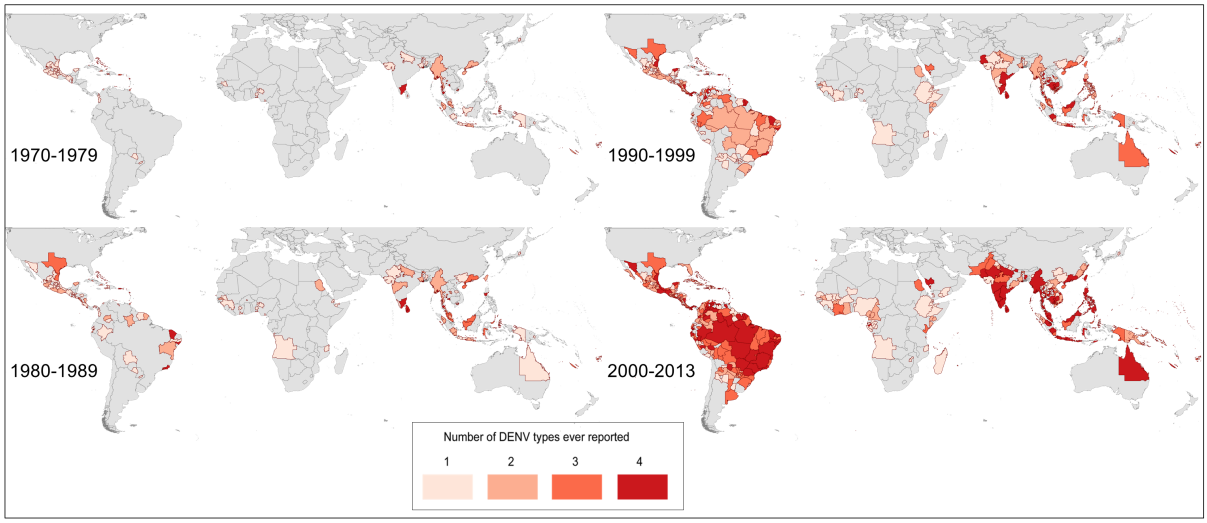
What little remaining protection the crumbling eradication programs provided was all but overcome by the mid-1980s. In many countries in Asia dengue was no longer just an urban disease, as nationwide year-round transmission of multiple serotypes saw the arrival of the first dengue hyperendemic countries (Fig. 1.2.) *(Messina et al., 2014; Monath, 1994; Pinheiro and Corber, 1997)*. A similar situation quickly developed in the Americas where the number of DHF cases jumped from less than 10,000 in the 1960s to over 250,000 throughout the 1990s *(Gubler, 2011)*. By the late 1990s dengue was becoming one of the leading causes of childhood hospitalisation in several countries *(Halstead, 1990)* and pressure was mounting to develop evidence-based guidelines for dengue treatment, surveillance and control that could help guide public health policy.



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In 1986 the World Health Organization (WHO) published its first set of international guidelines for dengue *(WHO, 1986)* which were quickly adopted and adapted by several WHO regional offices *(PAHO, 1994; Thongcharoen, 1993; WPRO, 1995)*. It was becoming increasingly clear that dengue morbidity and mortality rates could be drastically reduced with early clinical diagnosis and appropriate supportive treatment *(WHO, 1986)*. As a result these early guidelines had a heavy focus on dengue diagnosis and clinical care and gave little consideration to how to suppress transmission over the longer term. The paradigm had now shifted to containment and control instead of suppression or eradication.

As the recommendations of these guidelines began to be put into practice notable inconsistencies began to arise in different areas, particularly with the case definition of more severe clinical cases *(Gupta et al., 2010; Ng et al., 2007; Rigau-Perez, 2006; Srikiatkhachorn et al., 2010)* and the WHO has twice since revised its guidelines with significant modification to clinical diagnosis recommendations *(WHO, 1997*, *2009)*.

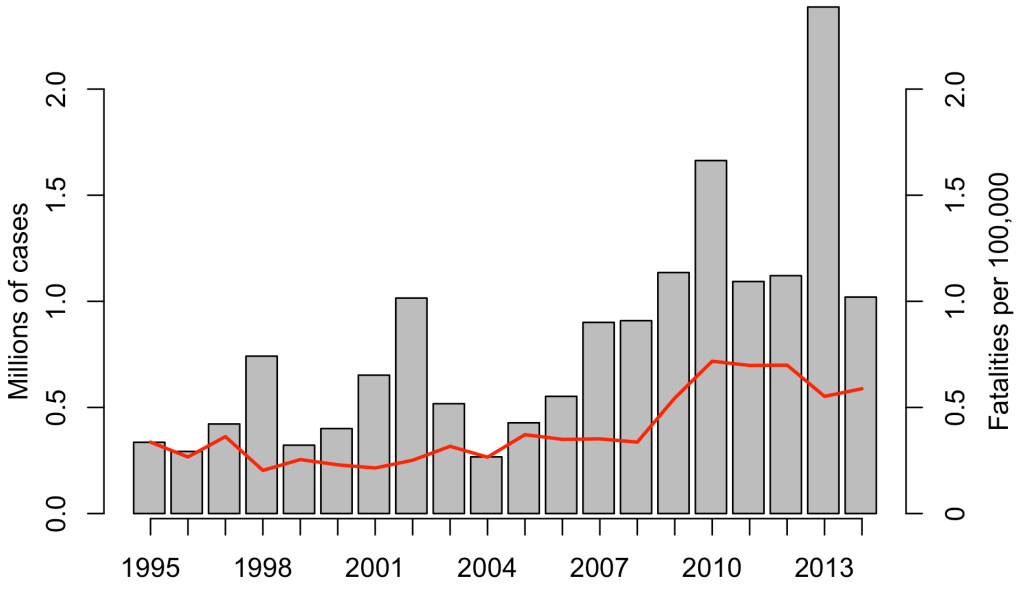

Figure 1.3. Number of cases and case fatality rate in the Americas. The bars show the number of dengue cases (clinically diagnosed) reported to PAHO *(PAHO, 2014)* and the red line shows the number of deaths per 100,000 cases.

Whilst over the last 20 years dengue case fatality rates (CFRs) have remained below 0.1% the number of reported cases has more than tripled (Fig. 1.3). High extra-seasonal surges in cases, or dengue outbreaks, have the capacity to severely overwhelm healthcare facilities with the resulting drop in the standard of care leading to excess morbidity and mortality rates (2013 in Fig. 1.3.). Among all infectious diseases only dengue, leishmaniasis and HIV have failed to see a fall in the number of deaths between 1990 and 2013 *(GBD, 2014)*. Existing policies have also failed to contain the expanding geographic distribution of dengue with expansion of hyperendemic transmission at both its northern and southern boundaries (Fig. 1.2.) *(Hakim et al., 2011; Masuh, 2008; Pandey et al., 2008)*. Sporadic transmission has also started to occur in areas such as southern Europe, the United States of America (USA) and Japan where diseases vectored by *Aedes* mosquitoes have been absent for over half a century *(CDC, 2010; La Ruche et al., 2010; ProMED, 2014)*.

Fortunately, current prospects for dengue control can be met with a degree of optimism. Despite unequal efficacy against the four serotypes, Sanofi Pasteur's live attenuated dengue vaccine has had promising phase 3b trial results in ten endemic countries *(Villar et al., 2014)*. An unparalleled financial commitment could now see the world's first dengue vaccine available as soon as the second half of 2015 *(Sanofi Pasteur, 2014)*. The WHO has firm commitments to reducing global dengue mortality by 50% and morbidity by 25% between 2010 and 2020 *(WHO, 2012)*. Efforts are also underway to improve the global quality, quantity and standardisation of dengue surveillance with the goal of producing a world dengue report with a framework similar to that pioneered by the World Malaria Report (Raman Velayudhan, personal

communication, November 2014) *(WHO, 2014)*. While it has long been recognised that control is a more realistic option than eradication, these new tools, commitments and optimisation strategies offer the potential to make the first measureable impact on global dengue burden since the eradication era.

1.2. Current Options for Dengue Surveillance

Public health surveillance is the systematic collection, analysis and interpretation of pathogen, host and vector data *(Thacker et al., 1989)*. A good surveillance system can i) identify areas that require additional control efforts, ii) measure the effectiveness of control and iii) suggest ways in which current control efforts may become more effective. Each of these three aims lead to different requirements in a surveillance system and questions such as "where, what and how often to sample?" are all affected by the spatio-temporal heterogeneity, visibility and response to control of the disease or vector in question. While it may be tempting to maximise surveillance in any given case, consideration must be given to the logistical constraints of reporting data of a consistent quality and the impact on resources, which are also required for control. Furthermore, disease surveillance is often composed of many different activities that measure different aspects of the disease, often in non-overlapping ways. This section describes and explores some of the methods available for dengue surveillance and how they can be combined to give a wider understanding of what is happening at each stage of transmission.

1.2.1. Surveillance of human dengue disease

Human DENV infection can result in a wide range of disease manifestations from completely asymptomatic through to mild febrile illness and up to severe or fatal disease *(Simmons, 2012)*. This complicates population-level surveillance of infected

individuals. Studies that account for every DENV infection in a population are extremely informative, but extremely expensive and, as such, rare. Other methods can detect some smaller proportion of DENV infections at a lower cost and are therefore used more commonly. The following section describes the different surveillance methods used for detecting DENV infected humans and gives a summary of their ubiquity, comparability and usefulness for different epidemiological purposes.

Total dengue virus infections

Accounting for total dengue infections remains the only way to fully describe the aetiology of transmission in the studied population. This information can only be obtained from a longitudinal cohort study, which follows a defined group of individuals who are repeatedly tested for dengue infection. This group sample is often chosen to be representative of the population in the area, but may be stratified by age/sex/location to further explore the details of transmission in specific groups.

Incidence can be estimated by testing paired sera collected over a period of time for evidence of newly developed anti-DENV antibodies. Enzyme-link immunosorbent assays (ELISA) and the hemagglutination inhibition (HI) test can be used to detect infection across DENV serotypes (i.e., they are not serotype-specific). The plaque reduction neutralization test (PRNT) can be used to identify the serotype of the infecting virus *(Clarke and Casals, 1958; Russell and Nisalak, 1967; Vaughn et al., 1999)*. A positive immunoglobulin M (IgM) ELISA result is indicative of a recent DENV infection and ELISA detectable immunoglobulin G (IgG) tends to predominate over IgM in secondary infections *(Simmons, 2012)*. These techniques have variable sensitivities and specificities and need to be calibrated across different DENV serotypes and closely related flaviviruses *(Houghton-Trivino et al., 2008)*, but remain the most appropriate methods available for detecting dengue infections in longitudinal cohort

studies. At the time of writing, worldwide only 57 dengue cohort studies have been conducted and published.

More commonly, a cross-sectional seroprevalence study may be conducted that measures DENV prevalence. An IgM response gives insight into prevalence at a given point in time, although it is not always easy to detect and is biased towards primary infections, while an IgG and/or PRNT response gives lifetime prevalence. While seroprevalence surveys are less informative about transmission dynamics of DENV, they do give a snapshot estimate of both recent (IgM) and historical (IgG) exposure to dengue. If such studies are age-structured they can be used to calculate force of infection (FOI), the rate at which susceptible persons are infected, which is an important metric for evaluating vaccine coverage and effectiveness *(Anderson and May, 1985)*. To date around 130 cross-sectional seroprevalence studies have been published globally.

Apparent dengue virus infections

An apparent dengue infection is defined as any DENV infection that disrupts the day-to-day activities of the infected individual. Apparent infections can include both clinical and sub-clinical manifestations (self-treatment) as well as mild to severe cases and must not be confused with these alternative measures. Apparent infections generally account for around 25% of total dengue infections *(Bhatt et al., 2013)*. Surveillance of apparent cases usually involves monitoring school or work-based absenteeism with follow-up visits and serological testing (i.e. active surveillance within a serological cohort study). Alternatively, healthcare officials may conduct active surveillance through household visits in the vicinity of a confirmed dengue case to detect episodes of fever, which are then referred for testing (reactive surveillance). Measuring the number and severity of apparent infections is important for quantifying the total

morbidity burden of dengue that might be underestimated when observing clinical cases alone. Because active fever surveillance lacks disease specificity, costly laboratory confirmation capacity is often a limiting factor. Only Singapore attempts routine active surveillance to consistently report apparent dengue infections at the country level, Australia and the USA report them during dengue outbreaks *(CDC, 2010; Gubler, 2002)* and around 70 other published studies have been conducted in smaller geographic units.

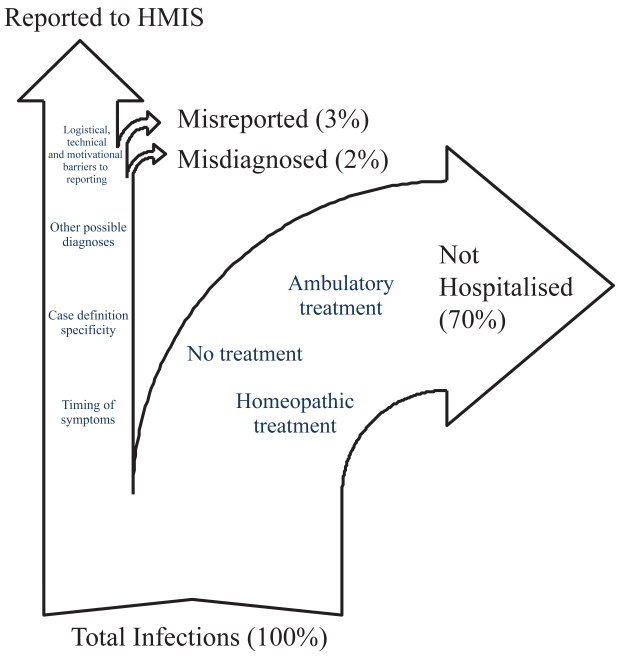

Figure 1.4. Hypothetical reporting chain of a dengue infection. Each branch represents different eventualities of apparent infections and the text highlights the main reasons why cases do not progress to the next level of detection and hence, are omitted from total cases reported by the health management information system (HMIS). Reproduced from Brady *et al.* (2014).

Hospitalised laboratory-confirmed dengue virus infections

Approximately 30% of apparent dengue infections are severe enough to require admission to official healthcare facilities (i.e., hospitals) as opposed to seeking unreported ambulatory treatment or no treatment at all *(Porter et al., 2005; Tien et al.,*

2010). This proportion varies widely depending on a number of factors but principally on treatment-seeking behaviours *(Huy et al., 2009)*. Despite passive surveillance capturing only a fraction of total infections (Fig. 1.4.), such data are useful for calculating the logistic and financial burden of dengue on healthcare systems. These data are more readily collected than apparent infection surveillance data, are currently available in all countries within PAHO and the Western Pacific Region Office (WPRO), and are becoming increasingly available in the South-East Asia Region Office (SEARO). The comparability of data on hospitalised confirmed infections, nevertheless, varies widely among countries, regions and seasons due to the varying availability of laboratory facilities and variation in the number of samples sent for testing.

Hospitalised clinically diagnosed dengue virus infections

Reporting hospitalised clinically diagnosed infections is less restricted by resource constraints. A clinical diagnosis of dengue is based on case definitions from the WHO *(WHO, 1997, 2009)* which are adapted in a number of incomparable ways to increase or decrease local sensitivity and specificity which will increase or decrease under and over diagnosis. The relative contribution of over- and under-diagnosis to the total number of reported clinically diagnosed cases also varies considerably through space and time, depending largely on the clinician's familiarity with diagnosing dengue and epidemiological circumstances that select for or against a differential dengue diagnosis, and must always be considered as a potential bias in this type of data.

Despite its variability, clinical disease classification is widely reported and contemporary case records exist for over 95 countries, constituting a major combined resource for analysing wide-scale patterns of dengue transmission.

1.2.2. Surveillance of mosquito vectors of DENV

DENV is transmitted by mosquitoes of the genus *Aedes*, with the vast majority of transmission due to the highly anthrophillic *Ae. aegypti*. More recently much attention has also been devoted to surveillance of *Ae. albopictus* following spread from its native range in North Asia across Europe and North America via the used tire trade *(Benedict et al., 2007; Reiter and Sprenger, 1987)*. While competent for DENV infection *(Gratz, 2004)*, it is hypothesised that a combination of poor viral dissemination to the salivary glands and inconsistent human biting behaviour mean that *Ae. albopictus* is usually an inefficient DENV vector and outbreaks vectored exclusively by the species (such as those in southern Europe and Japan) have been limited in duration and magnitude *(CDC, 2010; La Ruche et al., 2010; Lambrechts et al., 2010)*.

Surveillance of mosquito populations can be more complex than surveillance of human populations due to a number of factors. They exist in far greater number, have more variable spatio-temporal population dynamics and the tools available to measure important behavioural traits or changes in population dynamics are less sensitive, less precise and less consistent. In an attempt to distil the factors of a mosquito population that matter most for transmission, several models of "vectorial capacity" have been proposed based on measurable traits of a given vector combined with certain simplifying assumptions. By far the most commonly used and reproduced model of vectorial capacity was that originally proposed by George Macdonald *(Macdonald, 1952; Reiner et al., 2013; Smith et al., 2012)*:

$$V = \frac{ma^2p^n}{-\ln(p)}.$$

An intuitive restatement of this equation is that an infectious person will be subject to the attention of m adult female mosquitoes (assuming all hosts are equally attractive to mosquitoes) and will receive a bites. For those mosquitoes to become infectious they must survive the extrinsic incubation period (n) with probability p^n, where p is the daily

survival probability of the adult female mosquito. The adult female mosquitoes (on average) live for $1/-\ln(p)$ days biting and potentially infecting humans at a rate of a per day. The above equation combines these quantities to give the total potential infectious bites arising from one infected person for one day.

Measuring each of these parameters comes with its own inherent difficulties and sheds light on both the impreciseness of sampling techniques as well as some of the oversimplifying assumptions of transmission models.

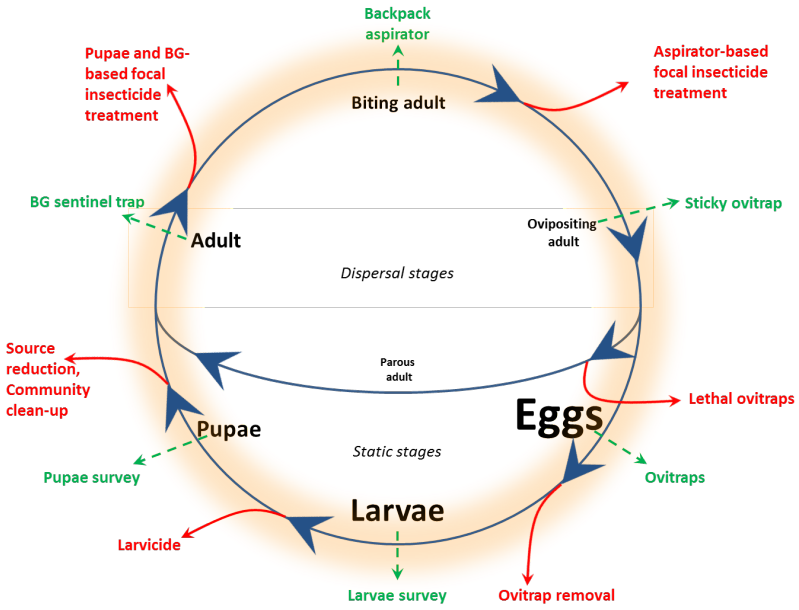

Figure 1.5. Surveillance and control in the mosquito life cycle. Black text and blue arrows display the key stages in the *Ae. aegypti* lifecycle with larger text indicating higher numbers. Green dashed arrows summarise the main surveillance methods while red arrows summarise the main control methods.

Measuring adult female mosquito density per human (m) is actually measuring the outcome of a complex multi life-cycle mosquito population dynamic process. While it is possible to directly collect adult mosquitoes within households using backpack aspirators *(Clark et al., 1994)*, odour-based traps *(Silva et al., 2005)*, or static fan-based Biogents traps *(Williams et al., 2006)*, it can often be an inconsistent measure depending on sampling effort, time of day of sampling and household

microenvironment *(Facchinelli et al., 2008; Morrison et al., 2004)*. Alternatively larval or pupal stages of the mosquito can be counted by surveying domestic water filled containers that form the typical habitats of the juvenile mosquito forms *(WHO, 2009)*. Correlation with adult population size will depend on the consistency of mortality in, and emergence from, these aquatic habitats and the relative importance of unsampled cryptic aquatic habitats in the community. Alternative methods using oviposition traps *(Mogi, 1988)* offer other ways of sampling adult and juvenile forms of the population, but their sampling rate is highly dependent on the availability of alternative oviposition sites *(Swanson et al., 2000)*. Perhaps the biggest issue with measuring adult population size is the lack of a gold standard dataset to validate sampling techniques, and it remains one of the hardest aspects of transmission to measure of any vector-borne disease despite the development of many sampling methods targeted at different life-cycle stages (Fig. 1.5.).

Measuring human biting rate (a) for *Aedes spp.* is more challenging than for other disease vectors as the prevalence and lack of prophylaxis for dengue make experimental human landing catches unethical. The arrival of cheap multiplex polymerase chain reaction (PCR) techniques have at least made it possible to identify the proportion of blood meals that are taken on human hosts (human blood-feeding habit) by sampling undigested blood from the mosquito midgut *(Siriyasatien et al., 2010)*. It is becoming increasingly clear that, especially in highly endemic areas, heterogeneous biting, where some individuals are bitten more frequently than others, is likely to be an important factor in transmission dynamics *(Smith et al., 2004)* and some specialist study designs have begun to quantify this *(Harrington et al., 2014)*.

Measuring mosquito survival (p) and duration of the extrinsic incubation period (EIP) (n) are complicated by environmental heterogeneity and the concordance between

results derived from the field and from the laboratory *(Brady et al., 2013; Chan and Johansson, 2012)*. These discrepancies are discussed at length in chapters 5 and 6.

Finally, each of these parameters can potentially vary in different environments, mosquito populations or seasons and can change in response to certain human interventions e.g., refractory behaviours in response to control. Given this variability, there is a need to consider the spatio-temporal scale over which each of these surveillance measures apply and what level of sampling would be required to accurately inform control efforts *(Smith et al., 2004; Smith et al., 2014; Smith et al., 2013)*. Quantifying how much these parameters change in response to a particular variable is also important for understanding why transmission occurs in some areas, but not in others. These variables can be either active interventions, such as the application of vector control, or passive, such as the effects of temperature on vector and virus population dynamics.

While many different methods are available to conduct entomological surveillance, their uptake for public health uses in the context of dengue has been limited and, at the time of writing, only Brazil and Singapore routinely collect entomological data for *Aedes spp. (Ministerio da Saude Brasil, 2012; Yoshikawa, 2013)*. This is likely due to the repeated lack of correlation between classic abundance measures and human dengue incidence, as well as the challenges of quantifying effectiveness of integrated intervention campaigns *(Bowman et al., 2014; Reiter, 2014)*. Despite this, a prominent case study remains where intensive vector control in Singapore suppressed dengue incidence for over 20 years between 1970 and 1990 (Fig. 1.6.) *(Ooi et al., 2006)*. As human herd immunity waned however, dengue outbreaks returned despite suppression of mosquito abundance measures.

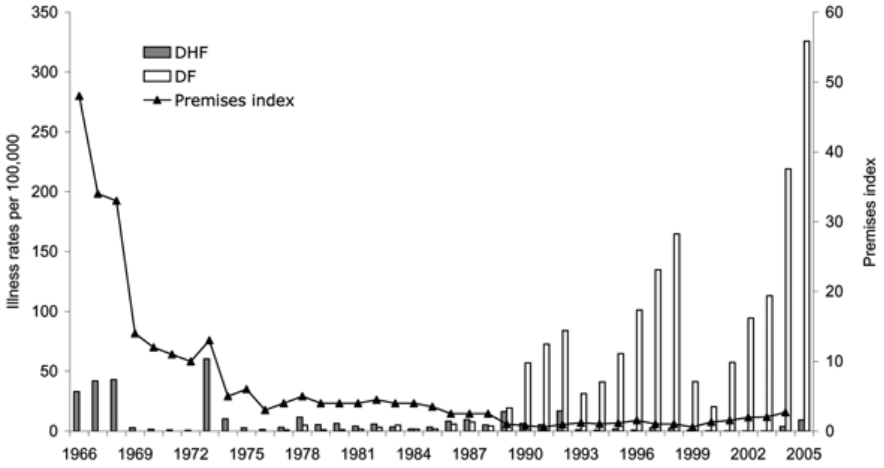

Figure 1.6. Dengue suppression in Singapore. The line shows the premises index (percentage of houses containing *Ae. aegypti* or *Ae. albopictus* pupae or larvae) suppression since 1970 and the reduction but eventual resurgence of reported dengue fever (DF) and DHF. Reproduced from Ooi *et al.* (2006).

The possible future prospects of a widely available vaccine that would boost herd immunity in combination with several new strategies for delivering vector control more efficiently in resource constrained settings could see the wider adoption and use of routine entomological surveillance.

1.3. The role of models and maps

While surveillance is informative about the current situation of vector populations or disease transmission, it does not tell us anything about the processes that have led to this outcome. Understanding these processes is important, as without it one cannot: i) understand why a particular change in incidence occurred e.g. in response to an intervention and ii) predict where or when such a change might occur again. Building a transmission model and evaluating it against surveillance data is one way of testing hypotheses about our understanding of transmission. Robustly evaluated models can then be used to make predictions under conditions for which data are unavailable.

Modelled-maps are spatially explicit ways of thinking about model predictions in different contexts.

The integration of models, that can inform us about how variables may change transmission, with maps, that explicitly target these predictions to relevant stakeholders, make an effective method of communicating information. A wide variety of model-based dengue maps are currently in use for different purposes at different spatial scales *(Brady et al., 2014)*. Their inherent value in maximising surveillance data is of benefit to both operational and research-based programs. The predictions from model-based maps can be used to guide further data acquisition, especially if accompanied by maps of model prediction uncertainty. This new data can then be used to improve model predictions, or generate hypotheses about why the predictions differ from a particular observation. This iterative process of prediction, testing and updating of understanding is where the role of model-based maps is highest in a public health context.

1.4. Aims of the thesis and description of the chapters

The aim of this introduction was to show that dengue is a rapidly expanding disease of increasing global public health concern. It has also shown that the tools available for measuring, monitoring and understanding this increasing threat can be limited in their scope and are often costly to implement. The combination of these factors has meant that global dengue surveillance efforts have lagged behind its increasing distribution, endemicity and public health burden. This discrepancy has implications for national and international policy makers who have to decide how much funding should be allocated to dengue control and how it should be spent.

This thesis critically assesses current global surveillance of dengue and its vectors and poses several new approaches to addressing long-term gaps in the evidence-base required for effective policy making. The specific objectives of this thesis are:

1. To develop new methods to map and quantify the global distribution and burden of human dengue cases.
2. To quantify the limits temperature places on the ability of *Ae. aegypti* and *Ae. albopictus* populations to transmit dengue in temperate areas.

The work will update imprecise, older estimates of the distribution and burden of dengue and identify specific contemporary surveillance gaps. Analysis of the entomological components of transmission will also highlight the potential for future spread and intensification of DENV transmission as well as building on our understanding of the factors that limit dengue transmission on a global scale.

In chapter 2 a framework is developed to map the global distribution of dengue based on disparate reporting data. The case burden within these countries is then estimated using a cohort-study-based cartographic approach described in chapter 3. The burden imposed by dengue outbreaks and how control approaches can best reduce it are discussed in the context of Brazil in chapter 4. Chapters 5 and 6 investigate the entomological components of transmission within the framework of the vectorial capacity equation. Chapter 5 improves estimates of a key parameter in transmission, the longevity of adult mosquitoes, and how this changes under certain environmental conditions. This is incorporated into a wider vectorial-capacity model in chapter 6 which evaluates the global limits temperature places on transmission of DENV in adult mosquitoes. Finally in chapter 7, general conclusions and implications of the combined research outputs are presented alongside a discussion of the strengths and limitations of the methods used in this thesis.

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Chapter 2 – Refining the known global distribution of dengue

2.1. Cover page

In 2012 the World Health Organization (WHO) and Centers for Disease Control and prevention (CDC) disagreed on dengue presence/absence status in 34 countries with many others having inconsistent or out of date evidence of dengue presence *(CDC, 2012; WHO, 2011)*. This first chapter aims to address this discrepancy and refine the global distribution of dengue by performing an audit of reported human DENV infections using a standardised and consistent methodology. This data is incorporated into a newly developed framework called "evidence-based consensus" that quantifies certainty on dengue presence or absence and identifies where specific surveillance or reporting activities are lacking. The original manuscript was published in *PLoS Neglected Tropical Diseases* in August 2012 *(Brady et al., 2012)*. The referred to supplementary information can be found in the Appendix section (page 171).

Since publication, the maps and methods developed here have been incorporated into further academic and operational research. First, ongoing dialogue with the WHO and CDC has resulted in the WHO adopting the maps presented here in their "Atlas of Health and Climate" in 2012 and the CDC adopting a hybrid map in their "Yellow book" in 2015 *(WHO, 2012)* (Michael Johansson personal communication August 2014). Second, the evidence gaps identified by this analysis have also been used to call for specific surveillance priorities, particularly in Africa, within the Eastern Mediterranean Regional Office (EMRO) and among isolated island communities *(Feldstein et al., 2015; Jaenisch et al., 2014)* (Raman Velayudhan personal communication February 2013). Finally, the approach developed here has become a core methodological

component of a wider effort to standardise how the Spatial Ecology and Epidemiology Group map a range of other infectious diseases *(Bhatt et al., 2013; Hay et al., 2013; Messina et al., 2014; Messina et al., 2015; Moyes et al., 2014; Pigott et al., 2014)*. The main advantage of the evidence consensus framework is that it summarises information in a standardised semi-quantitative manner that can be combined with quantitative disease models to estimate new metrics of transmission and disease burden.

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PLOS NEGLECTED TROPICAL DISEASES

Refining the Global Spatial Limits of Dengue Virus Transmission by Evidence-Based Consensus

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Abstract

Background: Dengue is a growing problem both in its geographical spread and in its intensity, and yet current global distribution remains highly uncertain. Challenges in diagnosis and diagnostic methods as well as highly variable national health systems mean no single data source can reliably estimate the distribution of this disease. As such, there is a lack of agreement on national dengue status among international health organisations. Here we bring together all available information on dengue occurrence using a novel approach to produce an evidence consensus map of the disease range that highlights nations with an uncertain dengue status.

Methods/Principal Findings: A baseline methodology was used to assess a range of evidence for each country. In regions where dengue status was uncertain, additional evidence types were included to either clarify dengue status or confirm that it is unknown at this time. An algorithm was developed that assesses evidence quality and consistency, giving each country an evidence consensus score. Using this approach, we were able to generate a contemporary global map of national-level dengue status that assigns a relative measure of certainty and identifies gaps in the available evidence.

Conclusion: The map produced here provides a list of 128 countries for which there is good evidence of dengue occurrence, including 36 countries that have previously been classified as dengue-free by the World Health Organization and/or the US Centers for Disease Control. It also identifies disease surveillance needs, which we list in full. The disease extents and limits determined here using evidence consensus, marks the beginning of a five-year study to advance the mapping of dengue virus transmission and disease risk. Completion of this first step has allowed us to produce a preliminary estimate of population at risk with an upper bound of 3.97 billion people. This figure will be refined in future work.

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Introduction

Despite increased interest in dengue in recent years, the global distribution of dengue remains highly uncertain. Estimates for the population at risk range from 30% [1] to 54.7% [2] of the world's population (2.05–3.74 billion) while the Centers for Disease Control (CDC) and the World Health Organization (WHO) currently disagree on dengue presence in 34 countries across five continents (Table S1). Clinical features of dengue virus infection include fever, rash and joint pain [3], which ensure the disease's misdiagnosis and mis-reporting among many other febrile illnesses. The diagnostic methods available also have limitations and a full complement of tests is not feasible in many healthcare settings. There is consensus, however, that dengue is a growing problem both geographically and in its intensity [4,5,6].

There is an urgent need to compile more extensive occurrence records of dengue virus transmission and assess them for contemporariness and accuracy. Evidence on dengue transmission comes in a wide variety of forms, with varying levels of spatial coverage and reliability. A global audit of dengue distribution therefore requires a transparent methodology to compile these disparate data types and synthesise an output map summarising the current consensus for each country. Such a methodology for compiling and assessing evidence must be robust, repeatable, able to evaluate a large variety of evidence types and incorporate expert opinion. An ideal output metric is a summary statistic (hereafter

Author Summary

Previous attempts to map the current global distribution of dengue virus transmission have produced variable results, particularly in Africa, reflecting the lack of accuracy in both diagnostic and locational information of reported dengue cases. In this study, instead of excluding these less informed points we included them with appropriate uncertainty alongside other diverse evidence forms. After assembling a comprehensive database of different evidence types, a weighted scoring system calculated "evidence consensus" for each country a continuous measure of the certainty of dengue presence or absence when considering the full aggregate of evidence. The resulting map and analysis helped highlight important evidence gaps that underlie uncertainties in the current distribution of dengue. We also show the importance of local knowledge through incorporating questionnaire-based responses that can help add clarity in uncertain regions. This analysis showed that presence/absence maps do not sufficiently highlight the uncertainties in the evidence base used to construct them. Mapping by evidence consensus not only encourages greater data inclusion, but it also better illustrates the current global distribution of dengue. Consensus mapping is thus ideal for a range of neglected tropical diseases where the evidence base is incomplete or less diagnostically reliable.

referred to as evidence consensus) that quantifies certainty on dengue virus transmission presence or absence given the accuracy and contemporariness of the evidence available. An evidence-based map of the current distribution of dengue virus transmission will have direct implications for design and implementation of dengue surveillance and, by showing gaps in contemporary knowledge, provide an advocacy platform for improved data.

Existing approaches to mapping the global limits of vector-borne diseases have used estimates of biological suitability of local environments, which have proved informative in the cases of some pathogens, such as *Plasmodium falciparum* [7,8] and *P. vivax* [9]. Several approaches have been used to map biological suitability for dengue using non-dengue-specific variables such as temperature, rainfall and satellite-derived environmental variables [1,10,11]. Although successive attempts have each increased predictive capacity and resolution, this approach produces variable results in Africa due to a scarcity of confirmed occurrence points across extensive geographic areas. An alternative approach has been to map evidence of dengue occurrence making no assumptions about biological suitability, as in Van Kleef *et al.*, who reviewed published literature to contrast historic, current and future limits of dengue [5]. To date dengue mapping has focussed on future scenarios, yet understanding of the current distribution of dengue virus transmission is far from complete and needs to be better evaluated before we can make predictions about forthcoming patterns and trends. In this study we combine evidence from large occurrence-point style databases used in biological suitability mapping approaches with a wider systematic review of various sources of evidence to create a more comprehensive dengue database. Using this database we then use the novel method of defining evidence consensus to evaluate the current level of certainty on dengue virus transmission presence or absence at national (and some sub-national) levels using a weighted evidence scoring system. Finally, we present these results as a series of global maps that explicitly identify surveillance gaps.

This study is the initial part of a five year project to collect, analyse and publicise global dengue virus transmission data. While the map presented here is the most extensive display of current dengue evidence available, we hope that continual data acquisition will result in more evidence from uncertain areas, increasing the resolution at which we can map evidence consensus in future advances.

Methods

Collection of dengue virus transmission evidence

Evidence for indigenous dengue virus transmission was obtained from four evidence categories: health organisations, peer-reviewed evidence, case data and supplementary evidence (Figure 1). The first three categories were used for all countries. For countries where some of these categories were not available and/or did not provide good consensus, the fourth category of supplementary evidence was used. Evidence was initially collected at a country level (Admin0), but resolution was improved to a state/province level (Admin1) or district level (Admin2) at the fringes of the distribution of detectable virus transmission when sufficient data were available.

Country dengue status as defined by health organisations was determined by consulting the WHO [12] and CDC [13] dengue distribution maps as well as the Global Infectious Diseases and Epidemiology Online Network (GIDEON) database [14]. GIDEON provides a collection of literature and case reports for a range of tropical and infectious diseases in 224 countries. Dengue status by country was recorded as present or absent.

The peer-reviewed evidence category contained evidence of dengue occurrence as determined by peer-reviewed sources where details of diagnostic techniques were given. Peer-reviewed journal (Google Scholar, PubMed, ISI Web of Science) and disease surveillance network (ProMED archives, Eurosurveillance archives) searches were conducted with search terms "country" or "Admin1/2" and "dengue". Sources were included for the period 1960–2012 and only if cases were confirmed as resulting from indigenous (i.e. not imported) transmission. The specialist regional journal collections African Journals Online (http://www.ajol.info/) and China National Knowledge Infrastructure (http://en.cnki.com.cn/) were also searched. Extra publications were found by searching using the location term in Genbank nucleotide records for dengue viruses isolated from human hosts. The search of peer-reviewed sources of evidence resulted in a total of 285 articles being selected for 123 countries where positive dengue occurrence records were identified. This included evidence from returning travellers who were diagnosed upon return to their often non-endemic home countries as opposed to the transmission setting. For these cases, evidence was attributed to the place to which they had travelled. The added value of returning traveller reports is that the travellers are often more immunologically naïve to dengue infections, and also that diagnosis is often pursued more rigorously. Therefore, the sensitivity of detecting an infection is increased. The results of our search were then cross-referenced against a dengue occurrence-point database compiled internally, in a separate exercise. Unlike our country-specific searches, this database of 2836 articles results from searches simply for "dengue", which were then geo-referenced using the article text. Full details are available in Protocol S1 and the geographic location of the occurrence points are displayed in Figures S1, S2, S3, S4, S5, S6. This cross-referencing resulted in the inclusion of an additional 16 articles in the current analysis and also provided increased justification for our choice of countries to evaluate at Admin1 level.

The case data category contained evidence of dengue outbreaks (minimum 50 infections) where evidence contained less diagnostic

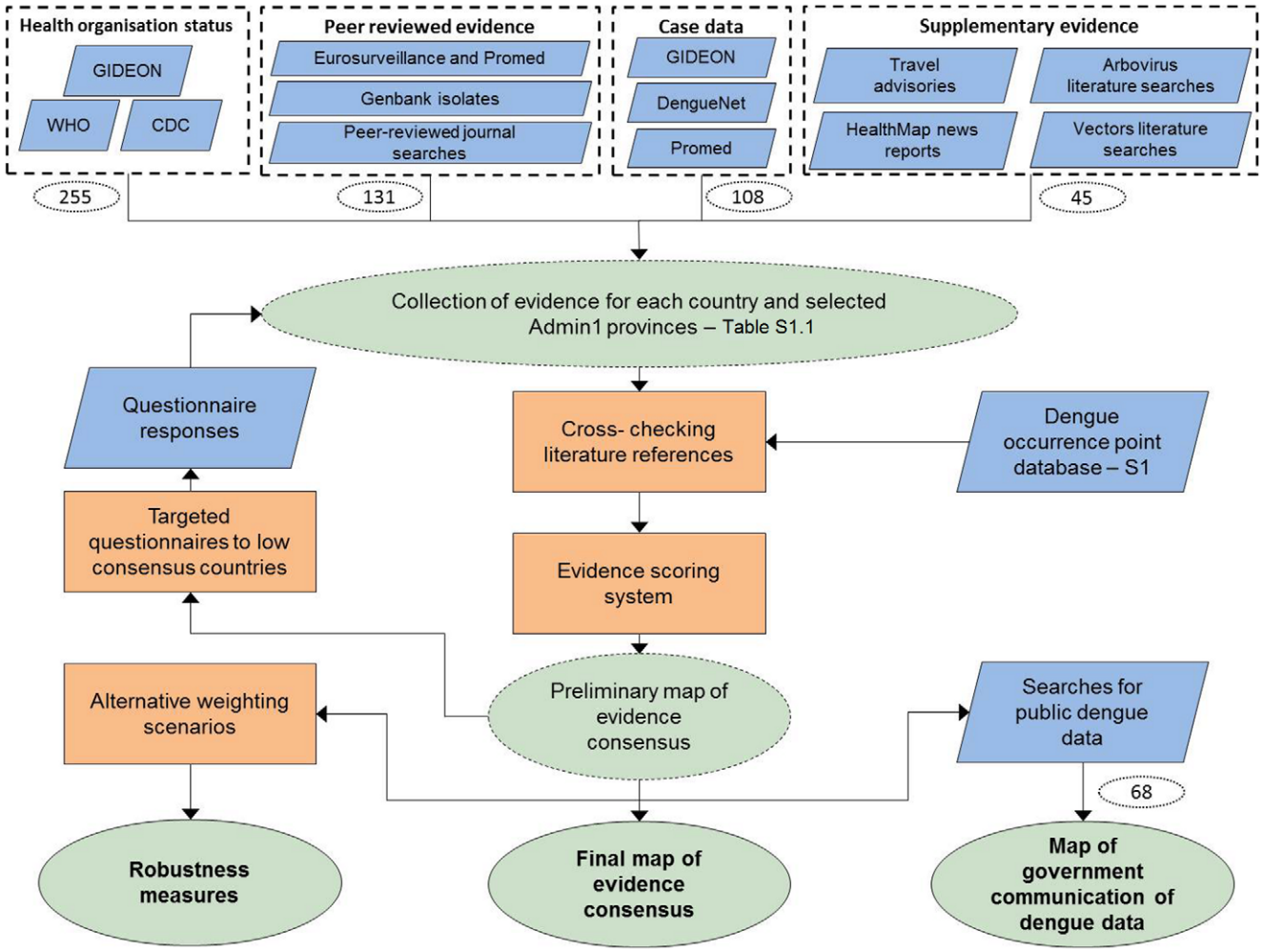

Figure 1. Schematic overview of the methods. Blue diamonds describe input data; orange boxes denote experimental procedures; green ovals indicate output data; dashed lines represent intermediate outputs and solid lines final outputs; dotted white ovals denote the number of countries for which data was available and added to the final output. Dotted rectangles identify the different evidence categories and their main data sources. S1 = Protocol S1.
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detail, but was more informative about the magnitude of dengue transmission occurring. Case data from the most recent outbreak were obtained from the Program for Monitoring Emerging Diseases (ProMED) archive search, WHO DengueNet data query [15] and from GIDEON which holds a detailed record of government-reported case numbers. This resulted in 100 countries with useful dengue case data.

In many resource-poor countries, both surveillance and researcher-generated reports are rare. Therefore, in countries where other evidence categories were sparse, we looked for supplemental evidence that suggested possible dengue virus presence. Supplemental evidence types included: presence of an established mosquito vector population of public health significance (*Aedes aegypti*, *Ae. albopictus* or *Ae. polynesiensis*) as documented by peer-reviewed literature, confirmed presence of multiple other rarely diagnosed arboviral diseases as documented by peer-reviewed literature, news reports of dengue epidemics found using GoogleNews archives (http://news.google.co.uk/archivesearch) and travel advisories from the National Travel Health Network and Centre (http://www.nathnac.org/ds/map_world.aspx) issued at a country-level. We included evidence of multiple other rarely diagnosed arboviral diseases, as these are informative about the ability of a country to detect any possible dengue infection. If other arboviral diseases are poorly reported, but documented by peer-reviewed literature as present, then it is possible that dengue is also underreported. In addition to this, we cross-referenced our dataset with the HealthMap database (www.healthmap.org/dengue/). This website-based application automatically geo-positions cases from websites with news reports and outbreak alerts related to dengue and contains data from a wide variety of sources dating back to 2007 [16,17]. This extensive database contributed important evidence especially at smaller spatial scales and in areas where translated articles are not so easily obtained. Supplementary evidence was used in evaluating dengue consensus in 45 countries.

While the categories are clearly defined here and in Figure 1, some overlap of evidence sources did occur, depending on the information content of each source. This meant evidence sources such as ProMED reports could be included twice, in both the peer-reviewed evidence and case data categories, if they contained information about diagnostic tests used for confirmation as well as overall outbreak case numbers. In this section we outline the main sources used for each category, but it should be noted that if evidence from a particular source fitted the criteria for a different

evidence category, it was not excluded, but rather included in that category.

Quantifying evidence with a weighted scoring system

In order to quantify evidence consensus, a weighted scoring system was developed that attributed positive values to evidence of presence and negative values to evidence of a lack of presence. The aim here was to use an optimal subset of evidence to accurately assess dengue status within a given area. By scoring the evidence categories mentioned above individually and then combining their respective scores, we were able to calculate "evidence consensus," a measure of how strongly the combined evidence collection supports a dengue-present or dengue-absent status (Figure 2). We defined a country as having "complete consensus" on dengue presence when the evidence base was comprised of contemporary forms of most or all of the following evidence types: 1) unanimous health organisations agreement, 2) a seroprevalence survey, 3) Polymerase Chain Reaction (PCR) typing of dengue virus or dengue viral RNA, 4) a foreign visitor to the area with a confirmed dengue infection upon returning to their home country, and 5) records of an epidemic of greater than 50 infections. Such a country has a consensus score of between 80% and 100%. A country with a complete consensus on dengue virus absence is characterised by all health organisations agreeing on dengue absence and high healthcare expenditure (as an approximate proxy for surveillance capability), therefore accounting for both the observed absence of dengue and the minimised possibility of any undetected dengue infections. Such a country scores between −80% and −100% on our scale. A country with no consensus on dengue virus status is characterised by conflicting evidence from different categories and scores close to 0%. Each evidence category was scored independently and category weights applied to reflect the level of detail each category provides: health organisation status (maximum score 6), peer-reviewed evidence (maximum 9), case data (maximum 9) and supplementary evidence (maximum 6). To support the choice of assigned category weights we performed a sensitivity analysis in which two alternative evidence weighting scenarios were applied to the same sources of data: 1) neutral (all categories hold the same weight) and 2) reversed (health organisation status and supplementary evidence hold weight 9, peer-reviewed evidence and case data hold weight 6). We then checked for any major deviations in overall country score resulting from such alternative scenarios.

Health organisation evidence. The data from the three health organisations (WHO, CDC and GIDEON) comprised discrete presence or absence answers. A consensus (+++ or −−−) scored 6 or −6 respectively, while a lack of consensus (++− or −−+) scored 3 or −3 respectively (Figure 2A). This gave a maximum score for this category of ±6.

Peer-reviewed evidence and returning traveller reports. These forms of evidence were each scored independently for contemporariness and accuracy. The date of occurrence was used for scoring as follows: between 2012–2005 = 3, 2004–1997 = 2 and pre-1997 = 1 (Figure 2B). This corresponded to a conservative estimate of the inter-epidemic period for dengue of three to five years [18]. This score was then added to a score for accuracy, whereby high accuracy, and a score of 3, was characterised by PCR methods, a Plaque Reduction Neutralization Test (PRNT), or a detailed case description of a complication of the disease. Complications of the disease were either dengue haemorrhagic fever (DHF) grades 1 and 2 or dengue shock syndrome (DSS) grades 3 and 4 under the old classification scheme [19] or severe dengue under the new classification scheme [3]. Medium accuracy methods including IgM- and IgG- based ELISA and Hemagglutination Inhibition (HI) assay approaches scored 2 because their calibration is sensitive to background immune responses [20], antibody response is variable over the course of an infection [21] and the test can cross-react with other non-dengue arboviruses [20]. A low accuracy score of 1 was used for articles that only reported case numbers with a non-dengue-specific case definition or a low participant number. Each included article was scored separately and then an average score was taken from all articles. This presented the possibility of devaluing the score of the most accurate and contemporary piece of evidence, so an extra score was added to reflect increased certainty provided by multiple forms of evidence. Evidence types 2) through 5) described above contributed to this extra score as such: if two types of evidence were present a score of 1 was added, three types = 2, four types = 3. This resulted in a maximum available score of 9 for peer-reviewed evidence.

Case data. This category was scored by contemporariness in eight-year intervals. The most frequent year in which an outbreak (over 50 cases or over 15 cases if the population is below 100,000) occurred was again scored in average inter-epidemic period intervals: 0–7 years since the last outbreak scored 9, 7–14 years = 6, 14–21 = 3, 21–28 = −3, 28–35 = −6, 35+ = −9 (Figure 2C). Where case data were unavailable, the distinction between true absences and inadequate surveillance was made using total annual healthcare expenditure (HE) per capita at average U.S. Dollar exchange rates (2011 WHO health statistics) [22]. Higher HE has been linked to better overall public health infrastructure, which includes high-quality diagnostic resources, greater healthcare coverage and higher levels of expertise, all of which may result in a more thorough characterisation of dengue status at the country-level [23,24,25]. Therefore, the lower the HE, the less certain we can be that an absence of case data accurately reflects an absence of dengue transmission. Class intervals for HE were chosen to reflect regional differences both within and between continents. Where information on HE was unavailable (Somalia, North Korea and Zimbabwe), low HE status was assigned. All overseas territories were assumed to have the same HE as their parent nations. The following criteria were used to derive the case scores in the absence of dengue case data: HE<$100 and reports of sporadic unconfirmed cases gave a score of 6, HE<$100 = low HE = 3, $100≤HE<$500 = medium HE = −3, HE≥$500 = high HE = −9 (Figure 2C). The maximum score for the case data category was ±9.

Supplementary evidence. This formed part of the evidence base if there was some suggestion of dengue presence, but the above three categories were insufficient to provide certainty on dengue status. If only two evidence types were available (see above), a score of 2 was given, three types = 4, four types = 6 (Figure 2D). Supplementary evidence carried a maximum score of 6.

Where a national score showed some uncertainty and an additional factor existed that was not captured by the default scoring system, an adjustment of up to ±3 was applied. For example, if multiple evidence categories suggested dengue presence in a country with high HE, but there was no case data, then the case data score was adjusted so as not to hold a disproportionate weight in deciding overall dengue status. This is termed the "ad hoc adjustment" (Figure 2E).

To derive an overall country evidence consensus score, the scores for all evidence categories were summed, and then divided by the maximum possible score and multiplied by 100. Evidence consensus was then mapped according to nine equal interval categories from 100% to −100% that differentiated evidence consensus worldwide, with evidence consensus being defined as

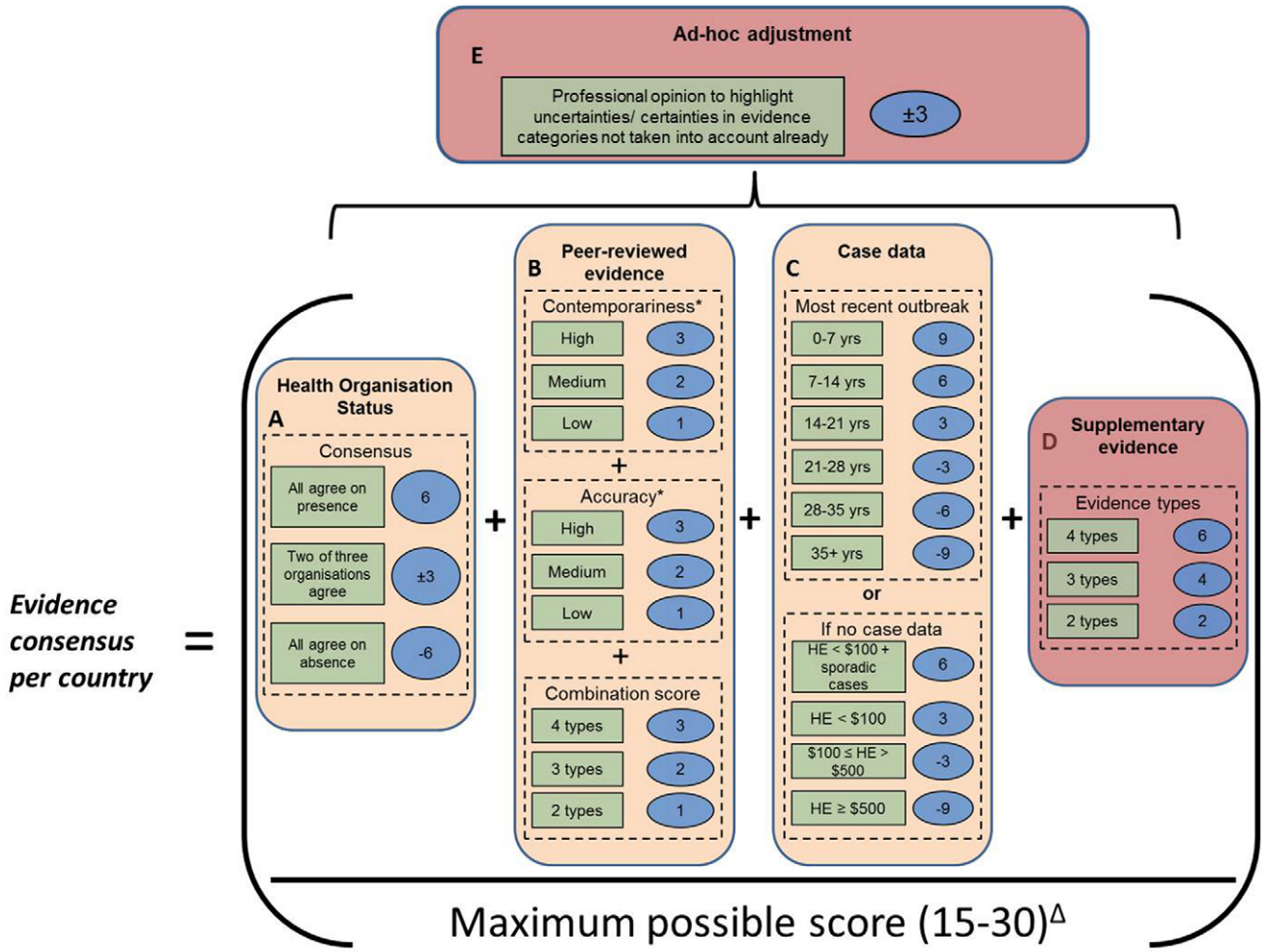

Figure 2. Overview of the evidence scoring system. Cream boxes represent mandatory categories while red boxes represent optional categories that are only used where required (see Methods). Dashed lines surround individual parameters that are assessed and totalled in the scoring system. Green boxes describe the level of evidence, with a given score in the blue oval. * Each individual piece of literary evidence is scored for contemporariness and accuracy before taking an average of the whole set then adding the combination score. Evidence consensus is calculated as the proportion of the maximum possible score from the dashed lined characteristics that are used. Δ Maximum possible score depends on which categories are included and can vary from 15 (Case data and Health organisation status, but no peer-reviewed evidence available) to 30 (all evidence categories included). Yrs = years. HE = total healthcare expenditure per capita at average U.S. $ exchange rates.
doi:10.1371/journal.pntd.0001760.g002

complete (±79% to ±100%), good (±57% to ±78%), moderate (±34% to ±56%), poor (±12% to 33%) or indeterminate (−11% to 11%). An odd number of intervals was chosen so as to highlight places where consensus is very low (indeterminate) and where improved surveillance is particularly needed. As such, the resulting classification of consensus scores should not be strictly interpreted, but rather taken as a general indication of the quality of dengue evidence in a given location. A full breakdown of the exact evidence included, individual scores and overall consensus percentages are given for each country in Table S1 and Figure S7.

Refining the evidence base and map with questionnaires targeted to consensus poor countries

In countries where evidence consensus was at best moderate, we attempted to increase consensus through targeted questionnaires. The questionnaire asked about endogenous surveillance and data collection. If available, diagnostic method(s) and summary results were requested. Any returned data or reports were then entered into their relevant evidence categories and scored in combination with existing evidence. Questionnaires were distributed to healthcare officials in the country of interest as well as selected offices of the Institut Pasteur. Questionnaire responses and expert comments are part of an on-going process that will lead to future modifications of this map.

Identification of countries that publically distribute dengue case data

To map public awareness of dengue worldwide, we searched the ministry of health websites of each of the 128 countries identified as dengue-present (evidence consensus positive but not indeterminate). A country was indicated as publicly displaying dengue data if national dengue case numbers were displayed annually or during epidemic years at a minimum.

Population at risk calculations

To calculate the maximum possible population at risk for dengue virus transmission we obtained total population counts from the Global Rural Urban Mapping Project (GRUMP) for the

128 countries identified as dengue-present. The GRUMP *beta* version provides gridded population count estimates at a 1×1 km spatial resolution for the year 2000 [26,27]. Population counts for the year 2000 were projected to 2010 by applying country-specific urban and rural national growth rates [28] using methods described previously [29]. As 2010 forms a landmark year for many national censuses, we were able to adjust these expanded population counts using the United Nations 2010 population estimates [30].

Results

Global distribution of dengue virus transmission based on evidence consensus

The global distribution of dengue virus transmission as defined by evidence consensus is shown in Figures 3–7. The mapped colour scale ranges from complete consensus on dengue presence (dark red) to indeterminate consensus on dengue status (yellow) then through to complete consensus on dengue absence (dark green). A full list of the evidence used for each area and their scoring is available in Table S1 and Figure S7. In total we identified 128 countries as dengue-present (i.e. positive values outside the indeterminate range), compared to 100 from the WHO, 104 from the CDC and 118 from GIDEON. Compared to the lists produced by the WHO and CDC, we identified 41 additional countries where evidence consensus for presence was outside the indeterminate range yet dengue-absent status was assigned by at least one of these health organisations.

Even after performing the sensitivity analysis described earlier, the number of countries defined by our methodology as dengue-present but defined by WHO/CDC as absent never dropped below 36 (Table 1). We therefore suggest that this list of 36 countries be subject to a review regarding their current health organisation dengue-absent classification. Of these countries, 31 had at least moderate consensus on dengue presence in our final analysis.

The majority of these newly identified dengue-present countries were in Africa and the evidence type that allowed greatest identification was returning traveller reports. These sporadic reports established preliminary evidence, which we improved with supplementary evidence and questionnaire retrieval to clarify dengue status if possible (Table 2). Outside of Africa, the remaining newly identified countries were almost exclusively islands in the Indian and Pacific Oceans and in the Caribbean. The reason for a lack of dengue presence identification by health organisations here is likely the longer interval between epidemics in small isolated nations, resulting in sparse data which different health organisations have interpreted inconsistently. Inclusion of less official surveillance evidence, such as ProMED reports, that detected background case loads alongside officially reported outbreaks allowed our distinction of these areas as in fact dengue-present.

A total of 3.97 billion people live in these 128 countries outside the indeterminate consensus class. Of these, 824 million live in urban and 763 million in peri-urban areas. These numbers therefore constitute plausible preliminary estimates for the maximum possible population at any risk of dengue transmission. We expect more comprehensive population at risk calculations to refine this figure and quantify levels of risk in our future work, allowing us to give a more accurate estimate.

Public display of dengue data varied by continent (Figure 8). In total, 46 of 128 dengue-present countries displayed annual dengue case numbers. Of these, the highest reporting coverage was observed in Asia and the Americas where 55% and 57% of countries respectively reported dengue publically. This figure was comparably worse in the Pacific (29%) and Africa, Saudi Arabia, Yemen and the western Indian Ocean islands (Africa+) where just 7% of dengue-present countries publicly report dengue and none on mainland continental Africa. There were no regional patterns in the level of dengue case data provided, although the publicising of epidemiological weeks in some Central and South American countries tended to provide higher levels of detail. Deaths due to DHF/DSS/severe dengue were far less commonly reported, although the data are available for some Central American countries. Even allowing for variable internet usage and endogenous public health systems, we highlight the magnitude of disparity in countries' provision of freely available dengue data.

The Americas

Dengue presence is well documented in the Americas with a continuous set of good- or complete- consensus countries from southern Brazil to the Mexico-U.S.A. border (Figure 3). However, a general regional classification was not producible as in some cases such as Montserrat and Saint Vincent and the Grenadines, where moderate rather than good consensus was found. With only 22% of dengue-present Caribbean countries displaying dengue data publically, dengue status in these small island nations that are characterised by longer inter-epidemic periods proved considerably more heterogeneous. This was mainly due to a lack of confirmed indigenous cases during recent epidemics.

Other regions of uncertainty reflect dynamic dengue status at the limits of the disease distribution. Lower consensus estimates in areas of Florida and Argentina result from reliance on smaller amounts of evidence from recent epidemics. Although the disease extent is better described in Florida (both in terms of resolution and consensus) due to greater data availability, uncertainty is still present due to the unknown persistence of recent events. A similar pattern of uncertainty exists in Texas but for different reasons, being that the occurrence evidence is older and six of seven counties have no record of occurrence since the late 1980s.

Africa+

A total of 58% of Africa+ countries had a good consensus or better but Africa still showed the highest levels of uncertainty in countries with poor consensus. Concentrations of higher consensus were identified in East and West Africa (Figure 4). Multiple seroprevalence surveys over several years [31,32,33,34,35] made the most significant contribution in defining East Africa's higher-consensus cluster which ranges from Sudan to Tanzania with only Uganda, Rwanda and Burundi exhibiting poor or worse evidence consensus. In addition to this, evidence of outbreaks in coastal areas of Yemen, Saudi Arabia and some evidence of spill-over into Egypt added certainty to the definition of the East Africa high-consensus cluster. Although not as contiguous a tract of countries, a higher-consensus region also exists in West Africa from Senegal to Gabon. Inclusion of reported dengue cases in travellers and soldiers returning from West Africa was available for 13 countries and proved the most useful information in this region.

Outside of these higher-consensus regions, evidence consensus is low and a series of countries with moderate or worse consensus can be identified from Chad to Mozambique with only the Democratic Republic of Congo exhibiting good evidence consensus. For many of these countries, there are sporadic reports of dengue occurrence combined with poor disease surveillance and a general lack of data. Dated seroprevalence surveys in areas where many other arboviruses are circulating did little to increase certainty. These factors result in a positive evidence consensus that is nevertheless highly uncertain in large portions of Africa. Even

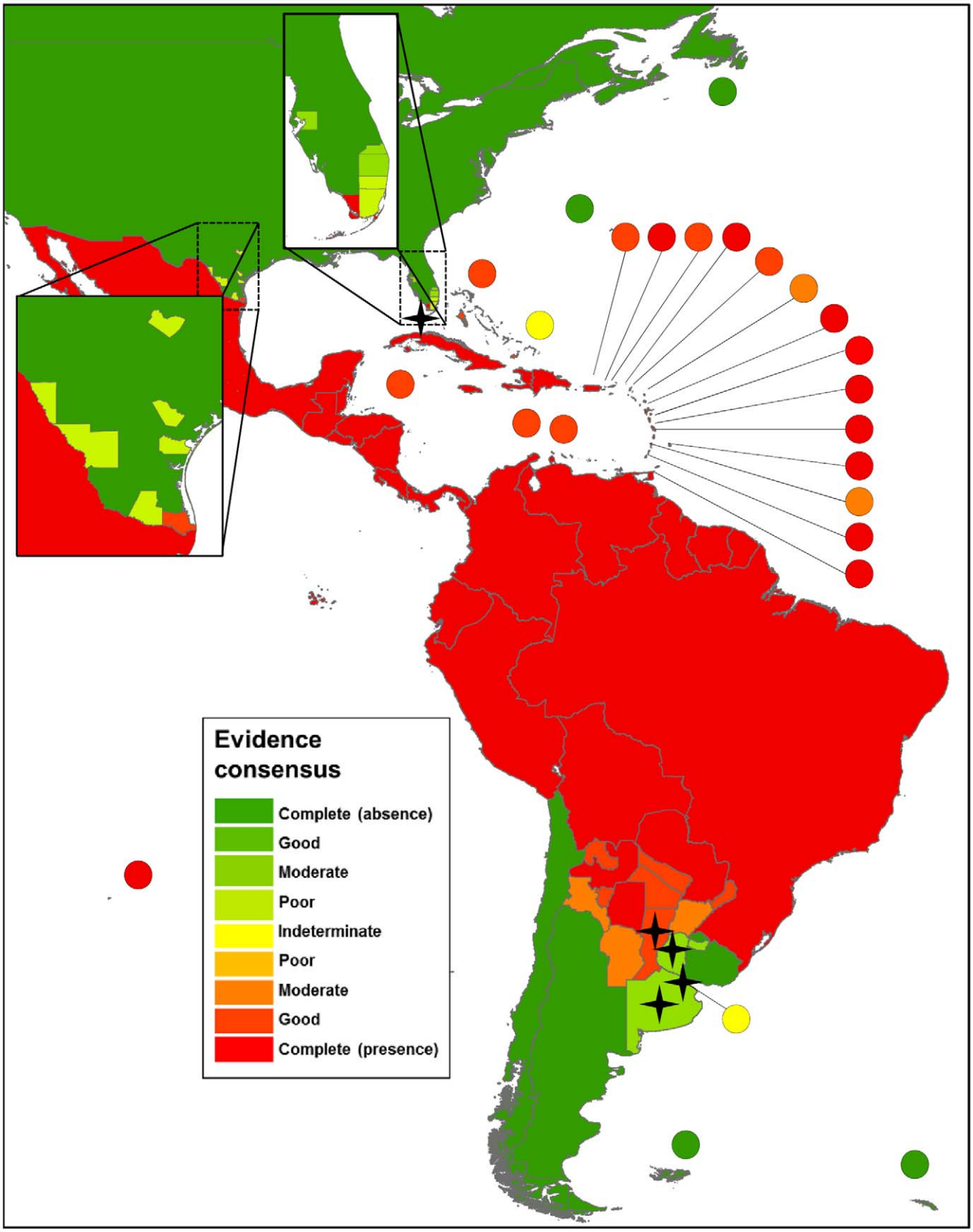

Figure 3. Evidence consensus on dengue virus presence and absence in the Americas. Figure 3 shows the areas categorised as complete evidence consensus on dengue absence in dark green, through to areas with indeterminate evidence consensus on dengue status in yellow, then up to areas with complete evidence consensus on dengue presence in dark red. Stars indicate one off indigenous transmission events with fewer than

50 cases. The map displays evidence consensus at Admin1 (state) level for Argentina and Uruguay, Admin2 (county) level for the United States of America and Admin0 (country) level for all other countries.
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where evidence was available from contemporary epidemics, such as in the case of the western Indian Ocean islands, it was often devalued because there was a lack of clinical differentiation between dengue and chikungunya despite epidemics coinciding. The lack of clear clinical distinction between the two diseases [36] makes the scale of dengue here difficult to identify and as a result, some countries (such as Reunion) were identified as having low consensus.

Despite the widespread uncertainty in dengue status in many African countries, we were able to differentiate multiple levels of uncertainty. Angola and Mozambique both show lower consensus due to dated evidence forms, yet they are still distinguishable from countries with no evidence or just sporadic occurrences such as Zambia or Congo.

Asia

A wide variety of contemporary evidence allowed us to display a near continuous distribution of good or complete evidence consensus countries from Indonesia to as far north as Pakistan and Zhejiang, China (Figure 5). Within this dengue-present area, 58% of countries publicly displayed dengue data (Figure 8) and many reported dengue case data with a high spatial resolution. Minor exceptions to this continuous distribution occur in southern China and North-East India largely due to a lack of contemporary evidence. In Gunagxi and Hainan there is little research interest or case data in recent years despite occurrences in urban centres further along the Chinese coast [37,38,39]. In North-East India, lower consensus was observed due to a lack of reported cases in recent years combined with the arrival of chikungunya in the area which complicates any potential dengue reporting [40].

Evidence consensus in Asia is lowest in central Asia where contemporary dengue occurrence records combined with low surveillance capacity results in an unclear boundary to the disease. While evidence for dengue presence in the lowland urban centres of Pakistan is accurate and contemporary, reports from the more remote north-west provinces are contemporary, but not accurate [41,42,43]. This makes determining the extent further north into remote and data-deficient areas of Afghanistan and central Asia

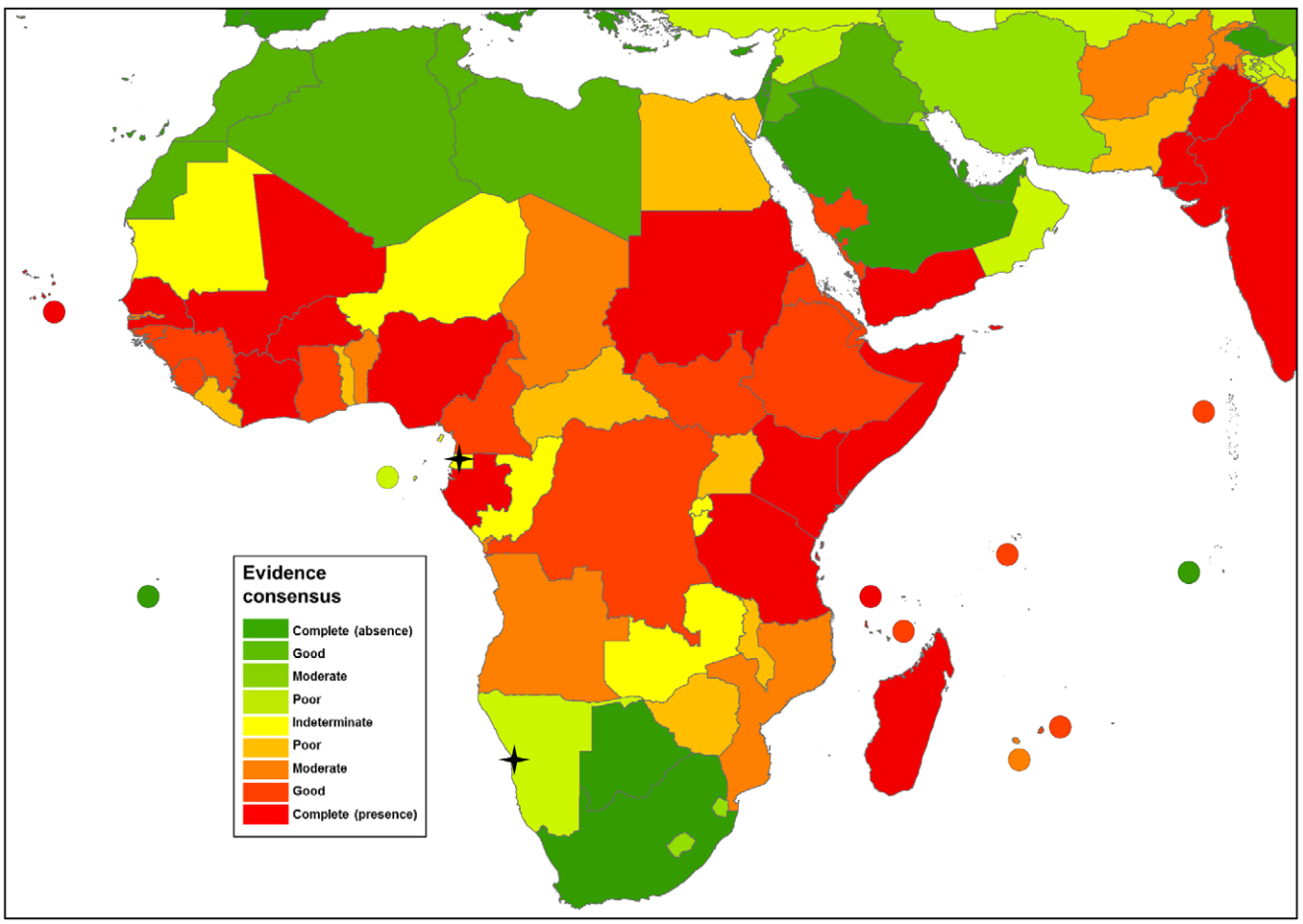

Figure 4. Evidence consensus on dengue virus presence and absence in Africa. Figure 4 shows the areas categorised as complete evidence consensus on dengue absence in dark green, through to areas with indeterminate evidence consensus on dengue status in yellow, then up to areas with complete evidence consensus on dengue presence in dark red. Stars indicate one off indigenous transmission events with fewer than 50 cases. The map displays evidence consensus at Admin1 (state) level for Saudi Arabia and Pakistan and Admin0 (country) level for all other countries.
doi:10.1371/journal.pntd.0001760.g004

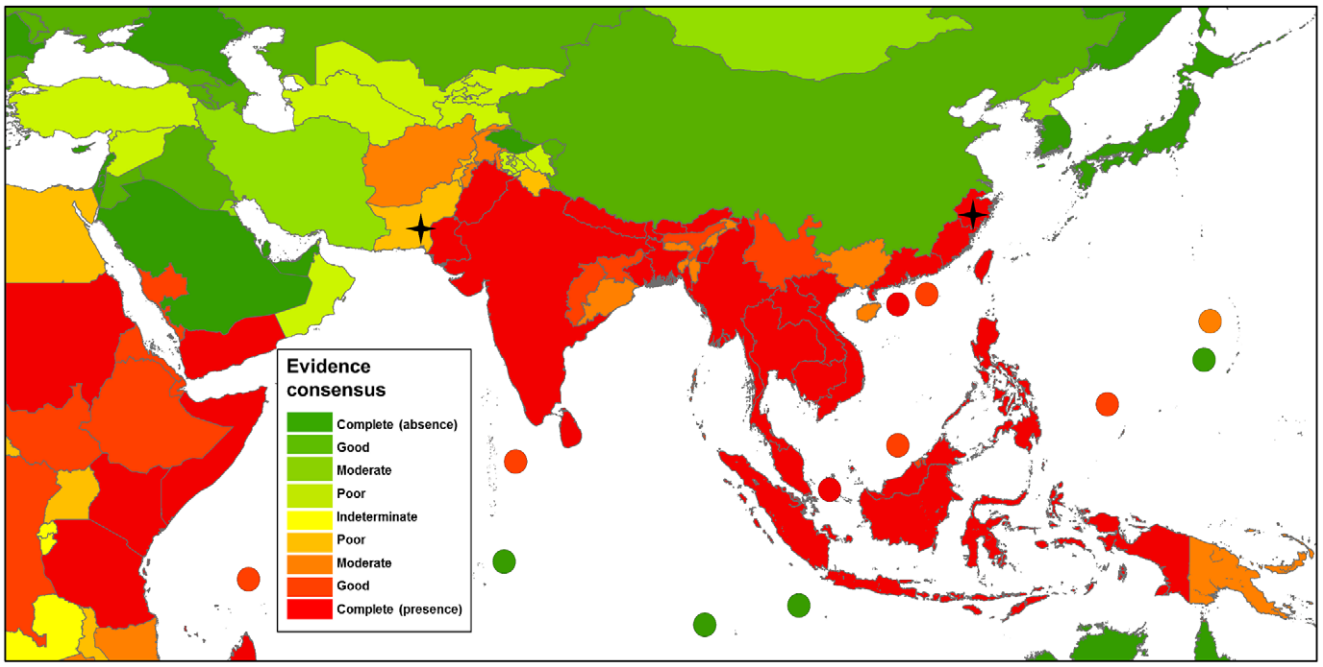

Figure 5. Evidence consensus on dengue virus presence and absence in Asia. Figure 5 shows the areas categorised as complete evidence consensus on dengue absence in dark green, through to areas with indeterminate evidence consensus on dengue status in yellow, then up to areas with complete evidence consensus on dengue presence in dark red. Stars indicate one off indigenous transmission events with fewer than 50 cases. The map displays evidence consensus at Admin1 (state) level for Saudi Arabia, Pakistan, India, China and South Korea and Admin0 (country) level for all other countries.
doi:10.1371/journal.pntd.0001760.g005

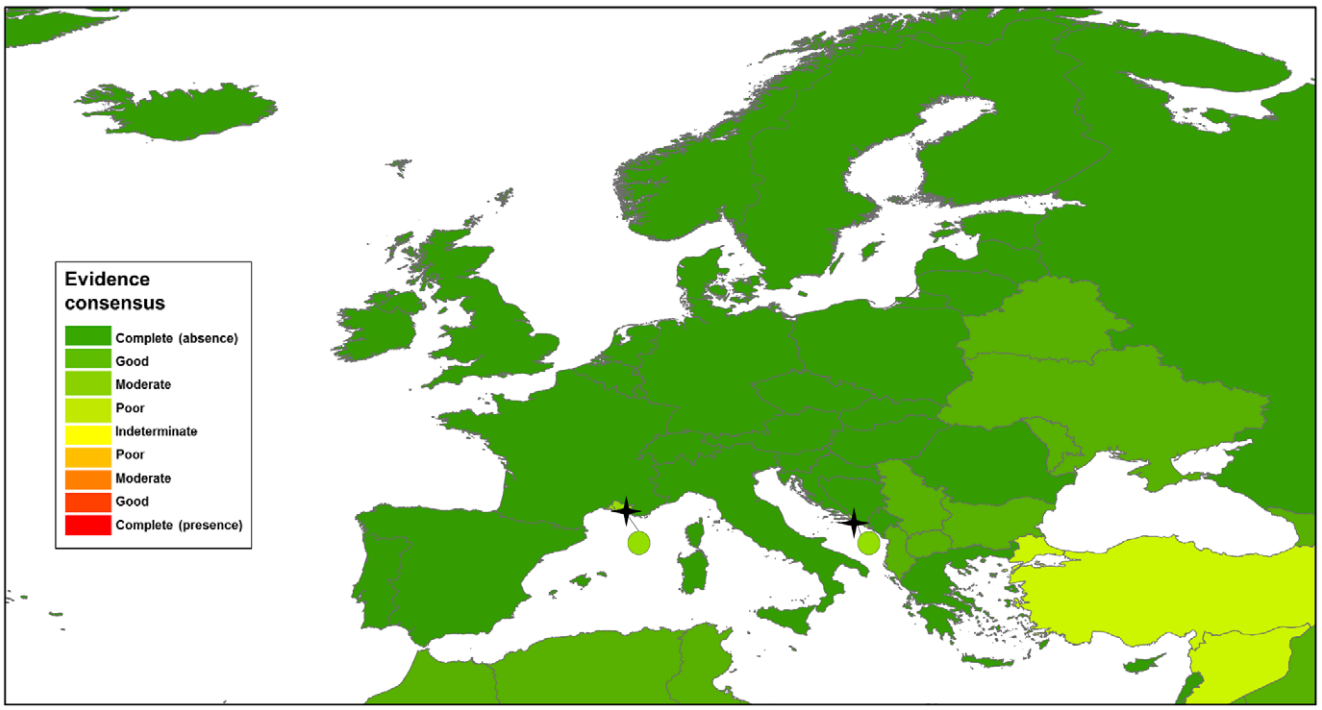

Figure 6. Evidence consensus on dengue virus presence and absence in Europe. Figure 6 shows the areas categorised as complete evidence consensus on dengue absence in dark green, through to areas with indeterminate evidence consensus on dengue status in yellow. Stars indicate one off indigenous transmission events with fewer than 50 cases. The map displays evidence consensus at Admin2 (county) level for France and Croatia and Admin0 (country) level for all other countries.
doi:10.1371/journal.pntd.0001760.g006

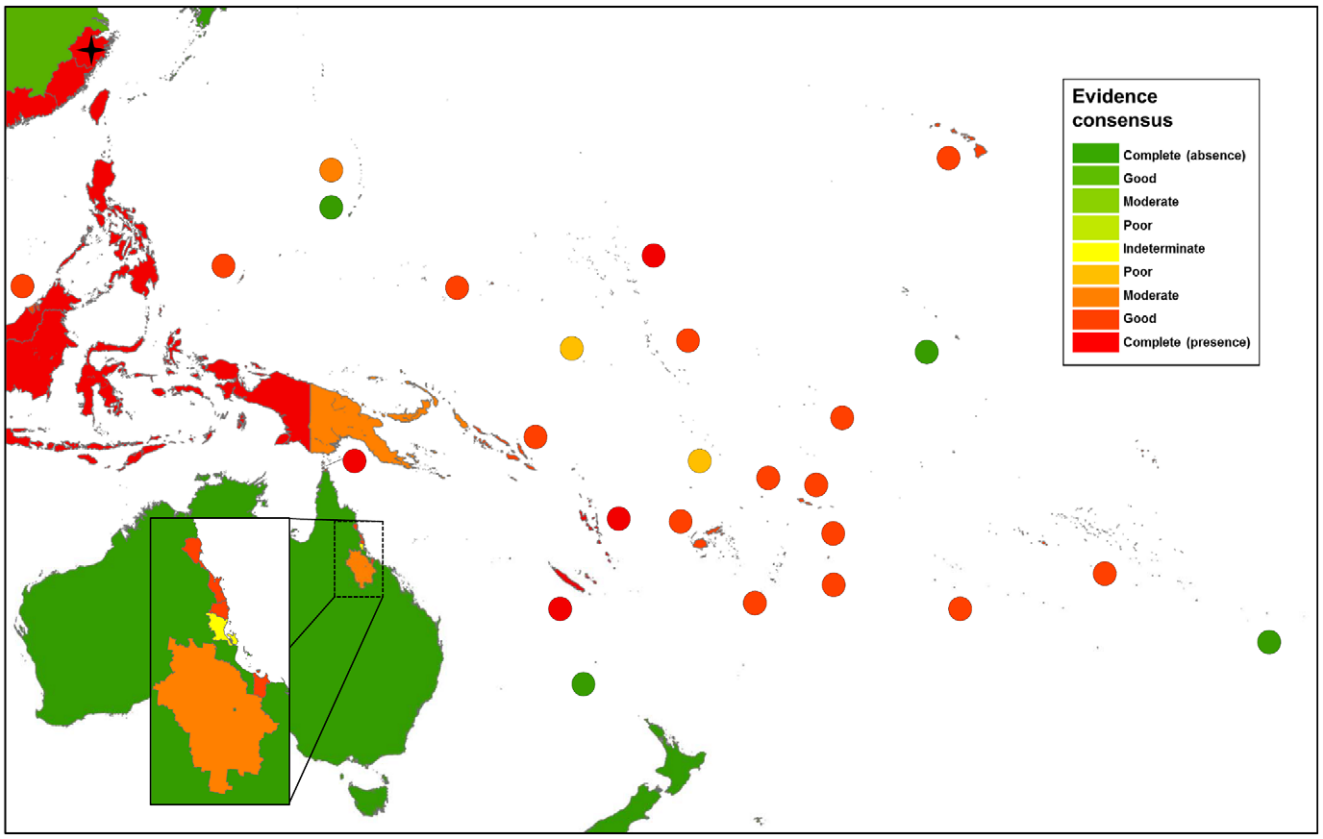

Figure 7. Evidence consensus on dengue virus presence and absence in Australasia. Figure 7 shows the areas categorised as complete evidence consensus on dengue absence in dark green, through to areas with indeterminate evidence consensus on dengue status in yellow, then up to areas with complete evidence consensus on dengue presence in dark red. Stars indicate one off indigenous transmission events with fewer than 50 cases. The map displays evidence consensus at Admin1 (state) level China, Admin2 (county) level for Australia and Admin0 (country) level for all other countries.
doi:10.1371/journal.pntd.0001760.g007

difficult to assess. We also found serologic evidence consistent with dengue presence in Turkey [44] and Kuwait [45], reducing evidence consensus for absence in these countries despite not belonging to any known cluster of dengue-present countries.

Europe

Although no countries in Europe were defined as dengue-present, sporadic indigenous transmission events have lowered consensus in some countries (Figure 6). Since the invasion and spread of *Ae. albopictus* along the Mediterranean coast [46], indigenous dengue transmission has been detected in Marseilles, France and Korčula, Croatia (both regions have moderate consensus on dengue absence) and chikungunya has been found in Italy (having good consensus on dengue absence) [47,48,49]. These isolated events do not in themselves confer dengue presence, but increased surveillance will be required in light of the *Ae. albopictus* invasion to maintain this status. This, combined with the lower levels of healthcare expenditure, has led to an observed greater uncertainty in some eastern European states.

Australia and Pacific Islands

In general, consensus on dengue presence and absence was well defined across Australia and the Pacific islands, with 85% of countries showing good or complete evidence consensus (Figure 7). Where low consensus was observed, it was largely due to a lack of contemporary evidence despite Pacific-wide dengue epidemics such as in Niue, Nauru, Tuvalu and Papua New Guinea. The duration between epidemics is typically longer in the Pacific and consensus is subject to continual change; for example, in the Marshall islands evidence consensus was upgraded from moderate to complete in the wake of the December 2011 epidemic, which came two decades after the last reported epidemic [50]. Such fluctuation is not entirely unexpected from remote, isolated communities, however. Even though evidence consensus decreases with time, it still remains positive, allowing for potential re-occurrence.

Lower evidence consensus was observed for Papua New Guinea due to a lack of reported case data since the 1980's, yet multiple literature sources suggest that dengue is still widespread [51,52,53]. While dengue occurrence is closely documented in some counties on the Australian coast, the serologic results from Charters Towers has contributed to uncertainty over the inland extent of the disease in Queensland [54]. Only the governments of Australia, New Caledonia and the Solomon Islands report dengue case numbers publicly. Considering the long intervals between epidemics in the Pacific, it is perhaps unsurprising that this is not a priority.

Discussion

Here we present the distribution of dengue virus transmission as assessed by evidence-based consensus. By emphasising the need for accurate, contemporary evidence through a weighted scoring

Table 1. Countries that require a reassessment of dengue status by health organisations.

Country	Evidence consensus (%)	Health organisations with dengue-absent status	Evidence included
American Samoa	Good (76)	CDC	2007 outbreak and SE
Aruba	Good (67)	WHO	2005 outbreak and PCR virus typing
Bahamas	Good (67)	WHO	2011 outbreak
Benin	Moderate (40)	WHO, CDC	Returning traveller reports, PCR virus typing and SE
Brunei	Good (75)	WHO	2010 outbreak, PCR virus typing
Cameroon	Good (76)	WHO	Seroprevalence surveys, returning traveller reports and questionnaire responzse
Cayman Islands	Good (69)	WHO	2010 outbreak and SE
Chad	Moderate (40)	WHO, CDC	Returning traveller reports and SE
Comoros	Complete (81)	WHO	2010 outbreak, seroprevalence survey and returning traveller reports
Cook Islands	Good (60)	WHO, CDC	2009 outbreak, PCR virus typing and SE
Djibouti	Good (75)	WHO	2005 outbreak, returning traveller reports and PCR virus typing
Eritrea	Good (63)	WHO	Returning traveller reports
Fiji	Good (69)	CDC	2012 outbreak and description of DHF
French Polynesia	Good (75)	CDC	2009 outbreak, PCR virus typing and description of DHF
Guinea-Bissau	Good (60)	WHO, CDC	Returning traveller reports, questionnaire response and SE
Kiribati	Good (71)	CDC	2008 outbreak and PCR virus typing
Liberia	Poor (29)	WHO, CDC	Reports of sporadic outbreaks and SE
Maldives	Good (71)	WHO	2011 outbreak and seroprevalence survey
Marshall Islands	Complete (80)	CDC	2011 outbreak
Mauritius	Good (65)	WHO	2009 outbreak, seroprevalence survey and PCR virus typing
Mayotte	Good (75)	WHO	2005 outbreak, seroprevalence survey and PCR virus typing
Micronesia	Good (69)	WHO, CDC	2011 outbreak, returning traveller reports, PCR virus typing and description of DHF
Netherlands Antilles	Good (75)	WHO	2008 outbreak and seroprevalence survey
Nauru	Poor (20)	CDC	PCR virus typing and SE
Niue	Good (65)	CDC	On-going-low level indigenous transmission with reports of sporadic outbreaks and PCR virus typing
Northern Mariana Islands	Moderate (54)	CDC	2001 outbreak and seroprevalence survey
Reunion	Moderate (43)	WHO, CDC	2010 outbreak, PCR virus typing and SE
Samoa	Good (68)	CDC	2001 outbreak, Returning traveller reports, PCR virus typing
Seychelles	Good (63)	WHO	2004 outbreak
South Sudan	Good (67)	WHO	PCR virus typing
Togo	Poor (30)	CDC	Returning traveller reports and SE
Tokelau	Good (60)	CDC	2001 outbreak
Tonga	Good (71)	CDC	2007 outbreak and returning traveller reports
Turks and Caicos Islands	Indeterminate (10)	WHO	Low level background case data, reported cases in peer-reviewed articles and SE
Tuvalu	Poor (30)	CDC	1998 outbreak, description of DHF and SE
Wallis and Futuna	Good (67)	CDC	1998 outbreak, PCR virus typing and SE

Table 1 shows countries for which we identified a consensus better than indeterminate on dengue-presence, but was listed as dengue-absent by the WHO or the CDC. WHO = World Health Organization, CDC = Centers for Disease Control, SE = supplementary evidence, PCR = polymerase chain reaction, DHF = dengue haemorrhagic fever.

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system, we were able to identify areas where dengue status was more uncertain, particularly in Africa and Central Asia, and identify evidence gaps where surveillance might be better targeted to more accurately assess dengue status. By including a wide variety of evidence we were able to cast doubt on dengue status in countries previously described by health organisations as dengue-absent.

While many studies have focussed on the future threat of dengue as a result of range expansion or climate change, this is the first to assess the entirety of knowledge regarding the extent of current virus transmission. We have found that evidence of dengue virus transmission is temporally dynamic and that a contemporary map must emphasise evidence by weighting it appropriately. By increasing temporal resolution to one inter-epidemic period, we have extended the approach of Van Kleef *et al.* [5] who used evidence from literature searches to produce distribution maps pre- and post- 1975. Focussing on a higher resolution timescale for dengue evidence is necessary if we are to infer changes in the evidence-based distribution of dengue.

The suggestion that dengue is an under-recognised problem in Africa is not a new one [55,56,57], but here we present a detailed

Table 2. Evidence consensus class changes in Africa as a result of including supplementary evidence and questionnaire responses.

Country	Evidence consensus class excluding questionnaires and supplementary evidence	Evidence consensus class including questionnaires and supplementary evidence
Equatorial Guinea	Poor (absence)	Indeterminate
Mauritania	Poor (absence)	Indeterminate
Niger	Poor (absence)	Indeterminate
Central African Republic	Indeterminate	Poor
Liberia	Indeterminate	Poor
Malawi	Indeterminate	Poor
Uganda	Indeterminate	Poor
Zimbabwe	Indeterminate	Poor
Angola	Poor	Moderate
Benin	Poor	Moderate
Chad	Poor	Moderate
Guinea-Bissau	Poor	Good
Cameroon	Moderate	Good
Côte d'Ivoire	Good	Complete
Nigeria	Good	Complete
Sierra Leone	Good	Complete

All classes refer to consensus on dengue presence unless otherwise stated. Supplementary evidence was available for all countries in this table, while questionnaire responses were received from Cameroon, Burkina Faso, Malawi, Guinea-Bissau, Gabon and Côte d'Ivoire.
doi:10.1371/journal.pntd.0001760.t002

summary of the specific gaps in evidence that exist in different regions. We show that consensus mapping is flexible to regional differences in evidence availability and as such can produce meaningful outputs in resource-high and low settings. The evidence that dengue is widespread in Africa implies that the continent is underrepresented by occurrence points in the model-based approaches that have been used to investigate the distribution of dengue so far [1,10,11]. If we are to estimate the burden of dengue in Africa with any fidelity, available data and their underlying assumptions need to be reassessed.

Evidence consensus maps provide a more informative alternative to existing country-level maps, such as those provided by the WHO [12] and CDC [58]. As presence or absence exists on a continuous scale of certainty, evidence consensus approaches are more adaptable to incorporating diverse forms of dengue evidence ignored by these organisations in producing their estimates. While we show that different evidence weightings in our scoring system do not significantly alter the result, we were unable to formalise a statistical validation of these weightings due to lack of a training dataset. Our results provide the best estimate thus far of where such data are most needed and comparisons with higher-consensus countries in similar settings should form the first step in directing regional surveillance. Development of methodologies to make approaches such as consensus mapping more reliable is needed as dengue status will increasingly rely on harder-to-quantify evidence types, such as internet search engine terms [59] and multi-language internet text-mining systems [60,61]. The success of automated disease surveillance systems such as HealthMap [16,17,62] and Biocaster [60,63] have already been demonstrated. We believe evidence consensus provides the best platform for integrating these diverse forms of information now available for disease occurrence to create an

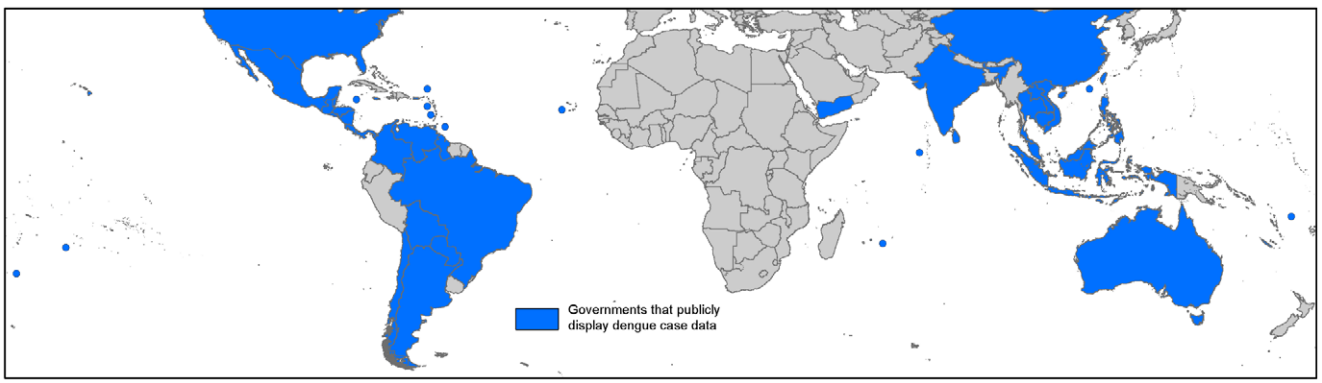

Figure 8. The worldwide variation in governments that publicly display dengue data. The map shows governments that at a minimum display dengue case data at a national level yearly via their official Ministry of Health website.
doi:10.1371/journal.pntd.0001760.g008

up-to-date, high-resolution map of dengue evidence, whilst retaining important assessments of certainty. We also intend to extend our own data collection and accessibility with a new website linked to the Global Health Network (http://globalhealthtrials.tghn.org/) that will allow evidence contribution from members and will provide a key platform for display of dengue data and consensus maps. Although the current approach was used to map the distribution of dengue, minor modifications to the scoring system would allow it to be utilised for a variety of diseases for which the quality of presence evidence is spatially variable.

In this work, our aim was to produce a standardised methodology that used the largest variety of evidence to assess country dengue status, whilst still being applicable in diverse healthcare settings and suitable at multiple spatial scales. We considered the stark contrast in evidence available in Africa as compared to the rest of the world. Our results show that the inclusion of supplementary evidence (used in 44% of African countries but only 11% of the rest), healthcare expenditure information (for case data absences) and questionnaires increased evidence consensus in these countries without impacting the methodology applied to the rest of the world. Similarly, we are aware that increasing resolution to Admin1 or Admin2 level may well reduce the evidence available for calculating evidence consensus in each area compared to country-level calculations. As a result, we carefully chose which countries should have increased spatial resolution based on whether sufficient evidence was available in smaller administrative units. We also limited the selection of these countries to those at the limits of the disease's distribution, as data deficiencies in these regions more accurately represent the uncertainty on dengue status given the dynamic nature of global dengue spread. Here we present the most flexible methodology available, to date, for overcoming these problems. We have demonstrated that a systemic approach with relevant optional categories has allowed us to utilise the maximum variety of evidence available for assessing dengue status in the widest variety of situations.

We also openly provide a full list of evidence for each country by category (Table S1). We intend to continue data acquisition by including more endogenous, local evidence through questionnaires and local language search methods, which we expect will allow us to further customise our methodology and assess dengue status in places where we are currently uncertain.

Mapping by evidence consensus is a useful approach to quantifying contemporary disease evidence and can be further integrated with geo-spatial modelling to produce worldwide continuous surfaces of dengue risk [64]. Current mapping approaches use presence/absence expert opinion maps to sample pseudo-presence or pseudo-absence points to increase the number of data points on which to base their prediction [65,66,67,68]. Pseudo-sampling could be improved by using the continuous scale of evidence consensus to either affect sample number or point weight within the geo-spatial model. This will lead to more robust, higher resolution dengue maps which are currently in progress [69]. By combining uncertainty assessment from consensus mapping with high-resolution predictions using geo-spatial modelling, we will be able to make more accurate predictions of disease burden with associated confidence intervals made explicit. This will then provide a series of up-to-date assessments of global dengue distribution, thus providing key information to assess dengue spread and the impact of control measures.

Supporting Information

Figure S1 Geographic locations of occurrence data globally. Country colouring is based on evidence based consensus (see main manuscript) with green representing a complete consensus on dengue absence and red a complete consensus on dengue presence.
(TIF)

Figure S2 Geographic locations of occurrence data in Africa+. Country colouring is based on evidence based consensus (see main manuscript) with green representing a complete consensus on dengue absence and red a complete consensus on dengue presence.
(TIF)

Figure S3 Geographic locations of occurrence data in Asia. Country colouring is based on evidence based consensus (see main manuscript) with green representing a complete consensus on dengue absence and red a complete consensus on dengue presence.
(TIF)

Figure S4 Geographic locations of occurrence data in the Americas. Country colouring is based on evidence based consensus (see main manuscript) with green representing a complete consensus on dengue absence and red a complete consensus on dengue presence.
(TIF)

Figure S5 Geographic locations of occurrence data in Australia. Country colouring is based on evidence based consensus (see main manuscript) with green representing a complete consensus on dengue absence and red a complete consensus on dengue presence.
(TIF)

Figure S6 Number of occurrence samples per year globally (a) and for Africa+ (b), Asia, (c) the Americas and Australia (d).
(TIF)

Figure S7 Map of evidence types used for each national and subnational area. Figure S7 shows the different evidence categories used in assessing evidence consensus for each country and Admin1/2 area. HO = health organisation status, L = literary evidence, CD = case data, SE = supplementary evidence, PO = -professional opinion.
(TIF)

Protocol S1 An outline of the dengue occurrence point database construction and content. Data sources, searches and exclusion criteria are outlined and the method of geo-positioning explained. The regional bias of available occurrence points is also given in the accompanying figures. Table S1 shows the collection of evidence used to assess evidence consensus for each country and Admin1 and Admin2 areas. Details of the scoring system can be found in the Methods section of the main manuscript. Scores for each category are highlighted in red. Evidence consensus is calculated as the percentage of the maximum possible score (see Fig. 2 in the main manuscript). HE = healthcare expenditure, DENV = dengue virus, DHF = dengue haemorrhagic fever, DSS = dengue shock syndrome, PCR = polymerase chain reaction, DF = dengue fever.
(DOC)

Table S1 The collection of evidence used to assess evidence consensus for each country and Admin1 and Admin2 areas. Details of the scoring system can be found in the

Methods section of the main manuscript. Scores for each category are highlighted in red. Evidence consensus is calculated as the percentage of the maximum possible score (see Fig. 2 in the main manuscript). HE = healthcare expenditure, DENV = dengue virus, DHF = dengue haemorrhagic fever, PCR = polymerase chain reaction, DF = dengue fever.
(DOC)

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Author Contributions

Conceived and designed the experiments: OJB PWG SIH TWS CLM AWF. Performed the experiments: OJB. Analyzed the data: OJB JPM CLM SB JSB AGH. Contributed reagents/materials/analysis tools: JSB AGH. Wrote the paper: OJB CLM AWF TWS PGW.

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Chapter 3 – Estimating the global burden of dengue

3.1. Cover page

Chapter 2 demonstrated how a semi-quantitative assessment of reported disease data could help refine the global distribution of dengue at a national and subnational level. I, along with a number of my colleagues in the Spatial Ecology and Epidemiology Group (SEEG), used the outputs of chapter 2 in combination with a number of environmental and socio-economic global covariate layers to fit a species distribution model for DENV transmission. This allowed us to refine the evidence-based national and sub-national map to a 5km x 5km surface of risk (Fig. 3.1., full details in the Appendix page 182) *(Bhatt et al., 2013)*:

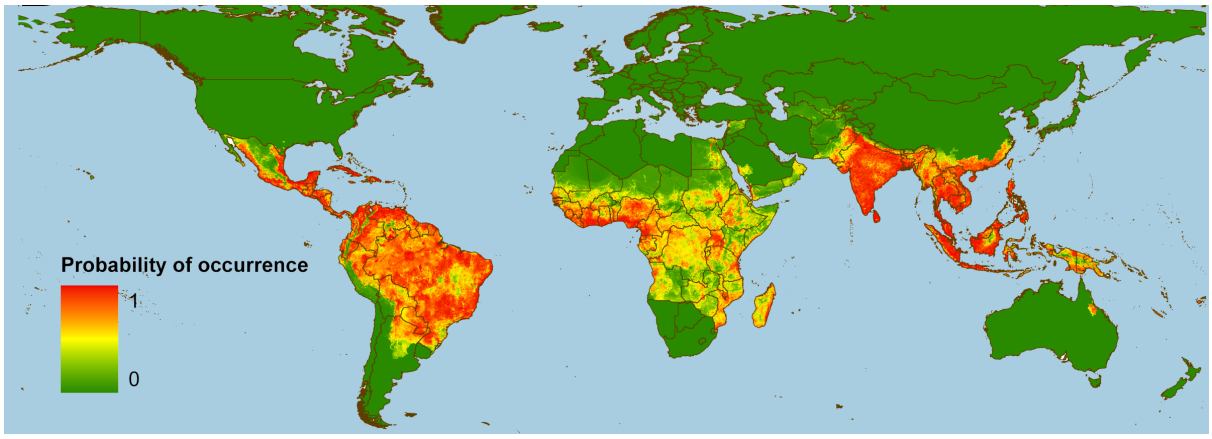

Figure 3.1. Modelled global distribution of dengue in 2013. The above map shows the predicted probability of one or more dengue cases occurring at a 5km x 5km pixel scale. Figure reproduced from Bhatt *et al.* (2013).

While details of the full project can be found in the appendix (page 182), this chapter specifically focusses on development of a model that converts the risk map in Fig. 3.1. into estimates of global apparent and inapparent case burden. This is a measure of significant public health importance and one that is widely used for disease funding prioritisation. The combined risk map and burden estimates were published in *Nature*

in April 2013 *(Bhatt et al., 2013)*. The risk mapping aspects of this project were primarily the responsibility of my co-author Dr. Samir Bhatt, while I was responsible for collecting and analysing the data from a collection of cohort studies and interpreting the results of the burden model as well as writing all sections of the manuscript that summarised these aspects. Background, methods, results and discussion sections of this burden estimation approach are outlined in detail in self-contained sections of the supplementary information from Bhatt *et al.* (2013) which are presented here as published with minor reformatting for consistency. The main manuscript and other parts of the supplementary information can be found in the Appendix section (page 182).

The resulting risk maps and burden numbers have sparked considerable interest and debate in the dengue community. From 2015 the global totals for symptomatic infections will become the WHO's official burden estimate (Raman Velayudhan personal communication November 2014) and the numbers have gone on to be used by a number of advocacy consortia, e.g. the Dengue Vaccine Initiative (DVI) *(Messina et al., 2013)*. The work has also stimulated ongoing discussion which the SEEG group are involved in on approaches to improve dengue burden estimation on the national and global scale. Among these projects are an in-depth study involving the New Caledonian ministry of health *(Brady, 2014)*, the development of a WHO handbook for burden estimation in partnership with Sanofi Pasteur and an effort to revise the dengue burden analysis for the Global Burden of Disease (GBD) project *(Murray et al., 2013)*.

3.2. Overview of burden model and past burden estimates

Despite the widely quoted figure of 50-100 million dengue infections per year *(Gibbons and Vaughn, 2002; Guzman and Kouri, 2002; WHO, 2012a)*, contemporary estimates

of the annual global incidence of dengue have a limited evidence base. The first estimate in 1988 suggested an approximate figure of 100 million infections per year, based on assuming a ten percent annual infection rate amongst a population-at-risk of one billion *(Halstead, 1988)* (Figure 3.2.). This annual infection rate was based on data from a small number of epidemics in the latter half of the 20th century. Although this was only ever intended as an approximate estimate, the figure of 100 million is still widely cited and reproduced, despite the realisation of a much larger population-at-risk and a more variable infection rate than was originally assumed.

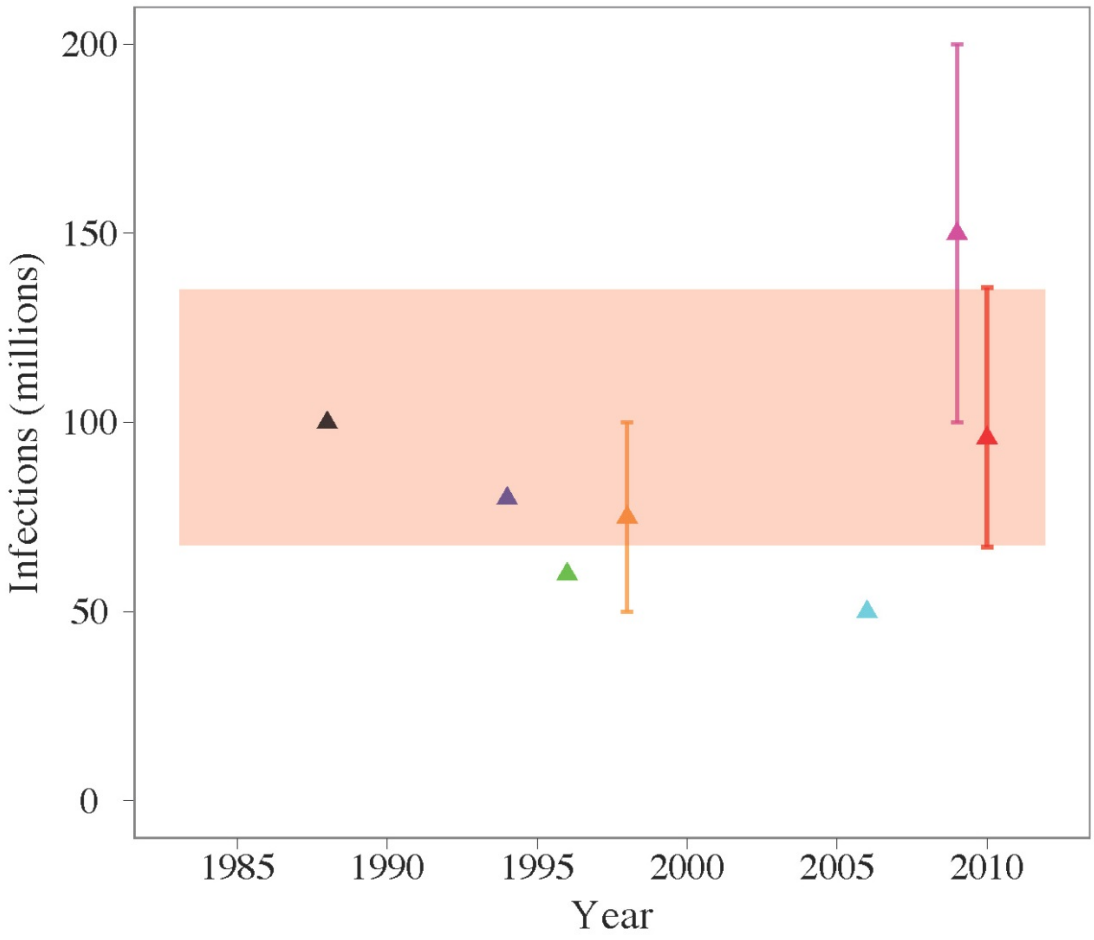

Figure 3.2. Global estimates of dengue infections. Comparison of previous estimates of total global dengue infections in individuals of all ages, 1985 to 2010: ☐ Halstead *et al.* 1988 *(Halstead, 1988)*, ☐ Monath *et al.* 1994 *(Monath, 1994)*, ☐ Rodhain *et al.* 1996 *(Rodhain, 1996)*, ☐ Rigau-Perez *et al.* 1998 *(Rigau-Perez et al., 1998)*, ☐ TDR/WHO. scientific working group 2006 *(TDR, 2006)*, ☐ Beatty *et al.* 2009 *(Beatty et al., 2009)*, ☐ apparent infections from this study. Estimates are aligned to the year of

estimate and, if not stated, aligned to the publication date. Red shading marks the credible interval of our current estimate for comparison.

A revision was made in 1994 using the same methodology *(Monath, 1994)*, after it became clear that dengue was far more widespread and there was increasing uncertainty over the proportion of inapparent infections. The evidence base for the four percent annual infection rate in this work is unclear and results in a lower estimated burden of 80 million infections per year. As more information became available concerning (i) the ratio of dengue hemorrhagic fever (DHF) cases to dengue fever (DF) cases and (ii) the ratio of deaths to DHF cases, a figure of 50-100 million infections globally gained more support *(Rigau-Perez et al., 1998; Rodhain, 1996)* (Figure 3.2.). This figure has since been adopted by the WHO and has been their estimate for the last 15 years.

In the absence of accurate or suitable estimates for the apparent-to-inapparent infection ratio and with a decline in completeness of global dengue reporting *(Suaya et al., 2007)*, progress on global burden estimation was hindered. Attention moved to estimating numbers of DHF and DF clinical cases using generic methods of "expanding/inflating" reported DF or DHF cases. The DHF case reporting in South East Asia (SEA) was considered to be the most accurate in the early 2000s, and therefore sex-specific DHF reported incidence values for SEA were extrapolated to give a global estimate of 0.4-0.5 million cases of DHF a year *(LeDuc, 2004)*. Using this figure and several estimates of the ratio of DHF to clinical DF cases, a global figure of 8 million cases of clinical DF was then suggested *(Cattand et al., 2006)*. A similar figure of 9 million clinical DF cases was presented in the WHO Global Burden of Disease 2004 update, which used reported dengue deaths and separate estimates of the DHF-to-

clinical DF cases ratio for SEA and the Americas *(WHO, 2004)*. Methods similar to these have also been widely used to estimate global deaths due to dengue.

While the apparent-inapparent link may, until now, have been insufficiently evidence-based, many attempts have been made to calculate national incidence estimates for the purpose of economic burden estimation *(Carrasco et al., 2011; Clark et al., 2005; Garg et al., 2008; Luz et al., 2009; Meltzer et al., 1998; Shepard et al., 2011; Standish et al., 2010; Suaya et al., 2009; Wichmann et al., 2011)*. These vary in their thoroughness, but the best approach has been to use locally relevant cohort studies to derive sex- and age-specific estimates for the apparent-inapparent ratio and then apply these to multi-year reported clinical DF datasets. A more common approach is to gather a range of these ratios, which range from 1:0.3 to over 1:100, and then apply them to national clinical DF case data of variable reliability. The major problem with this approach is geographical variation in dengue transmission intensity, treatment-seeking behaviour and healthcare treatment and reporting capacity. A factor that is often overlooked is that many of the cohort studies employ active fever surveillance in their study population which reduces barriers to healthcare access, thus modifying treatment-seeking behaviour *(Gething et al., 2010)*. This ensures simply converting nationally reported numbers of clinical dengue cases to infections using a common factor is likely to give a significant underestimate of true infection incidence.

Although the inflation factor approach is unsuitable on a global scale, this method of inference based upon cohort studies has reinforced the estimate of 50-100 million infections. Of the 2.2 million clinical dengue cases reported to the WHO in 2010, 1.7 million of these were reported from the Americas *(WHO, 2012c)*. Therefore, using an average range of previously-used expansion factors for this region (6-27) *(Armien et al., 2008; Meltzer et al., 1998; Standish et al., 2010)* would suggest that between 10 and 46 million dengue infections occur in the Americas alone *(Meltzer et al., 1998)*.

The variability in national clinical dengue case data has prompted the latest estimate, which excludes it altogether. Beatty *et al. (Beatty, 2009a)* redefined a dengue-endemic country as having a published record of dengue occurrence or sharing a significant border with one that does. This suggested a population-at- risk of 3.5 billion from which average inflation factors suggest a total of 100-200 million dengue infections and 36 million cases of clinical DF per year *(Beatty, 2009a; Beatty, 2009b)*. This generalized single factor global approach is unlikely to give accurate national case burden estimates, however, their focus on creating an evidence base for the global extent of the disease creates a better basis for initial burden estimates than approaches that use reported case data of variable or unknown quality.

Considering the variable data sources on which these estimates are based, it is perhaps surprising that confidence intervals are not presented. With the exception of Rigau-Perez *et al. (Rigau-Perez et al., 1998)* and Beatty *et al. (Beatty, 2009a; Beatty, 2009b)*, no existing estimates have conveyed the uncertainty present in dengue burden estimation (Figure 3.2.), yet this is considered a vital step for accurate interpretation of these estimates.

This study has produced the first cartographic based burden estimate for dengue. This involved assembling an extensive data set of 54 cohort studies that defined incidence rate in person years with estimated the inapparent-to-apparent ratio. A hierarchical Bayesian model was then used to model a relationship between the cohort incidence data and the previously generated boosted regression tree map of the probability of occurrence of dengue infection (see Appendix section (page 182)), following a rubric applied previously to malaria *(Patil et al., 2009; Snow et al., 2005)*. Using this relationship, it was possible to provide estimates of annual inapparent and apparent dengue infections with confidence intervals at the national, continental and global scales.

3.3. Assembly of cohort studies

3.3.1. Existing incidence data

Estimating incidence of dengue fever is complicated by a spectrum of clinical manifestations *(2009)* and the variable reporting capacity of different healthcare systems. Therefore, the most accurate way of comparing incidence rates in different locations worldwide is through serological cohort studies that follow a standard methodology. Due to the high proportion of inapparent dengue infections *(Endy et al., 2011)*, active case detection must go beyond improved clinical surveillance. Estimates of total cohort dengue infections must, therefore, observe immune responses to dengue virus antigens before and after the dengue transmission season *(Kao et al., 2005)*. Dengue virus-infected humans exhibit dengue-specific Immunoglobulin M (IgM) and Immunoglobulin G (IgG) immune responses. High IgM titres can be observed in primary infections as soon as four days *(Simmons, 2012)* after the onset of fever and persist for another 30-90 days *(Cuzzubbo et al., 2000)*. IgM responses can also be seen in secondary dengue infections, but the response is often slower, weaker and shorter lived *(Innis, 1997)*. In contrast IgG levels rise around seven days *(Simmons, 2012)* after the onset of fever in primary infection and can be observed for life *(Innis, 1997)* thus providing a good indicator of previous dengue exposure. The total number of primary infections can be obtained by observing the number of IgG-negative individuals that seroconvert to IgG-positive status over the course of the transmission season. The number of post primary dengue infections can be estimated by identifying individuals with both IgG and IgM responses after the dengue transmission season. This is likely to under-estimate post primary infections due to weaker secondary IgM responses *(Innis, 1997)* and the 30-90 day window for IgM detection *(Cuzzubbo et al., 2000)*. Experimental protocols often assume infection is the result of certain

predominant serotypes that may be circulating in a given study region at the time and thus monitoring may be type-specific, resulting in further underestimation if multiple serotypes are circulating. IgM and IgG serologic surveys also cross-react with other flaviviruses *(Allwinn et al., 2002)* and therefore need situation-specific controls to estimate sero-conversions to dengue alone. However, despite these limitations, serological cohort studies represent the best possible method for estimating dengue infection incidence at a given site.

Cohort studies are often difficult to compare due to different focus demographics. For dengue, higher than normal incidence of infection and clinical disease have been reported in both paediatric and non-paediatric populations in different settings *(Vong et al., 2012)*. Therefore, having no robust function with which to adjust for age and no clear demographic basis for exclusion of available information, our assemblage of cohort studies comprised incidence in all age groups. The inclusion criteria developed for the cohort study database are described below.

3.3.2. Inclusion criteria

(i) The study took place during or after 1960 to coincide with the date range of our occurrence database.

(ii) Surveys were longitudinal and involved active case detection of sero-conversion to dengue type-specific antibodies in a defined cohort.

(iii) Monitoring of sero-conversion through paired blood samples was undertaken at least before and after each dengue transmission season for IgG immune responses, or at least every 90 days for IgM immune responses *(Chungue et al., 1989; Cuzzubbo et al., 2000)*.

(iv) Data was presented in a way that enabled the total number of infections and the number of person-years of observation to be obtained.

(v) All dengue infections were identified from blood samples that distinguished primary and secondary infections using all or one of the following methods: (a) Hemagglutination inhibition (HI), (b) plaque reduction neutralization test (PRNT) or (c) enzyme-linked immunoabsorbant assay (ELISA).

(vi) Surveys were conducted over at least a 12 month period, or else over a clinically defined period of transmission with blood samples taken before and after this period.

The location, total number of infections, person-years of observation and the ratio of inapparent to apparent (I:A) infections was recorded. Definitions and methods for detecting apparent infections varied from study to study but adhered to the following general criteria: (i) an apparent case was any manifestation of febrile illness accompanied by fever greater than 38^{o}C; (ii) such infections were detected either through enhanced clinical surveillance, retrospective cohort participant questionnaires, or systematic surveillance of school or workplace absentees. Therefore our definition of an inapparent infection is an infection that does not have any impact on the day-to-day life of the subject. As such, an inapparent infection will not modify a person's regular schedule e.g. attending school, register as an extraordinary period of ill-health that can be recalled when later questioned, nor will it prompt any treatment-seeking beyond self medication. While each of the separate symptom detection methods have the potential to underestimate apparent infections, they allow us the best possible estimate of the I:A ratio.

3.3.3 Summary

A search for "dengue cohort study" in PubMed, subsequent reference tracking, and personal requests enabled the identification of 54 geographically unique locations from 38 cohort studies in 19 countries in a variety of regions. Estimates of the I:A ratio were available for 40 locations (27 cohort studies, 15 countries). We excluded one cohort study by Teixeira *et al. (Teixeira Mda et al., 2002)*, as both the reported incidence and I:A ratio were an order of magnitude outside the others observed. Excluding this study, the mean reported incidence across all cohort studies was calculated as 129.7 per 1000 person years (standard deviation ±135) and the mean I:A ratio as 4.3 (standard deviation ± 2.8). The incidence and I:A ratio from Teixeira *et al. (Teixeira Mda et al., 2002)* was 706 and 1555.55 respectively, which we deemed implausible given the other cohort studies.

3.4. Relationship between incidence and probability of occurrence

We determine the relationship between incidence and probability of occurrence using a Hierarchical Bayesian linear model *(Gelman et al., 2013)*. We chose a Bayesian formulation due to statistical robustness, explicit handling of uncertainty, transparent variable and model selection and the ease of incorporating complex nonlinear functions *(Gelman et al., 2013)*. The Bayesian hierarchical model is defined as a tiered structure where, at the first level, a likelihood function defines the probability distribution that generates the data (the data model - *[data|process,parameters]*) , at the second level, prior distributions define the parameters of the likelihood function (the process model - *[process|parameters]*), and at the third, and final level, hyper prior distributions define the prior parameters (the parameter model - *[parameters]*). The end result of the product of these three distributions is proportional to the posterior distribution which is the distribution of the process and the parameters *[process,parameters|data]*.

3.4.1 Data model

Modelling count data (incidence per 1000 person years) imposes restrictions on the choice of probability distribution as an event count is the realization of a non-negative integer-valued random variable *(Cameron and Trivedi, 1986; Winkelmann, 2000)*. The foundation building block in this modelling framework is the Poisson regression model where the variance of a random variable is constrained to equal the mean *(Winkelmann, 2000)*. However, a more broadly applicable and general specification – the negative binomial model *(Hilbe, 2011; Patil et al., 2009)* can be used to include the case when the variance exceeds the mean. We choose this distribution to ensure maximum flexibility in modelling our data:

$$p(x|f(x), r(x)) = \frac{\Gamma(x + \mathrm{V})}{x!\,\Gamma(V)}\left(\frac{V}{f(x)+V}\right)^{V}\left(\frac{f(x)}{f(x)+V}\right)^{x} \quad (1)$$

where x is the incidence per 1000 person years, $f(x)$ is the mean or rate function defining how the mean value of incidence changes with the probability of occurrence and $r(x)$ is the noise or dispersion function, representing the variance in population-wide levels of incidence.

3.4.2. Process model

We model the negative binomial rate function, $f(x)$, using a Gaussian process *(Patil et al., 2009; Rasmussen, 2004)*. The Gaussian process model ensured that no parametric form was imposed on $f(x)$, thereby allowing for a data-driven approach. In this context, the Gaussian process was parameterised with two components: a mean function ($M(x)$, controlling the central tendency of the function at a given value of x) and a covariance function ($C(x, x')$), controlling the second order characteristics of the

function, such as differentiability. For *M* a quadratic function: $M(x) = Ax + Bx^2$ was used and for *C* we chose a highly smooth Gaussian kernel *(Banerjee et al., 2004)* characterised by two parameters that control for the scale (*Scale*), and amplitude (*Amp*) of the covariance *(Rasmussen, 2004)*.

We included several constraints to prevent biologically implausible scenarios from being included in the model. First the Gaussian process was constrained to include positive values only if $(f(x) > 0 \; \forall \; x)$ to prevent impossible negative incidence values. Second the Gaussian process was conditioned to include the assumption that at zero probability of occurrence, there is zero incidence $(f(0) = 0)$. Third, the Gaussian process was constrained to have at most one inflection point (excluding a saddle inflection point), thereby allowing only ecologically simple models without multiple peaks, troughs or saddles.

Previous approaches have modelled the dispersion or noise function *(Patil et al., 2010)* as a quadratic function incorporating specific prior biological knowledge about the disease being modelled *(Patil et al., 2009)*. For our dengue incidence data we lack any such prior knowledge, and therefore to prevent any bias and unjustifiable complexity we parameterise the dispersion function as constant, that is $r(x) = V$.

3.4.3. Parameter model

Hyper priors for the Gaussian process rate function parameters, $\theta_{f(x)} \in \{A, B, C, Scale, Amp\}$, were set to uninformative *(Gelman et al., 2013)* uniform distributions with sensible ranges. This was done to express vague prior knowledge about these parameters. The hyper parameter for the dispersion function, $\theta_{r(x)} \in \{V\}$, was set to an uninformative hyper prior *(Gelman et al., 2013; Patil et al., 2010)* requiring no defined ranges.

3.4.4. Posterior inference

The model was fitted and the posterior characterised using markov chain monte carlo (MCMC) sampling *(Gelfand and Smith, 1990; Gilks et al., 1996; Patil et al., 2010)*. The final models were run over one million iterations, sampling every 500 iterations to prevent autocorrelation effects between samples *(Gelman et al., 2013)*. Additionally, the first 200,000 samples were discarded ("burn in") to ensure that the posterior was drawn from the equilibrium distribution of the Markov chain. This gave a total of *n*=1600 posterior samples for all the model parameters. Visual inspection of the MCMC trace and Geweke plots *(Geweke, 1991; Patil et al., 2010)* were used to check model convergence. The output of the burden model consists of samples from the joint posterior distributions for all the model parameters $\theta_i \in \{f(x)_i, r(x)_i, A_{i,}, B_i, C_i, Scale_i, Amp_i, V_i\}$ where $i = 1,,,n$. For all individual combinations of these parameter samples (θ_i) individual curves can be constructed, using the data model (equation 1), that represent a realised relationship between incidence and the probability of occurrence (Ω). The full set of these realisations $(\overline{\Omega} = [\Omega_1, \dots, \Omega_n])$ represents a joint model for the data and unknown parameters.

The entire model fitting procedure was performed separately for apparent and inapparent infections, resulting in two separate burden models derived from the I:A ratio measured in the serological cohort studies. It should be noted that only 39 of the 54 cohort studies provided information on these ratios and, therefore missing ratio values were imputed in the MCMC *(Patil et al., 2010)*. The results of the burden models are shown in Figure 3.3.

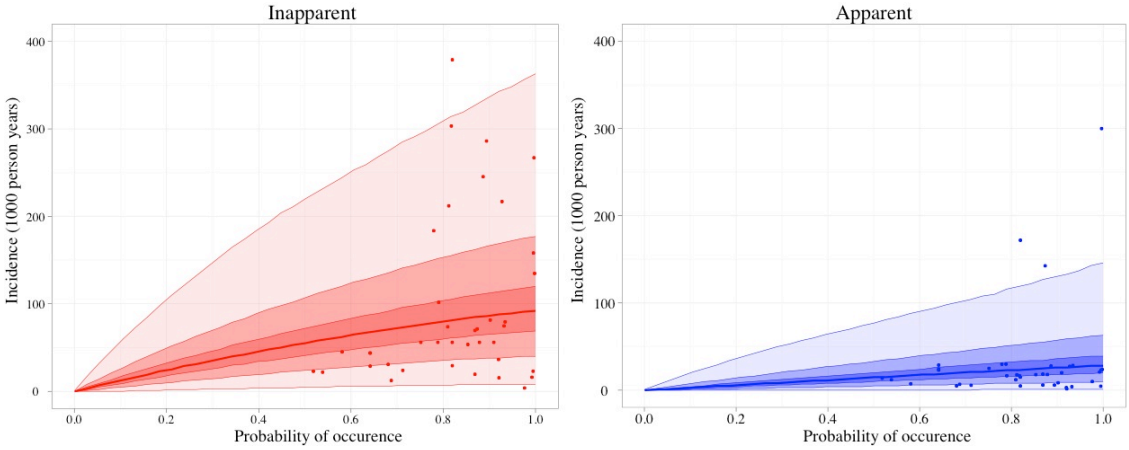

Figure 3.3. Bayesian modelled relationship between the probability of occurrence and incidence for inapparent and apparent number of infections. The data are the points, the bold lines are the medians and the envelopes are the 0.25, 0.5 and 0.95 credible intervals centred on the median displayed with progressively lighter shades.

3.5. Overview of map generation and burden estimates

Each burden model (apparent and inapparent) provided a posterior set of relationships ($\overline{\Omega}$) between the probability of occurrence and incidence. From each realised relationship (Ω_i) the probability of occurrence map was translated into an incidence map. We did this for all relationships in $\overline{\Omega}$ generating a distribution of incidence maps, reflecting the posterior predicted relationships. For each of these maps, using a human population surface for 2010 *(Gething et al., 2011)*, infection numbers were estimated on a global scale. Stratification of these estimates into national and subnational divisions is discussed in section 3.4.1. These are compared to surveillance-based burden estimates of dengue cases reported to the WHO *(WHO, 2010*, *2012b*, *2012c*, *2012d)*. We explain how the order of magnitude difference between these two estimates is feasible if we consider the systematic underreporting introduced at

multiple stages along the pathway that separates an apparent infection in the community from a reported clinical dengue case at the national level. We then go on to explain and quantify each of these steps in detail using a comparative analysis of previously published estimates for each step to reconcile the two differing burden estimates.

3.5.1. Surveillance-based burden data sources

National annually reported numbers of diagnosed dengue cases were taken from the WHO regional office websites *(WHO, 2010*, *2012b*, *2012c*, *2012d)*. While DengueNet *(WHO, 2011)* remains the central repository for dengue data within the WHO, its accessible database contains only sporadic case numbers since 2005. By obtaining case numbers directly from WHO regional offices, we were able to obtain a more contemporary estimate of reported national burden. For this analysis we used the average annual case numbers from the three most recent complete years available — 2009-2011 for the Pan American Health Organization (PAHO) and the Eastern Mediterranean Region Office (EMRO), 2008-2010 for the South-East Asia Region Office (SEARO) and the Western Pacific Region Office (WPRO). Case numbers presented by PAHO (47 countries) and SEARO (11 countries) are based exclusively on country reports to these offices, which are themselves based around the WHO clinical guidelines *(WHO, 2009)*. WPRO case numbers come from country reports to WPRO (23 countries) and country Ministry of Health (MoH) websites (4 countries). Case numbers reported by EMRO (1 country) are from the WHO country offices and are only available in sporadic reports. Imported cases were excluded when a differentiation between imported and indigenous cases was made. There is no legal obligation for countries to report annual case numbers to the WHO, nor is any attempt made to standardise what countries report beyond issuing standard guidelines *(WHO,*

2009). While diagnostic limitations and a lack of standardisation make international comparisons difficult for dengue, we believe that comparing country reports of diagnoses based on WHO guidelines forms the most reliable and internationally comparable source for annually reported diagnosed dengue cases.

Here we are interested in comparing surveillance-based estimates of total symptomatic clinical dengue cases (dengue fever (DF) + dengue hemorrhagic fever (DHF) + dengue shock syndrome (DSS) or dengue (DW^-) + dengue with warning signs (DW^+) + severe dengue (SD)) with our own cartographic burden estimates of total apparent infections *(WHO, 1997*, *2009)*. While countries are instructed to report only laboratory confirmed cases large case numbers often make it prohibitively expensive to laboratory confirm every suspected clinical case. It is therefore not uncommon for countries to report suspected dengue (based on clinical diagnosis) and confirmed dengue (based on laboratory diagnosis) cases as a single combined figure. The distinction into DF+DHF+DSS or DW^-+ DW^++SD is then often made using a country-specific adapted WHO case definition which may or may not be reported in the WHO figures (Table 3.1.) *(Ng et al., 2007; Rigau-Perez, 2006)*. In this analysis we took the broadest spectrum of clinical manifestations available in the WHO data, however it is well known that reporting fidelity and standardisation of the clinical forms is far from globally consistent and our comparison with cartographic burden estimates must take these regional differences in surveillance into account (Table 3.1.).

Table 3.1. Levels of reporting in the four World Health Organization (WHO) regions that display dengue data. PAHO = Pan American Health Organization, WPRO = Western Pacific Region Office, SEARO = South East Asian Region Office, EMRO = Eastern Mediterranean Region Office, DF = dengue fever, D = dengue, SD = severe dengue.

WHO region	Total DF/D	DF (suspected/ confirmed)	SD	Deaths	Percentage of global dengue cases reported 2008-2010

PAHO	✓	✓	✓	✓	73
WPRO	✓	✓		✓	14
SEARO	✓	✓*		✓	12
EMRO	✓			✓	1

* Available for some countries for some years.

3.5.2. Country-level burden estimates

In 2010, the total global apparent dengue infection burden estimated in this study (96 million, credible interval = 67-136, Fig. 3.8.) is substantially larger than the global number of reported clinical dengue cases (2.2 million). However, the burden rank of each country is largely consistent in both estimates and the differences in absolute burden estimates often show a common factor that suggests an intrinsic loss to underreporting.

In the countries of the Americas, both approaches predict a concentration of the burden in four countries: Venezuela, Colombia, Mexico and Brazil (Figure 3.5.). However, in contrast to the WHO figures, our estimate suggests an additional sizable contribution from Peru, Guatemala and the Caribbean islands of Cuba and the Dominican Republic. Outside of these countries both approaches agree on a uniformly low burden. Our estimate for total apparent infections in the Americas (13.3 million) is around seven times that reported by the WHO.

WHO estimates were not available for the African regions, with the exception of Cape Verde (Figure 3.6.). For Cape Verde, our estimate of 10,515 cases is comparable with the WHO reported figure of 10,760. Elsewhere, we predict the highest burden in the areas of high population density in the countries of Nigeria, Egypt, Democratic Republic of the Congo, Ghana and Uganda. In addition, total apparent burden is

considerable in a large number of countries, with 32 having a burden of over 50,000 apparent infections per year, contributing an additional 15.7 million apparent infections.

In Asia, both approaches predict a high burden in South East Asia and the Indian subcontinent (Figure 3.7.). Our estimates from countries in South East Asia are consistently around 40 (20-60) times those reported to the WHO. Despite a large number of countries falling within this interval, our figures for China (6.5 million) and India (32.5 million) are far above what is reported to the WHO. In both cases, large populations are combined with large areas of high suitability for dengue. We suggest that under-reporting of dengue in these two countries is a significant part of reconciling the gap between our global burden estimates and cases reported to the WHO. China and India contribute 58% of the total burden for Asia (66.8 million apparent infections per year). It is clear that reducing the uncertainty in the estimates for these two countries is important for reducing uncertainty at the global level. Achieving this will require better evidence consensus in low consensus areas, more occurrence points, particularly where our data is currently sparse, and additional cohort studies across a range of transmission intensities.

In Oceania, both approaches predict a low burden, with our predictions suggesting that only Papua New Guinea faces a burden of over 50,000 apparent infections a year (Figure 3.4.). Considering their population size, Fiji and Samoa also contribute a sizable portion of Oceania's total 178,000 apparent infections. Our predictions are, on average, 40 times greater than the WHO estimates, although this is variable due to low reported case numbers.

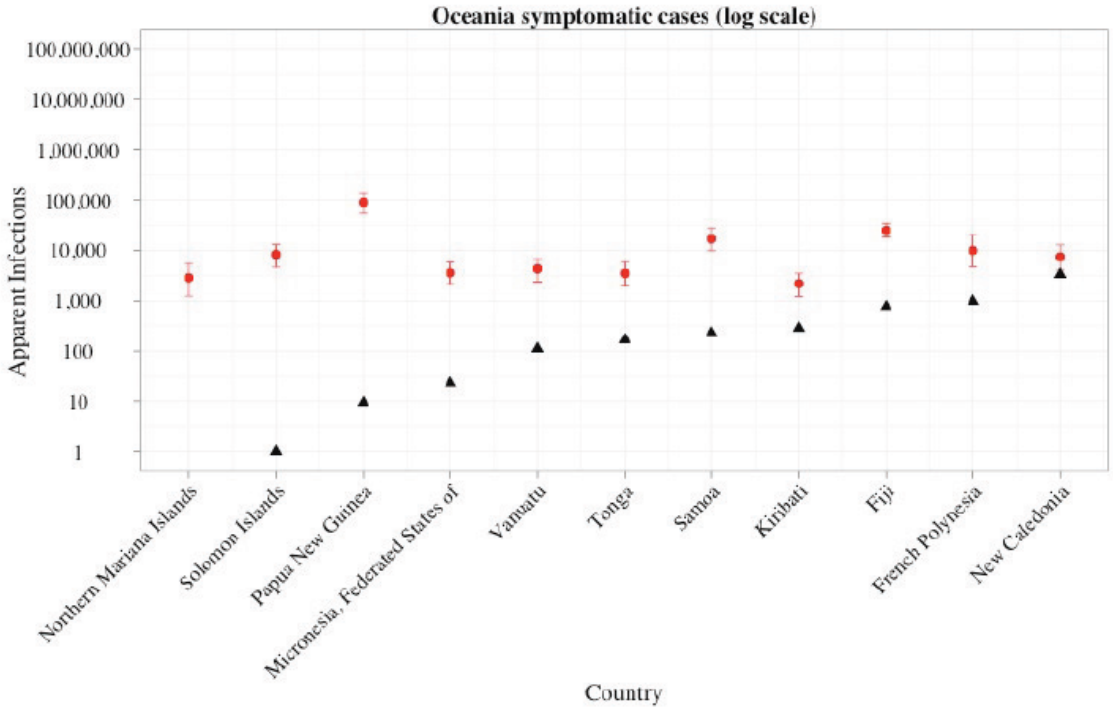

Figure 3.4. Number of apparent infections (red) and number of diagnosed dengue cases reported to the WHO (black) per country in Oceania.

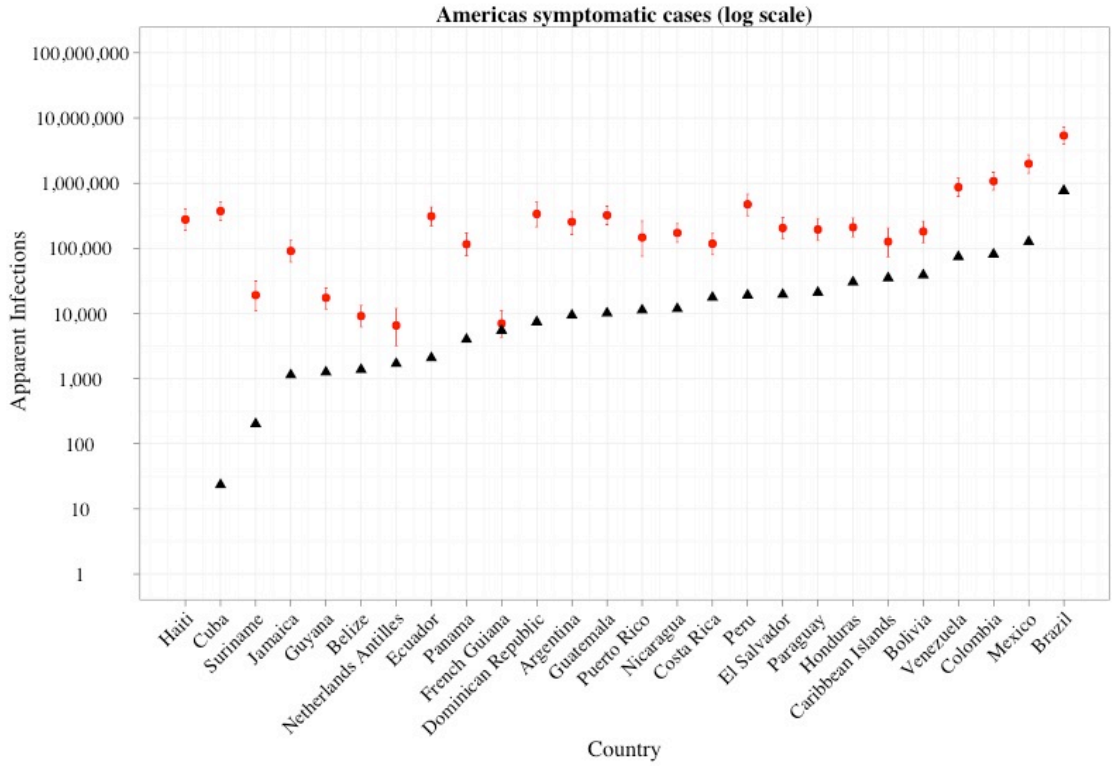

Figure 3.5. Number of apparent infections (red) and number of diagnosed dengue cases reported to the WHO (black) per country in the Americas.

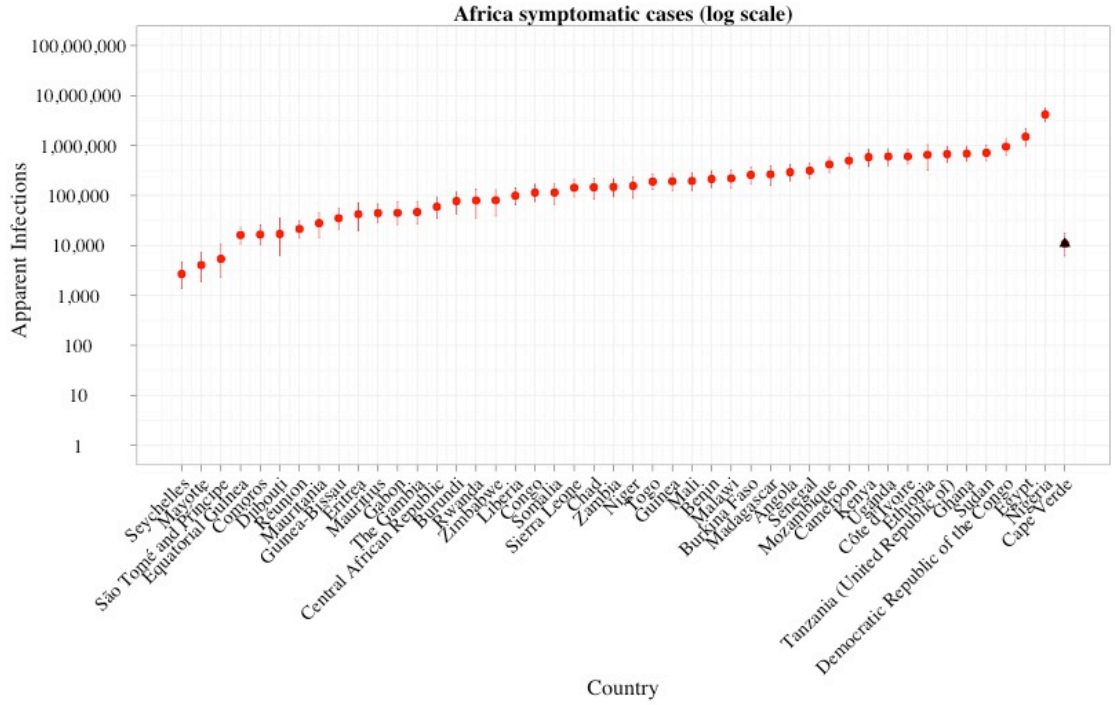

Figure 3.6. Number of apparent infections (red) and number of diagnosed dengue cases reported to the WHO (black) per country in Africa. Only Cape Verde provided any reported cases to the WHO.

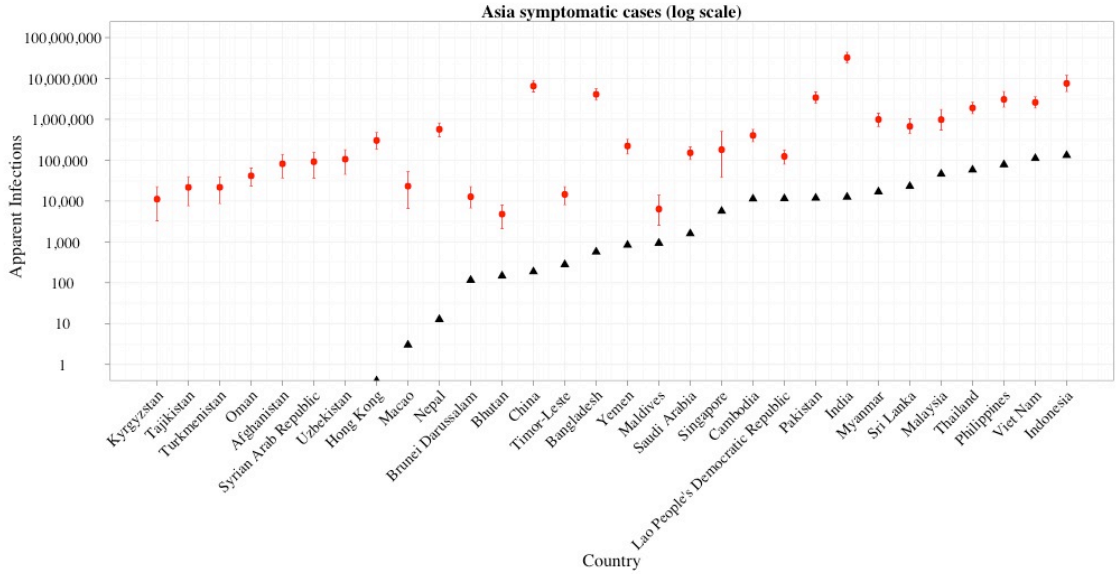

Figure 3.7. Number of apparent infections (red) and number of diagnosed dengue cases reported to the WHO (black) per country in Asia.

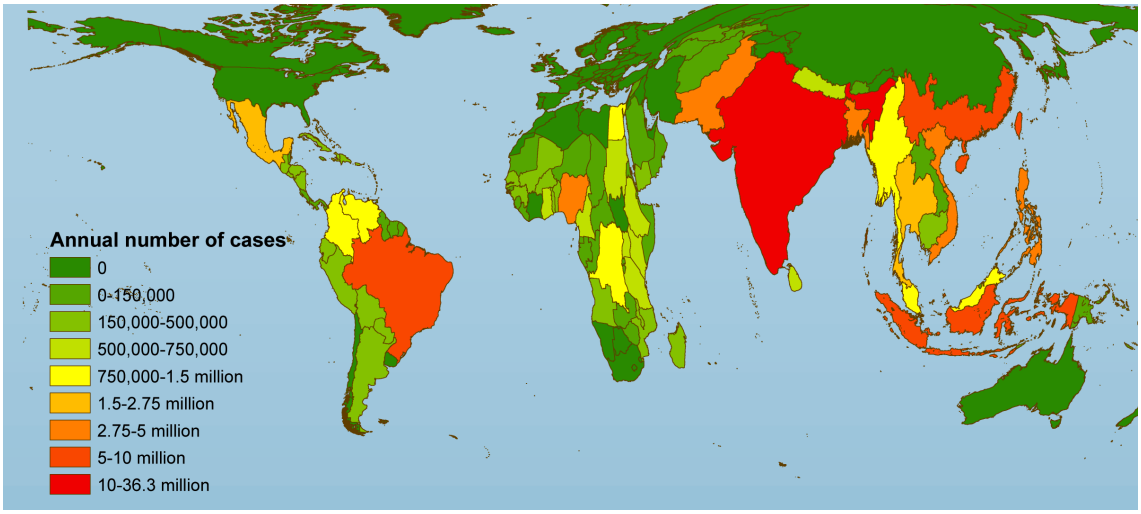

Figure 3.8. Cartogram of the annual number of infections for all ages as a proportion of national or subnational (China) geographical area.

Country Name	Apparent Mean	Apparent 2.50%	Apparent 97.50%	Inapparent Mean	Inapparent 2.50%	Inapparent 97.50%	WHO. estimates	CI Ratio Rank	CI Absolute Rank
Aruba	3,165	1,451	5,820	9,715	5,174	16,299	1,762	22	116
Afghanistan	81,687	36,803	138,574	255,681	128,224	399,333		33	63
Angola	292,178	196,712	419,141	896,115	643,138	1,210,316		107	37
Anguilla	519	216	1,006	1,571	770	2,767	1,418	16	133
Netherlands Antilles	6,546	3,199	11,930	19,765	10,998	32,919	1,690	24	104
Argentina	254,470	162,631	370,798	787,499	532,735	1,082,768	9,337	85	41
American Samoa	2,414	935	4,883	7,316	3,421	13,350	373	12	119
Antigua and Barbuda	3,226	1,592	5,800	9,739	5,467	16,031	3	28	118
Burundi	77,023	42,810	119,252	238,713	145,122	345,040		62	71
Benin	213,030	143,875	311,527	651,489	469,940	894,900		96	46
Burkina Faso	257,950	167,531	370,996	797,426	548,974	1,084,392		95	42
Bangladesh	4,097,833	2,952,879	5,608,456	12,581,091	9,519,133	16,359,636	568	135	7
Bahamas	10,971	6,306	18,003	33,335	21,183	50,405	2,336	53	101
Belize	9,128	6,208	13,270	27,878	20,264	38,061	1,368	101	108
Bolivia	181,219	122,119	260,474	555,702	399,308	751,368	38,640	106	53
Brazil	5,371,268	3,952,287	7,283,317	16,404,160	12,672,363	21,111,729	765,769	139	4
Barbados	9,398	4,753	16,653	28,343	16,213	46,077	1,239	32	100
Brunei Darussalam	12,732	6,776	22,541	38,421	22,836	62,606	116	35	94
Bhutan	4,793	2,109	7,946	15,042	7,343	23,177	147	38	111
Central African Republic	59,436	34,672	93,206	183,305	117,623	266,963		63	74
China	6,523,946	4,683,881	8,919,000	20,062,625	15,083,630	26,126,115	186	133	3
Cote d'Ivoire	603,431	427,796	845,552	1,843,448	1,383,217	2,435,397		122	27
Cameroon	497,871	350,920	701,594	1,521,543	1,132,524	2,022,270		120	29
Democratic Republic of the Congo	947,801	629,617	1,356,953	2,917,767	2,062,475	3,941,733		103	15
Congo	114,173	75,677	168,494	347,147	246,770	480,451		88	67
Cook Islands	712	225	1,766	2,155	827	4,711	60	3	129
Colombia	1,073,891	783,699	1,465,285	3,281,089	2,515,002	4,243,729	80,634	137	17
Comoros	16,538	10,228	25,414	50,733	34,230	72,452		73	96
Cape Verde	10,879	6,126	17,651	33,547	20,972	50,226	10,760	55	102
Costa Rica	117,677	79,912	168,993	361,243	261,580	488,074	17,524	109	69
Cuba	372,825	266,724	518,849	1,132,115	856,787	1,489,340	23	123	34
Cayman Islands	1,987	1,058	3,404	6,016	3,599	9,452	3	41	126
Djibouti	16,946	6,291	35,062	52,407	23,568	97,101		11	86
Dominica	2,327	1,286	3,880	7,052	4,347	10,850	227	47	125
Dominican Republic	336,410	213,296	519,207	1,017,290	698,134	1,466,630	7,383	76	30
Ecuador	310,448	220,963	430,732	951,375	713,884	1,248,114	2,066	124	40
Egypt	1,499,568	965,744	2,164,954	4,645,241	3,186,358	6,312,307		91	13
Eritrea	42,184	19,704	70,386	131,736	68,901	202,693		39	75
Ethiopia	651,184	320,718	1,041,952	2,037,422	1,089,989	3,038,520		48	16
Fiji	24,969	18,152	34,109	76,371	58,437	99,072	759	136	93
Micronesia, Federated States of	3,567	2,158	5,701	10,872	7,153	16,136	23	61	121
Gabon	44,792	25,942	74,225	135,942	86,746	208,083		51	77
Ghana	687,110	486,967	963,366	2,093,455	1,571,708	2,765,615		121	22
Guinea	192,067	125,712	275,871	593,001	412,489	803,659		97	50
Guadeloupe	17,466	10,027	28,528	52,680	33,409	79,826	14,754	56	89
The Gambia	46,476	27,000	76,385	141,685	90,603	214,567		54	76
Guinea-Bissau	35,011	20,615	55,465	107,616	69,781	157,739		60	82
Equatorial Guinea	16,166	10,729	23,256	49,799	35,252	67,404		100	99
Grenada	3,723	1,891	6,526	11,246	6,444	18,119	78	34	114
Guatemala	322,243	231,037	443,795	988,330	745,518	1,289,753	10,073	127	39
French Guiana	7,024	4,325	11,090	21,312	14,285	31,274	5,449	68	109
Guyana	17,416	11,728	24,596	53,779	38,306	71,810	1,249	114	98
Hong Kong	304,782	184,690	475,819	924,234	613,579	1,342,712	0	69	32
Honduras	209,834	149,848	291,525	641,409	483,737	841,072	30,134	125	52
Haiti	276,581	188,402	403,229	844,925	613,842	1,152,883		98	38
Indonesia	7,590,213	4,798,222	11,944,976	23,009,108	15,724,054	33,745,901	130,575	70	2

India	32,541,392	23,809,852	44,196,670	99,692,319	76,480,648	128,730,948	12,484	138	1
Jamaica	90,807	61,553	132,005	275,459	199,486	376,774	1,132	99	73
Kenya	583,960	376,348	843,317	1,807,001	1,234,459	2,461,954		92	24
Kyrgyzstan	11,135	3,300	22,437	35,093	12,827	63,199		10	88
Cambodia	404,533	282,589	568,752	1,243,325	918,191	1,649,357	11,247	119	33
Kiribati	2,173	1,215	3,475	6,712	4,170	9,958	280	57	127
Saint Kitts and Nevis	1,845	968	3,134	5,581	3,312	8,766	23	42	128
Lao People's Democratic Republic	124,006	79,970	178,093	383,905	263,083	521,000	11,431	94	64
Liberia	98,678	65,906	140,777	304,137	216,716	408,522		108	72
Saint Lucia	6,144	3,655	9,698	18,609	12,168	27,335	226	65	110
Sri Lanka	673,544	445,991	1,027,403	2,042,226	1,447,987	2,910,693	22,902	80	18
Macao	23,158	6,626	52,502	69,833	26,037	140,928	3	5	78
Madagascar	264,443	159,191	393,246	821,514	526,343	1,148,447		79	35
Maldives	6,372	2,557	13,981	19,735	9,298	38,567	933	9	103
Mexico	1,987,320	1,422,381	2,730,919	6,102,891	4,582,683	7,964,033	125,217	128	10
Marshall Islands	1,891	757	4,195	5,774	2,687	11,424		8	122
Mali	194,903	125,686	281,231	602,552	411,989	820,354		93	48
Myanmar	992,954	669,765	1,408,977	3,056,420	2,191,868	4,098,171	16,824	111	14
Northern Mariana Islands	2,862	1,235	5,493	8,704	4,406	15,167		18	117
Mozambique	418,090	287,800	586,770	1,285,737	935,627	1,707,800		118	31
Mauritania	27,859	14,145	44,508	86,922	48,185	129,140		50	85
Montserrat	178	73	342	545	269	952	1	17	137
Martinique	14,845	8,099	25,136	44,766	27,271	69,900	12,918	44	92
Mauritius	44,471	28,232	68,107	134,755	92,595	192,540		78	81
Malawi	220,050	139,738	319,955	680,097	461,604	931,479		84	45
Malaysia	983,619	546,225	1,746,771	2,969,671	1,817,360	4,835,731	45,664	37	12
Mayotte	4,049	1,886	7,286	12,445	6,734	20,417		25	112
New Caledonia	7,423	3,988	12,665	22,609	13,639	35,448	3,301	43	105
Niger	155,313	87,805	237,801	482,943	291,858	693,142		67	51
Nigeria	4,153,338	3,004,606	5,700,852	12,698,054	9,670,162	16,510,850		132	6
Nicaragua	172,439	124,002	239,068	526,486	399,249	689,249	11,763	126	60
Niue	36	17	64	108	59	178	1	23	139
Nepal	571,773	377,060	813,702	1,769,014	1,232,877	2,386,338	13	105	26
Nauru	303	62	812	915	260	2,138	1	2	134
Oman	41,524	23,179	63,745	129,341	77,707	185,832		66	80
Pakistan	3,414,749	2,455,183	4,680,780	10,481,756	7,898,303	13,642,888	11,787	131	8
Panama	115,465	77,189	171,413	349,933	250,842	488,023	3,979	87	66
Peru	472,445	316,552	677,480	1,454,164	1,038,618	1,964,217	19,005	104	28
Philippines	3,076,863	1,990,758	4,810,993	9,339,425	6,493,806	13,584,794	77,598	74	5
Palau	715	248	1,562	2,168	920	4,207	72	7	130
Papua New Guinea	89,943	53,076	134,815	279,597	175,330	394,470	9	77	70
Puerto Rico	146,564	75,342	262,390	441,460	256,280	727,231	11,201	29	43
Paraguay	194,400	131,147	287,580	591,779	426,866	820,674	20,880	90	47
French Polynesia	9,879	4,734	20,300	29,763	16,034	55,211	968	13	95
RŽunion	21,329	13,990	31,231	65,378	46,176	89,873		89	91
Rwanda	79,509	35,540	132,872	248,565	124,383	383,373		36	65
Saudi Arabia	152,009	103,604	213,847	468,868	337,790	624,191	1,584	115	61
Sudan	713,990	488,578	1,002,006	2,200,977	1,587,613	2,923,357		116	20
Senegal	314,220	215,380	449,280	962,422	703,216	1,295,220		112	36
Singapore	180,895	38,032	506,530	543,970	153,362	1,338,288	5,631	1	23
Solomon Islands	8,250	4,572	12,834	25,552	15,642	37,037	1	59	107
Sierra Leone	143,041	92,780	212,462	439,787	306,502	607,668		82	59
El Salvador	205,242	141,902	295,506	624,014	458,412	843,641	19,427	110	49
Somalia	114,617	67,014	173,889	354,808	224,893	504,903		71	62
Sao Tome and Principe	5,372	2,326	10,714	16,289	8,203	29,361		14	106
Suriname	19,114	10,933	31,511	57,875	36,560	88,136	201	52	87
Seychelles	2,677	1,375	4,589	8,173	4,772	12,922		40	123
Syrian Arab Republic	91,973	36,485	156,775	289,260	128,876	455,438		26	58
Turks and Caicos Islands	563	230	1,095	1,746	849	3,051	8	15	132
Chad	145,525	85,538	218,800	451,621	286,219	637,502		75	56

Togo	189,659	132,343	268,471	578,418	429,007	770,252		117	54
Thailand	1,903,694	1,373,605	2,621,098	5,823,012	4,424,859	7,596,099	57,589	130	11
Tajikistan	21,693	7,680	39,389	68,289	27,976	113,063		19	83
Tokelau	32	8	76	96	32	205		4	138
Turkmenistan	21,770	8,698	39,089	68,326	31,473	111,794		21	84
Timor-Leste	14,586	8,141	22,488	45,345	27,395	65,380	278	64	97
Tonga	3,494	1,938	5,852	10,584	6,528	16,323	166	46	120
Trinidad and Tobago	48,180	31,084	72,602	145,572	101,375	205,512	1,255	81	79
Tuvalu	155	48	348	488	188	968		6	136
Tanzania (United Republic of)	674,056	456,225	954,435	2,075,066	1,483,724	2,778,664		113	21
Uganda	602,260	395,846	859,315	1,862,790	1,297,641	2,511,403		102	25
Uzbekistan	105,943	45,117	180,283	332,456	158,412	521,236		30	55
Saint Vincent and the Grenadines	3,267	1,994	5,033	9,979	6,659	14,331	63	72	124
Venezuela	866,172	625,770	1,193,623	2,634,742	2,011,455	3,434,002	73,796	129	19
Virgin Islands, British	664	298	1,252	2,014	1,056	3,445	338	20	131
Virgin Islands, U.S.	3,885	1,900	6,977	11,748	6,571	19,306		27	113
Viet Nam	2,603,443	1,890,174	3,578,852	7,965,912	6,081,413	10,371,255	110,217	134	9
Vanuatu	4,274	2,365	6,764	13,222	8,155	19,401	111	58	115
Wallis and Futuna	488	242	860	1,487	843	2,408	4	31	135
Samoa	16,759	9,671	28,084	51,096	32,418	78,612	226	49	90
Yemen	222,930	141,959	324,076	689,860	465,642	945,429	833	86	44
Zambia	148,229	94,049	215,850	458,423	310,077	628,782		83	57
Zimbabwe	80,075	38,569	129,836	250,485	131,542	377,858		45	68

Table 3.2. Apparent and inapparent mean and confidence (95%) burden estimates per country. The CI ratio rank is calculated as the ranked index of the apparent confidence interval difference divided by the mean *(Hay et al., 2010)*. The CI absolute rank is calculated as the ranked index of the difference in the apparent confidence interval. Only countries with evidence consensus > -25 are included.

3.6. Comparing cartographic and surveillance-based burden estimates

In the previous section we presented two estimates of dengue burden that were different by an order of magnitude. In this section, we reconcile these estimates by discussing and reasonably quantifying each step in the surveillance-based reporting system (Figure 3.9.).

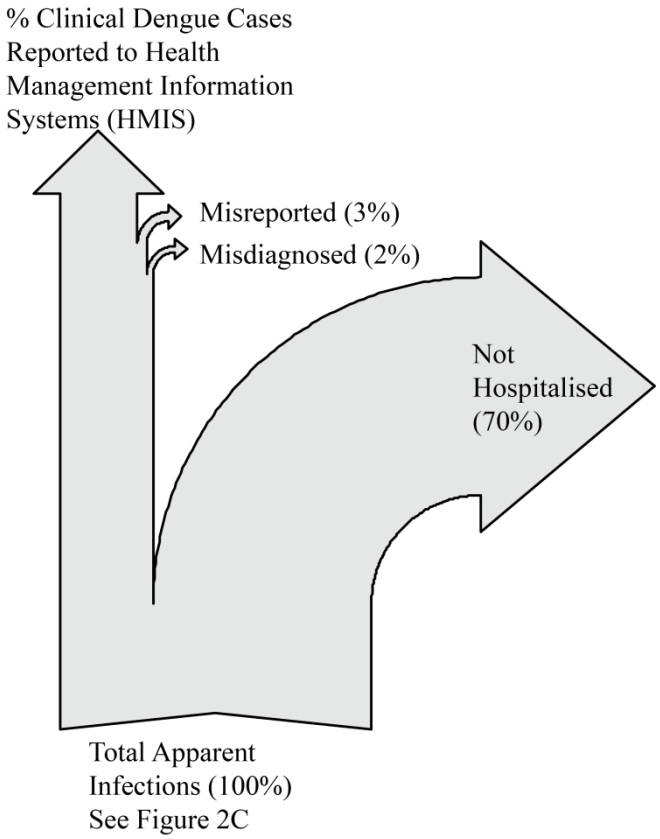

Figure 3.9. Hypothetical reporting chain of a dengue virus infection. Of the total number of *apparent infections* only around 30% will seek treatment at official healthcare facilities as opposed to alternative treatment options. Of the formally *hospitalised infections*, a large proportion are misdiagnosed, although total *diagnosed infections* is nearly counter balanced by non-dengue illnesses receiving a dengue diagnosis. Finally, technical, political and logistical barriers exist between the hospital and the governing bodies that result in fewer *reported infections*. These steps collectively ensure that the reported burden of dengue cases is only a small proportion of the total volume of apparent infections. The loss arrows represent an average estimate of the proportion of total apparent infections reported at each level, based on a comparative analysis discussed in section 3.5.

In our estimates we define an apparent dengue infection as any infection that results in visible symptoms, for example nausea or vomiting, rash, aches and pains, mucosal bleeding or restlessness *(WHO, 2009)*, sufficient to disrupt to the individual's daily routine (see section 3.2.2.). Our analysis suggests a mean figure of 96 million (CI = 67-136) apparent dengue infections per year which captures the complete spectrum of dengue infections from mild to severe. However, of these total apparent infections,

there is a wide range in severity *(Endy et al., 2002; McBride et al., 1998; Yew et al., 2009; Yoon et al., 2012)* which affects the treatment-seeking behaviours of infected individuals. The individual may choose to present to formal healthcare facilities operated by ministries of health, or the private or non-profit sectors. It is also the case, however, that many care-seekers will choose other options such as homeopathic medicine *(Jacobs et al., 2007)* or ambulatory clinics *(Wichmann et al., 2011)*, or will simply use over-the-counter medicines if they seek treatment at all. These decisions depend on a myriad of factors including the availability of the various healthcare facilities and the cost associated with each option. As such, treatment-seeking behaviour for dengue is likely to vary considerably in time (e.g. epidemic versus inter-epidemic periods) and in space (e.g. country to country), as well as by socio-economic, cultural, age and gender groupings. Whilst some of these alternative treatment options may sometimes result in dengue reporting *(Kantachuvessiri, 2002)*, this is likely to be the exception rather than the norm, and in most cases will leave no formal record for inclusion in national health statistics *(Chaudhuri, 2013; Wichmann et al., 2011)*. Approximations of the fraction of dengue infections that present to formal healthcare facilities can be made from cohort studies where the hospitalised case numbers are recorded in parallel to the incidence of apparent infections in the general community. From the nine studies in section 3.2.2. that measured both of these parameters *(Endy et al., 2002; Endy et al., 2010; Graham et al., 1999; Porter et al., 2005; Thein et al., 1997; Tien et al., 2010; Tuntaprasart et al., 2003; Vong et al., 2010; Yoon et al., 2012)* an average of 30% (range 18-60) of apparent infections presented to formal healthcare facilities. If generalisable, this would suggest only 28.8 million of the 96 million apparent infections would present to formal healthcare facilities. This is an upper-bound estimate as these studies minimise financial, educational and logistic barriers to healthcare access thus modifying treatment-seeking behaviour. This is an important

factor in reconciling these estimates because it accounts for the single biggest loss of dengue infections between total apparent infections and cases reported to the WHO.

Of the apparent dengue infections that do present to an official healthcare facility, a wide spectrum still exists between the mildest and the most severe clinical cases *(Dinh The et al., 2012; Simmons, 2012; Sirivichayakul et al., 2012)*. This complicates accurate diagnosis of dengue, particularly in the febrile phase where up to 12 other major pathogens may be implicated in the differential diagnosis *(Simmons, 2012)*. This creates two potential problems: (i) clinical diagnosis of dengue infections as other febrile illnesses (under-diagnosis) and (ii) diagnosis of non-dengue febrile illnesses as clinical dengue (over-diagnosis). Under-diagnosis of dengue is common in situations where the disease has a less familiar clinical presentation compared to other febrile illnesses, for example misdiagnosis as malaria in Africa *(Blaylock et al., 2011)*. Even in cases where dengue is a common diagnosis, often the rigidity of the case definition neglects the broad spectrum and transitory nature of symptoms presented *(Bandyopadhyay et al., 2006; Deen et al., 2006; Deparis et al., 1998; Gupta et al., 2010; Kalayanarooj, 2011; Ng et al., 2007; Rigau-Perez, 2006; Srikiatkhachorn et al., 2010)*. The extent of under-diagnosis can be estimated by comparing independent attempts to calculate the positive predictive value of a particular clinical dengue case definition. The positive predictive value gives the probability that the case definition used by the hospital will accurately diagnose a true dengue infection. A comparison of case definitions based on the 1997 WHO clinical guidelines *(WHO, 1997)* for diagnosis of DF revealed an average positive prediction value of 57% (range 16-87) *(Deparis et al., 1998; Diaz et al., 2006; Dietz et al., 1990; Kalayanarooj, 1999; Kalayanarooj et al., 1997; Lima et al., 1999; Premaratna et al., 2009; Rodrigues et al., 2002)*. Over-diagnosis of dengue is likely to occur in the later stages of an epidemic when healthcare services are overwhelmed and a diagnosis of dengue is more likely to be

suspected over other clinically similar infections *(Klaucke, 1994)*. It is also possible to quantify over-diagnosis by observing the proportion of true dengue infections, as determined by laboratory confirmation, out of the total number of clinical dengue diagnoses. This information was extracted from a collection of 29 published clinical outcome studies available on request. We found that, on average, true dengue infections make up only 60% of total clinically diagnosed dengue cases. Of course, over-diagnosis can be reduced when laboratory confirmation is available for clinically-diagnosed samples; however, this makes up only a small proportion of globally-reported dengue cases (6.9% of total cases reported to WHO regional offices in 2010) *(WHO, 2010*, *2012b*, *2012c*, *2012d)*. If we again generalise these figures globally, the suggested 28.8 $\left({}^{30}/_{100}\times 96\right)$ million presenting to official healthcare facilities is further reduced to 16.4 $\left({}^{57}/_{100}\times 28.8\right)$ million in light of under-diagnosis, 27.4 $\left({}^{100}/_{60}\times 16.4\right)$ if over-diagnosis is included as well, and 26.6 $((1-0.069)\times 27.4+0.6\times 0.069\times 27.4)$ million taking into account both under and over-diagnosis with laboratory confirmation available for 6.9% of clinical samples.

Once a clinical or laboratory diagnosis of dengue has been given, the remaining step in the reporting pathway is for this case to be incorporated in the national health management information systems (HMIS) and then to the WHO. The systems in place to transfer this data from the hospital to the WHO vary widely in their use of technology and frequency of reporting. While many health system records are now computer-based, much of the primary reporting is still gathered over the telephone or by fax *(Beatty, 2008)* which creates inherent standardisation issues. Furthermore, gaps in weekly reporting schedules can be observed during national holidays or during temporary shortages of available staff and/or funding. These logistical errors are compounded by political conflicts of interest in what is reported and how it is used to avoid accusations of liability. Thus, communication through HMIS systems will

inevitably result in minor data losses. Although a lack of available data makes this step difficult to quantify, we were able to estimate the data loss at the final step of HMIS reporting to the WHO. A comparison of reported DF cases on ministry of health (MoH) websites with those reported to the WHO from 2009-2011 found that 95% of total cases were reported to the WHO *(WHO, 2010*, *2012b*, *2012c*, *2012d)*. No persistently under-reported countries were identified, but one-off years where data was not reported were observed. If we assume a 95% reporting capacity across *both* steps (local hospital to MoH and MoH to WHO) our suggested figure of 26.6 million diagnosed dengue infections would translate to 24.0 $\left(26.5\times {}^{95}/_{100}\times {}^{95}/_{100}\right)$ million potentially reportable cases per year or, overall approximately one quarter of the total apparent dengue infections that occur each year.

This figure of 24.0 million potentially reported cases is an absolute upper bound of what we might expect to be reported to the WHO globally. This is because the studies we have used all estimate the upper bound of reportable cases. The hospitalisation rates are heavily influenced by increased healthcare provision. Case-definition positive predictive values are calculated under controlled situations that do not accurately reflect the strains on the healthcare system that vary both through space (e.g. equipment deficits in rural areas) and time (e.g. resource constraints during epidemics). Furthermore, estimates of over-diagnoses often take a very broad, and hence less specific, clinical definition of dengue so as to maximise study participant number. This increases the number of falsely clinically-diagnosed dengue patients. Finally, the losses through HMIS and between HMIS and the WHO were calculated from countries that have already been shown to proactively use their reported data (in so far as they display it on their MoH website). In countries where there is no motivation to use this data at a national level, it is easy to see how further data losses

could occur, meaning an adjustment factor of only 5% loss at this stage is likely to be extremely conservative.

PAHO, with 76% of total reported dengue cases in 2010, provides the most accurate and consistently reported dengue data (Table S1). PAHO reported 1.7 million dengue cases in 2010 *(WHO, 2012c)*. For this region our cartographic burden-estimation approach predicts 13.3 million apparent dengue infections suggesting a clinical burden of 3.99 million cases, 3.68 million clinical dengue diagnoses (with over and under reporting and laboratory confirmation) and 3.32 million cases reported to the WHO. The potential for over-estimation of parameters discussed above, in particular the over-diagnosis of dengue due to other febrile illnesses, is likely to be a specific source of over-estimation in the Americas. Considering this, parameter overestimation of as little as 10% is enough to reduce our burden estimate to levels comparable with WHO regional office reports (1.77 million compared to 1.7 million).

In summary, the aim of this section is not to quantitatively estimate the number of cases at each stage in the healthcare system, but rather to show that our cartographic burden estimates of apparent infections are plausible when compared to surveillance-based estimates of clinical dengue cases once the inevitable underreporting of the latter approach is considered. We have shown that the biggest under-reporting step between apparent infections and reported cases occurs during treatment-seeking. Further losses occur due to difficulties in diagnosis and through errors in reporting. A detailed understanding of the link between total apparent infections and total reported cases is an important consideration if the true burden of dengue is to be estimated at various levels.

3.7. References

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Chapter 4 – Assessing the applicability of dengue outbreak response guidelines

4.1. Cover page

While chapter 3 focussed on improving estimates of the long-term average burden of dengue across the globe, at any one particular location both incidence and burden can vary considerably over time. Comparatively rare dengue outbreaks, or cases in significant excess of what is expected or prepared for, may account for only a small proportion of total infections but are responsible for a disproportionately high morbidity, mortality and political burden *(Garg et al., 2008; Grais et al., 2007; Najera, 1999; WHO Ebola Response Team, 2014)*. Therefore any attempt to produce a long-term strategy for burden measurement and reduction must include ways to detect and respond to dengue outbreaks. While standard WHO advice on outbreak response (which is primarily vector control-based) is frequently implemented *(Harrington et al., 2013; WHO, 2009)*, determining when these activities should be triggered is not clearly defined. A wide variety of methods are used for outbreak detection that vary widely in sensitivity and specificity of outbreak detection. Outbreak response guidelines are most effective if applied early on in a large outbreak and deploying them earlier or later, or in response to bigger or smaller outbreaks, may reduce their effectiveness. In this chapter the consistency of the most commonly-used methods for outbreak detection are evaluated, including those recommended by specific countries and international health organisations. The manuscript presented here has recently been resubmitted to the journal *Epidemics* following favourable peer-review. The referred to supplementary information can be found in the Appendix section (page 188).

As well as having implications for outbreak response, this analysis is equally relevant for the evaluation of forecasting / early warning models that attempt to predict when an outbreak will occur and its magnitude. This analysis emphasises that forecasting systems need to be explicit about the characteristics of the event they are predicting, be it excess cases above a seasonal mean, detrimental impacts on the healthcare system, incidence at a particular point in time, etc. and exactly how the models' recommendations should inform control actions. In all cases these models must offer a predictive improvement over simple statistical summaries of the average seasonal variation. An interesting test case of this converged around the 2013 World Cup in Brazil where a number of research groups, including SEEG, made estimates of dengue risk in the 12 host cities. Our predictions based-solely on seasonal averages gave largely similar estimates to those from other groups despite very different approaches of differing levels of model complexity and data requirements *(Hay et al., 2013; Lowe et al., 2014; Massad et al., 2014; van Panhuis et al., 2014; Wilson et al., 2014)*.

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Dengue disease outbreak definitions are implicitly variable

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Abstract

Infectious diseases rarely exhibit simple dynamics. Outbreaks (defined as excess cases beyond response capabilities) have the potential to cause a disproportionately high burden due to overwhelming health care systems. The recommendations of international policy guidelines and research agendas are based on a perceived standardised outbreak defined as a prolonged, high-caseload, extra-seasonal surge. In this analysis we apply multiple candidate outbreak definitions to reported dengue case

data from Brazil to test this assumption. The methods identify highly heterogeneous outbreak characteristics in terms of frequency, duration and case burden. All definitions identify outbreaks with characteristics that vary over time and space. Further, definitions differ in their timeliness of outbreak onset, and thus may be more or less suitable for early intervention. This raises concerns about the application of current outbreak guidelines for early warning / identification systems. It is clear that quantitatively defining the characteristics of disease is an essential prerequisite for outbreak response. More work is needed so that definitions of disease outbreaks can take into account the baseline capacities of treatment, surveillance and control. This is essential if outbreak guidelines are to be effective and generalisable across a range of epidemiologically different settings.

Keywords

Outbreak; Response; Dengue; Decision-making; Policy

Introduction

While much progress has been made in our ability to treat and reduce the long-term burden of many infectious diseases *(Lim et al., 2013)*, unexpected surges in case numbers above the seasonally expected mean can frequently derail progress or push already stretched healthcare resources to breaking point *(Cotter et al., 2013; Garg et al., 2008; Hay et al., 2003a; Hay et al., 2003b)*. Disease outbreaks often develop rapidly, are difficult or impossible to predict and cause a disproportionately high burden due to the lack of response capabilities *(Garg et al., 2008; Grais et al., 2007; Najera, 1999; WHO Ebola Response Team, 2014)*.

As a result of the clear importance of disease outbreaks to wider control efforts, research agendas and subsequent policy guidelines have heavily focussed on methods to predict outbreaks (early warning), how to identify them once they are occurring (early detection), how to respond to them appropriately (outbreak response protocols) and how to better plan for future outbreak occurrences (effective healthcare, surveillance and control resource allocation) *(Farrar et al., 2007; Hutwagner et al., 2003; Myers et al., 2000; WHO, 1999*, *2005*, *2009)*. Optimisation of each of these individual goals is dependent on an unambiguous quantitative definition of exactly what the term “outbreak” refers to in terms of frequency, duration, amplitude and burden.

One common method for defining outbreaks is to use epidemiological criteria, where any temporal anomaly from the expected number of cases is classified as an outbreak *(Stroup et al., 1993; Wagner et al., 2001)*. Distinguishing the expected number of cases (seasonal variation in incidence) from excessive case numbers (outbreaks) can be difficult for many communicable diseases that exhibit complicated transmission dynamics that are imperfectly sampled by health systems. Dengue, for example, is composed of four serotypes with complex patterns of cross-immunity in humans

(Simmons, 2012; Wearing and Rohani, 2006) that are further complicated by highly heterogeneous environmentally-driven variations in each serotype's distribution and force of infection *(Messina et al., 2014; Reiner et al., 2014)*. In addition to this, treatment-seeking, diagnosis and reporting of dengue is highly variable *(Brady et al., 2014; Endy et al., 2011; Simmons, 2012)*, making interpretation of the seasonal signals in reported case data difficult *(Hay et al., 2013)*.

Despite the complex heterogeneities in transmission of dengue virus (DENV) and many other infectious diseases, methods to distinguish baseline transmission from outbreaks remain simple, often attributed to the capacity of health systems personnel to implement them. Many methods restrict their analysis to intra-annual trends by calculating a monthly or seasonal mean to define the baseline, often referred to as an endemic channel *(Cullen et al., 1984)*. Outbreak thresholds are then often arbitrarily set at two standard deviations in excess of the endemic channel *(Hutwagner et al., 2003; Stroup et al., 1993; Wagner et al., 2001; WHO, 2009)*. For many diseases, the remaining variation not accounted for by these methods means that i) determining the endemic channel is highly uncertain and ii) that the outbreak threshold line is exceeded frequently, briefly and sporadically *(Badurdeen et al., 2013)*. These brief outbreaks may lead to considerable outbreak response measures being deployed, despite minimal excess cases. In public health or operational terms, such an occurrence would be considered as a false alarm for an outbreak.

The inappropriateness of some outbreak response plans to the original case data-based outbreak definitions for dengue has led many to adapt the endemic channel plus two standard deviations method to increase or decrease sensitivity and specificity, for example by requiring two successive weeks above the threshold before response activity is triggered *(Harrington et al., 2013)*. This has led to many different dengue outbreak definitions being employed in different countries and regions *(Badurdeen et*

al., 2013; Harrington et al., 2013). These individual definitions are at odds with international efforts to produce standardised, evidence-based outbreak response strategies *(Farrar et al., 2007; Hutwagner et al., 2003; Pilger et al., 2010; WHO, 2009)* which are focussed on optimising responses to an idealised extra-seasonal surge-type outbreak that may or may not be relevant to the types of outbreaks identified by these locally adapted definitions.

In this paper we use a dataset of reported dengue cases from Brazil (figure 1) to test a wide range of 102 existing outbreak definitions based on five endemic channels and their various parameterisations. This allows us to assess their comparability, consistency over a range of DENV transmission settings and timeliness in outbreak detection.

Methods

Dengue case data

We chose dengue in Brazil as a case study for testing outbreak definitions for a number of reasons. First, Brazil has one of the most comprehensive dengue surveillance systems of any country, with over 200 million people under observation and data disaggregated monthly over ten years or more across 5,570 municipalities. This data is also readily and freely available over long time periods from the Brazilian Ministry of Health surveillance system SINAN *(Ministério da Saúde; Ministério da Saúde)*. Second, Brazil is also epidemiologically representative of a wide range of DENV transmission settings: In the tropical urban northeast, year-round transmission enables relatively consistent transmission levels with some seasonal patterns (figure 1); in the densely populated cities of the southeast much of the case burden is concentrated in outbreaks that occur only once every few years and in the interior western regions, dynamics are dominated by rare large outbreaks that may only occur once every ten years. Third, Brazil already dedicates significant resources towards vector control, including dengue, with over one billion USD being spent in 2008 *(WHO, 2012)*, as well as, having an active research community interested in optimising how this money is spent. Brazil, therefore, is one of the most suitable countries to test and evaluate the usefulness of current dengue outbreak policy with a view to informing international policy guidelines on dengue outbreaks.

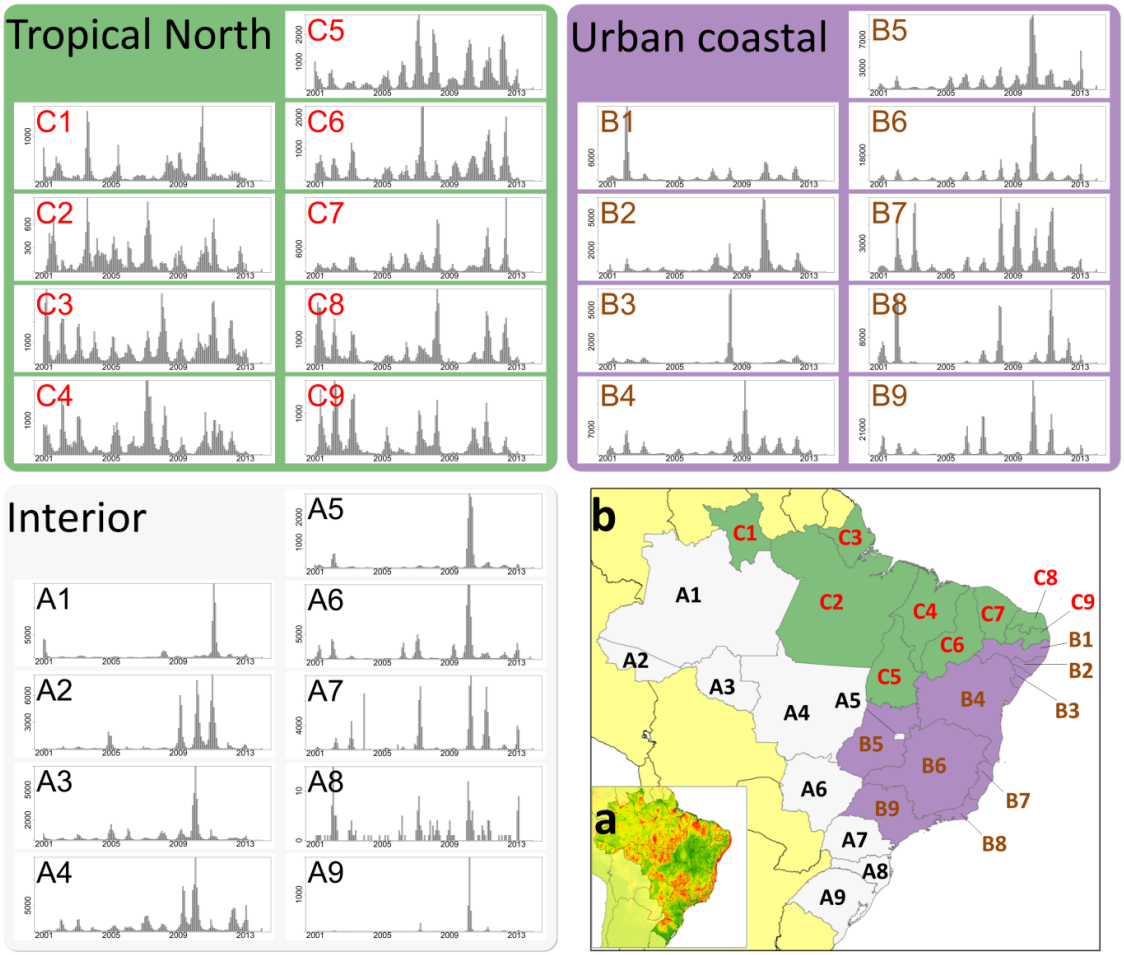

Figure 1. Reported dengue case data in Brazil. Each bar chart displays monthly reported dengue cases (suspected and confirmed) at a state level (n = 27) between the start of 2001 and the end of 2013. Map a) shows the long-term average probability of dengue occurrence as determined by Bhatt *et al. (Bhatt et al., 2013)*. Map b) shows the division of the 27 states into epidemiologically defined groups based on the epidemiological characteristics of their time series. States are divided as follows: Amazonas (A1), Acre (A2), Rondônia (A3), Matto Grosso (A4), Districto Federal (A5), Matto Grosso do Sul (A6), Santa Caterina (A7), Paraná (A8), Rio Grande do Sul (A9), Pernambuco (B1), Alagoas (B2), Sergipe (B3), Bahia (B4), Goiás (B5), Minas Gerais (B6), Espírito Santo (B7), Rio de Janeiro (B8), São Paulo (B9), Roraima (C1), Pará (C2), Amapá (C3), Maranhão (C4), Tocantins (C5), Piauí (C6), Ceará (C7), Rio Grande do Norte (C8), Paraíba (C9).

Monthly total dengue cases were extracted from January 2001 to December 2013 for 27 states (admin1 level *(Food and Agriculture Organization of the United Nations, 2008)*) from the Brazilian Ministry of Health surveillance system SINAN *(Ministério da Saúde; Ministério da Saúde)*. Total dengue cases comprised of hospital reported, suspected and confirmed cases of dengue fever, dengue haemorrhagic fever and dengue shock syndrome. We chose to aggregate the municipality-level data to state-level as, in endemic settings, a more reliable seasonal signal can be obtained over this scale, meaning it is the more frequent scale at which outbreak identification for strategic planning occurs *(Harrington et al., 2013)*. The robustness of our results to our choice of spatial and temporal scale is examined in the Supplementary Information with evaluation at the municipality level, in the state of São Paulo, and using simulated weekly dengue cases.

Outbreak definitions

Existing outbreak definitions are composed of two components, i) an endemic channel which aims to replicate a historical trend of expected cases and ii) a set of criteria that determines what level of variation above this endemic channel is classified as an outbreak. In this analysis we aim to test the comparability of all endemic channels and their various parameterisations that make up the variety of outbreak definitions that are currently in use *(Badurdeen et al., 2013)*.

There are five main methods used to calculate an endemic channel: recent mean, monthly mean, moving mean, cumulative mean and fixed incidence threshold, the calculation of which is summarised in table 1 *(Cullen et al., 1984; Stroup et al., 1993;*

Wagner et al., 2001). These methods are included in core policy documents published by the World Health Organization (WHO) *(WHO, 2009)* and Centers for Disease Control (CDC) *(Hutwagner et al., 2003)* and are widely used across a range of different diseases at different levels of transmission intensity *(Badurdeen et al., 2013; Harrington et al., 2013; Hay et al., 2002)*. Calculation of each definition and its typical uses are given in table 1. For our analysis, the fixed incidence threshold was set at 100 or 300 cases per 100,000 individuals *(Badurdeen et al., 2013; Lowe et al., 2014; Rigau-Perez et al., 1999)* with population data for each state obtained from the Instituto Brasileiro de Geografia e Estatística (IBGE) *(IBGE, 2010)*. For all other methods the following variations on parameters used to define the base dataset for endemic channel calculation were explored: i) the number of historical years to include: five (current) or all available (long-term); and ii) the inclusion of outbreak years in the base dataset, yes or no. Outbreak years within the base dataset were defined by the total annual number of cases exceeding two standard deviations of any combination of three or more historical years. This is a practical approach to "trimming", an approach that minimises the effect of long-tailed distributions on the historical mean and is often implemented by excluding arbitrarily or quasi-quantitatively determined "outbreak years" to increase sensitivity of the contemporary outbreak definition. While "outbreak years" and "outbreaks" do not necessarily have to overlap, there may be some circularity in defining outbreaks using a dataset that has already had outbreak years arbitrarily defined and removed.

Table 1. Endemic channel definitions. Equations calculate the mean (μ), standard deviation (σ) and critical threshold (T_c) for observations at day i for each of the five methods. Selected methods can be modified by changing the number of years (b) in the baseline dataset, or by altering the number of standard deviations (k) that define the critical threshold.

	Recent mean (EARS C1 and C2)	Monthly mean (Historical limits method)	Moving mean (Smoothed mean)	Cumulative mean	Fixed incidence threshold
Countries using method	USA for respiratory illnesses *(Hutwagner et al., 2003)*. Similar methods for dengue in Indonesia *(Badurdeen et al., 2013)*	Colombia, Dominican Republic, Peru and Vietnam for dengue *(Badurdeen et al., 2013)*	Brazil, Malaysia and China for dengue *(Badurdeen et al., 2013; Zhang et al., 2014)*. USA for multiple diseases including dengue *(Rigau-Perez et*	USA for *Salmonella (Hutwagner et al., 2003)*. Proposed for malaria in Thailand *(Cullen et al., 1984)*. Ros River virus in Australia *(Pelecanos et*	Puerto Rico and Brazil for dengue *(Badurdeen et al., 2013; Lowe et al., 2014; Rigau-Perez et al., 1999)*
Typical uses	Diseases with little seasonal pattern and limited surveillance data	Diseases with a consistent seasonal cycle	Diseases with a seasonal cycle, the timing of which shifts year on year	Diseases with sporadic outbreaks	Diseases where response capacity is set to a particular fixed level of incidence
Method of calculation	The overall mean of a small set of recent observations *(Hutwagner et al., 2003)*	The mean of the corresponding months in the base dataset *(Cullen et al., 1984)*	The mean of the corresponding months and three months either side in the base dataset *(Cullen et al., 1984)*	The mean of the corresponding months yearly cumulative case count *(Hutwagner et al., 2003)*	A chosen fixed value of cases per 100,000 individuals in the population *(Lowe et al., 2014)*
Equation	$\mu_i = \frac{\sum_{b=s}^{b=t}(x_{(i-b)})}{7}$ $\sigma_i = \sqrt{\frac{\sum_{b=s}^{b=t}(x_{(i-b)} - \mu_i)^2}{6}}$ $T_{c,i} = \mu_i + k\sigma_i$ Where the data window is defined by: $s = 1$ and $t = 7$ for C1 and $s = 3$ and $t = 9$ for C2	$\mu_i = \frac{\sum_{b=1}^{b=b}(x_{(i-12b)})}{b}$ $\sigma_i = \sqrt{\frac{\sum_{b=1}^{b=b}(x_{(i-12b)} - \mu_i)^2}{(b-1)}}$ $T_{c,i} = \mu_i + k\sigma_i$	$\mu_i = \frac{\sum_{b=1}^{b=b}(x_{(i-12b-1)} + x_{(i-12}}{b}$ $\sigma_i = \sqrt{\frac{\sum_{b=1}^{b=b}(x_{(i-12b-1)} - \mu_i)^2}{}}$ $T_{c,i} = \mu_i + k\sigma_i$	$\mu_i = \frac{\sum_{b=1}^{b=12b}(x_{(i-b)})}{12b}$ $\sigma_i = \sqrt{\frac{\sum_{b=1}^{b=b}(x_{(i-12b)} - \mu_i)^2}{(b-1)}}$ $T_{c,i} = \max\left(0, [\sigma_i((k+0.5) - T_{c,(i-1)}) + \mu_i]\right)$	$T_{c,i} = 0.001 \; or \; 0.003$

The following parameters that determine the level of variation above the endemic channel that defines an outbreak were also explored: i) the number of standard deviations above the historical mean: 1 or 2 and ii) the number of monthly observations above the threshold that would trigger the start of an outbreak: 1, 2 or 3. The four dynamic endemic channel methods, combined with the four base dataset parameters and the six outbreak threshold parameters gave a total of 96 (= 4 x 2 x 2 x 2 x 3) definitions which combined with the six definition variations of the fixed incidence method (two thresholds that just vary by the number of observations above the threshold to trigger an outbreak), gave a total of 102 different outbreak definitions.

Each outbreak definition was calculated annually at the beginning of years 2006-2013, then its performance was evaluated over the following year, with the exception of the recent mean method which was evaluated on a rolling monthly basis. The following measures were collected: the number of outbreaks identified and the proportion of total cases in the time series that exceeded the outbreak threshold.

To quantify the relative variation in outbreak characteristics identified by each definition, a kernel estimation method was used. The two variables, number of outbreaks identified and the proportion of total cases in the time series that exceeded the outbreak threshold, were first scaled to equivalent maximum ranges (0-10). Using the "*hdrcde"* package in R, a two dimensional highest density region method was used to estimate the area of the kernel that contained the most concentrated 50% of the density of the combined distribution *(Hyndman, 1996)*. This allowed us to measure relative variation in outbreak characteristics, between for example two different states, by taking into account the spread of the most central results. A dispersal index, D, quantified this relative dispersal with higher values indicating more varied outbreak characteristics.

Results

Different outbreak definitions are incomparable even when applied to the same data

To compare the outbreak characteristics identified by each different outbreak definition we applied every definition to each state in Brazil. To compare outbreak characteristics we measured the total number of unique outbreaks identified by each definition and the percentage of total cases across the time series that were classified as outbreak cases. The distribution of these results for each state is shown grouped by the three epidemiological categories in figures 2a-c and the aggregated results showing variation around the mean for each state is shown in figure 2d.

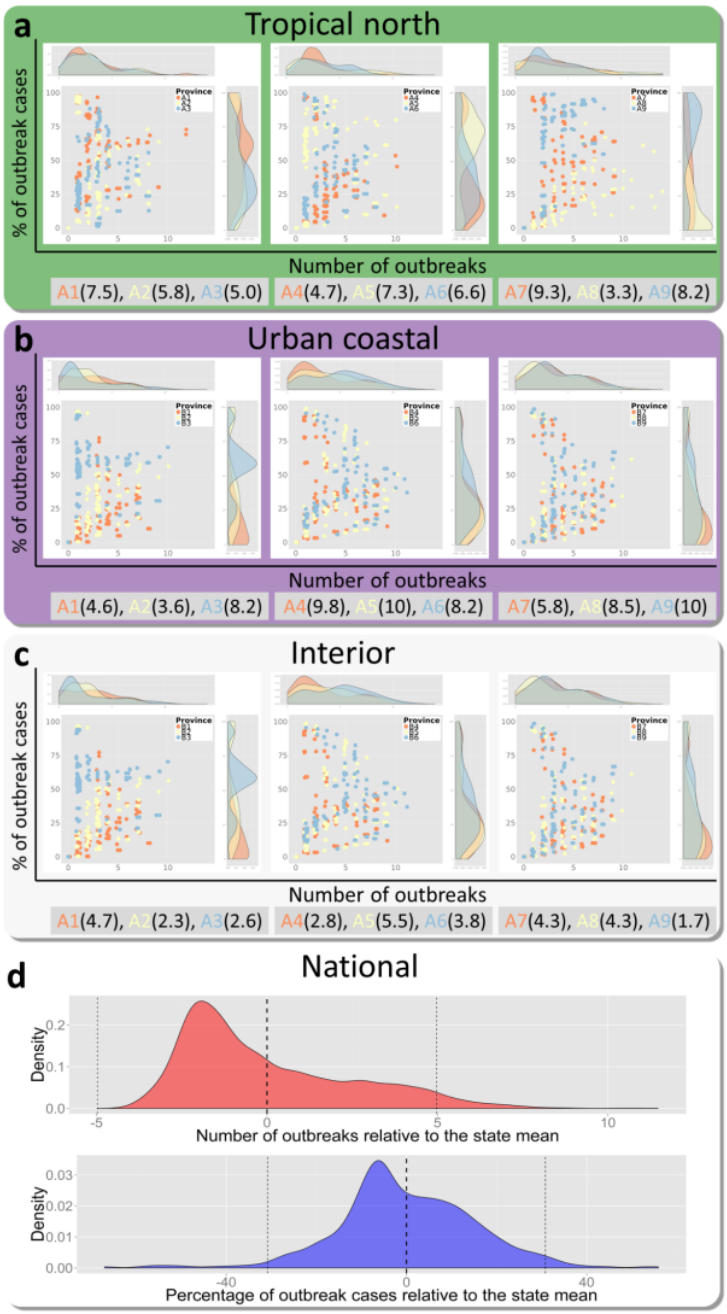

Figure 2. Variability between all outbreak definitions applied to grouped states. Figures a-c show the distribution of the number of outbreaks and percentage of outbreak characteristics identified (the two axes) when each definition (102 same coloured points) is applied to the same state (different colours, grouped three states per plot). This variance around the mean for each state is aggregated to give the distribution at the national level in d. Dotted black lines in d show the mean and 95% confidence intervals.

Overall outbreak characteristics are highly variable (figure 2d). Among the majority of definitions (95%), the total number of outbreaks identified by any given pair of definitions could differ by as many as 10 outbreaks and the total proportion of outbreak cases could differ by as much as 65% over just a nine-year time series. This variability is highest in the urban coastal regions (average dispersion metric $\widehat{D} = 7.6$), still high in the tropical north regions ($\widehat{D} = 6.4$), and lowest in the interior region ($\widehat{D} = 3.6$) (figure 2). For the interior states variability mainly occurs in the number of outbreaks identified (figure 2c) due to definition sensitivity in already low transmission settings, while variation in the tropical north mainly occurs in the proportion of cases identified as outbreaks (figure 2a) due to the similarities between seasonal trends and the small outbreaks they experience (figure 1). In the urban coastal region we see high variation in both of these axes (figure 2b) highlighting the difficulty in distinguishing frequent large outbreaks from seasonal trend, such as in Espírito Santo (B7, figure 1)

Even if outbreak definitions are standardised, outbreak characteristics are inconsistent in different states

While outbreak characteristics are clearly variable between different outbreak definitions, thus hampering inter-definition comparability, in many cases a particular single definition will be chosen and applied over a wide number of areas, such as a nationally standardised definition. To test the consistency and comparability of such an approach we measured variation in outbreak characteristics when applying a single definition to all 27 Brazilian states (figure 3).

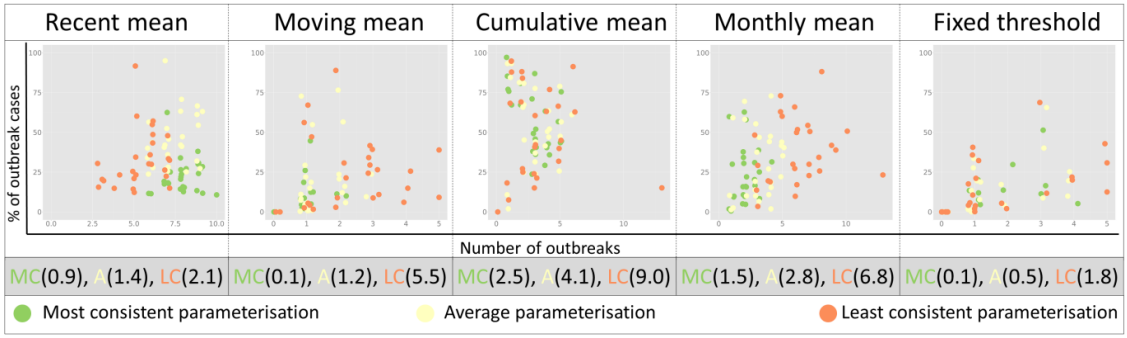

Figure 3. Variability of outbreak characteristics when one outbreak definition is applied to all 27 states. The scatterplots show the outbreak characteristics of each state (shown by 27 same coloured dots) when the most consistent (green), least consistent (orange) and an average (yellow) definition is chosen from each endemic channel.

Endemic channel	Parameterisation	Years of baseline data	Number of consecutive observations above threshold	Outbreak years included	Standard deviations above mean	Relative consistency (D)
Recent mean	Most consistent	5	1	No	1	0.9
	Average	All	1	Yes	1	1.4
	Least consistent	All	2	Yes	1	2.1
Moving mean	Most consistent	All	3	Yes	2	0.1
	Average	5	1	No	2	1.2
	Least consistent	All	1	No	1	5.5
Cumulative mean	Most consistent	5	3	Yes	1	2.5
	Average	All	3	Yes	1	4.1
	Least consistent	All	1	Yes	2	9.0
Monthly mean	Most consistent	5	3	No	2	1.5
	Average	All	2	No	2	2.8
	Least consistent	All	1	Yes	1	6.8
Fixed threshold	Most consistent	-	3	-	0.01 fixed	0.1
	Average	-	2	-	0.01 fixed	0.5
	Least consistent	-	1	-	0.01 fixed	1.8

Table 2. Parameter details of the most and least consistent definitions shown in Figure 3. Parameters are explained in the methods section. Fixed threshold methods use fixed values of incidence (100 or 300 cases per 10,000) instead of standard deviations above the mean.

No one single definition identified consistent outbreak characteristics when applied nationally (figure 3). While some outbreak definitions clearly identified outbreaks with more variable characteristics than others, the general trend was that even the most consistent outbreak definition offered little improvement in consistency over the average definition and there were no common themes among the parameters that made definitions more consistent (Table 2). Where outbreak definitions did reach consistency, such as the most consistent moving mean definition (figure 3), this was often due to identifying no outbreaks, or outbreaks of limited duration. As the choice of outbreak definition parameterisation is often justified by improved geographic relevance, this result suggests little improvement in consistency is gained by minor changes in definition parameterisation.

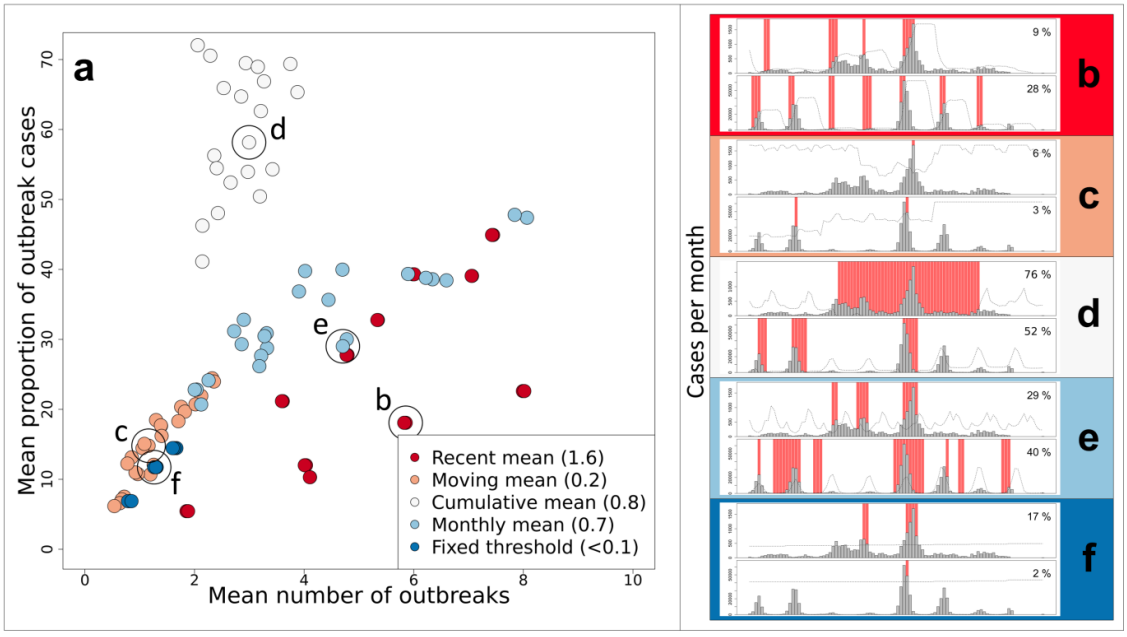

Figure 4. Outbreak characteristic variability by endemic channel parameterisation. Figure 4a shows the mean outbreak characteristics across all states of each endemic channel parameterisation (n = 24 except fixed threshold where n = 6 unique coloured dots). Representative examples of each of the endemic channel

definition types applied to low transmission (Roraima, C1, upper panel) and high transmission (São Paulo, B9, lower panel) environments are shown in figures 4b-f. Grey bars indicate monthly case numbers 2006-2013, dotted lines show the endemic channel for each year and red background indicates outbreak months identified by the given definition. The percentage figure in the top right shows the percentage of total cases that are identified as outbreak cases.

Outbreak definitions based on cumulative mean and monthly mean endemic channels tended to identify a greater proportion of total cases as outbreaks, while monthly mean and recent mean methods tended to identify the highest number of outbreaks (figure 4). Fixed mean and moving mean endemic channels tended to identify outbreaks of smaller magnitude, but also fewer outbreaks (figure 4). Recent mean, cumulative mean, and monthly mean-based definitions appeared to be the most flexible and could be tweaked to identify outbreaks of different magnitudes and frequencies ($\widehat{D} = 1.6$, 0.8 and 0.7 respectively). Representative examples from each endemic channel type are explored in more detail in figures 4b-f where each definition is applied to a low incidence (Roraima, C1, upper panel) and high incidence (São Paulo, B9, lower panel) setting. The types of outbreaks identified in these two contrasting DENV transmission settings vary considerably in terms of i) the characteristics of each outbreak (how many cases and over how long) and ii) outbreak frequency and intra-annual timing. This suggests that even if a standardised definition of an outbreak was adopted, the actual characteristics of an outbreak, and thus approaches and resources needed to respond, would be significantly different in different states.

Finally, it is also worth noting that even when one definition is applied consistently in one state, the characteristics of the outbreaks identified across time are inconsistent

(figures 4B-F). The outbreaks identified vary in size, length and in the times of year in which they occur.

The timing of onset of an outbreak varies significantly depending on outbreak definition

For the purpose of early detection and rapid response to disease outbreaks, the definitions need to identify the onset of an outbreak, and trigger appropriate response activities before the bulk of cases occur. The earlier response measures are enacted during the onset of an outbreak, the greater the chance interventions will have at reducing the number of excess cases. To test the variation in sensitivity of timeliness of outbreak detection, we analysed the predictions of all 102 definition combinations against outbreaks in an interior state (Matto Grosso do Sul, A6, 2010), an urban south eastern state (Rio de Janeiro, B8, 2011) and a northern state (Maranhão, C4, 2007) all with differing DENV transmission dynamics (figure 5).

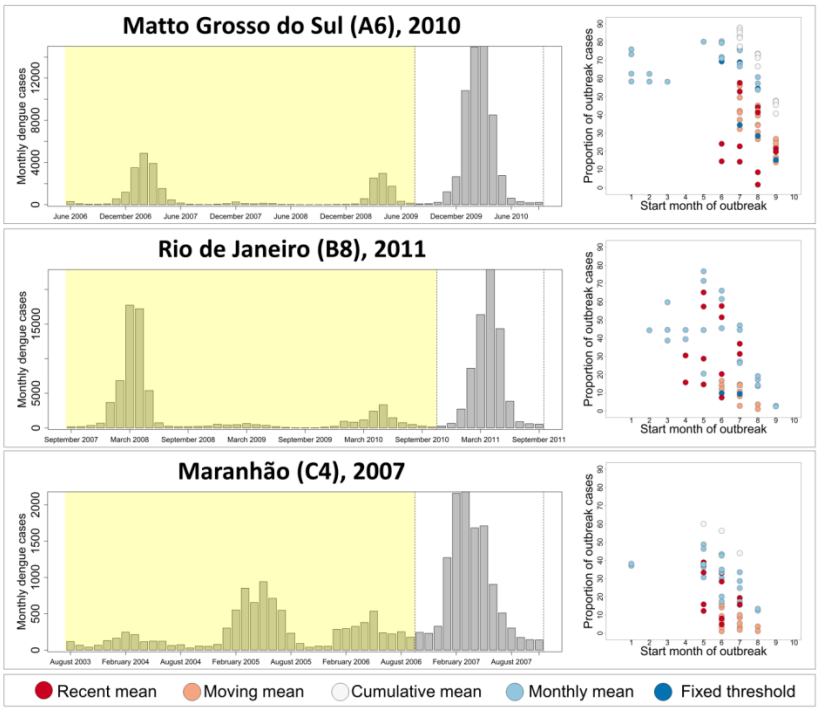

Figure 5. The difference in time of onset and overall outbreak size for different outbreak definitions applied to different example outbreaks. The longitudinal plot (left) shows the monthly reported case number in the four years (shaded yellow) building up to an extra-seasonal surge in cases (unshaded) in three different states with differing DENV transmission dynamics. For each outbreak, the graph on the right shows the variability in timeliness of detection (number of months since outbreak onset, x-axis) and outbreak size as a proportion of total cases (y-axis) when fitted to data in the yellow shaded region and applied to the unshaded region. Only definitions that identified an outbreak are shown.

When focussing on a single outbreak there is considerable variation in timeliness of detection and resultant outbreak size between different outbreak definitions. In all three states different definitions identified outbreaks that varied in their timing of outbreak onset by as much as seven or eight months, with the majority of outbreaks still varying by as much as four months (figure 5). Generally, monthly mean and recent mean methods proved the timeliest, while moving mean methods were consistently the least timely of all methods tested. Over all three outbreaks it can be observed that even definitions that lag behind those that identify outbreaks early, can still identify the bulk of cases in a given outbreak. If outbreak response is primarily reactive, these less sensitive definitions would be more suitable, however if outbreak response is preventative, the additional lead time afforded by more sensitive definitions may be of benefit. As a final point, it should be noted that at least the majority of definitions did identify an outbreak in these three selected examples and while the timing and scale of responses would likely be very different depending on which outbreak definition was used, at least a response would have been triggered.

Conclusions and discussion

While the concept of a disease outbreak is undoubtedly important, it is clear there is no consensus on how to define such a term. Moreover, the range of existing definitions is incomparable, inconsistent and highly variable in their ability to permit effective early response. The magnitude of this incomparability also increases with outbreak size, and thus disease burden. These findings present a real concern for existing and planned policy guidelines, as well as operational early warning and early detection systems, that have given insufficient consideration to the heterogeneous outbreak characteristics identified in real time in a variety of different DENV transmission settings *(Farrar et al., 2007; Harrington et al., 2013; Hutwagner et al., 2003; Myers et al., 2000; WHO, 1999*, *2005*, *2009)*.

In this analysis we have shown that even if a single outbreak definition was adopted from among the range of different definitions currently available, exactly what constitutes an outbreak would still be inconsistent both across space and through time. This has implications for national level outbreak response guidelines as it does not present a standardised measure with which to compare sub-national healthcare and control needs, nor does it allow the monitoring of progress towards long-term reductions in disease burden. Furthermore, even using a standardised definition within a single state to optimise outbreak response strategies may be inadequate as the characteristics of outbreaks may change over time despite only subtle changes in DENV transmission dynamics over the same time period.

As an additional limitation, we showed that currently-used outbreak definitions vary widely in their capacity to enable effective preventative outbreak measures such as early detection and early response *(Chowell et al., 2008; Lowe et al., 2014)*. This may make the recommendation of such activities obsolete under certain conditions with

particular outbreak definitions, particularly those with reduced sensitivity that require more than one observation above a given threshold to trigger an outbreak. In such a situation, preventative control may be more heavily reliant on early warning systems that predict outbreaks based on temporal anomalies in epidemiological and environmental warning signals *(Degallier et al., 2010; Lowe et al., 2014; Teklehaimanot et al., 2004a; Teklehaimanot et al., 2004c)*. Detecting these temporal anomalies in different kinds of data may well encounter similar issues with inconsistency and variability that have been highlighted here. We would therefore recommend the use of techniques that are more complex than simple interpretations of historical means or fixed thresholds to detect these temporal anomalies. Particular care must be taken in making arbitrary decisions about the data used to define the historical baseline, such as excluding ”outbreak years”. Even with the use of more flexible statistical frameworks *(Box et al., 2013)* and a wealth of new data *(Ministério da Saúde; Ministério da Saúde; PAHO, 2014)*, the identified associations may well be highly dependent on how disease outbreaks are defined and a clear definition of a disease outbreak is an essential prerequisite for the evaluation of the sensitivity and specificity of forecasting, early warning, or early detection. Without a meaningful and operationally relevant fixed definition, instead of just arbitrary thresholds *(Lowe et al., 2013; Lowe et al., 2014)*, there is no way to compare the performance of predictive models, nor is it possible to construct guidelines based on their outputs. These criteria need to be rigorously assessed before any predictive or early warning system can become a practical tool for outbreak identification and response.

The recommendations presented here come from the analysis of reported, suspected and confirmed dengue cases in Brazil. This was done to maximise the available data and to avoid the effects of discrepancies in laboratory diagnostic capacities between different states. While spatial and temporal variation in misreporting are likely to affect

the ability of outbreak definitions to identify consistent outbreaks, it is likely that the common propensity for over-reporting of dengue cases during outbreaks and underreporting at other times would only reinforce the consistent distinction between outbreak periods and non-outbreak periods *(Teutsch and Churchill, 2000)*. It is possible, however, that mis-reporting could affect the timelines of outbreak detection, which was not tested here.

We chose a subset of the wide variety of outbreak definitions available *(Chen and Chang, 2013; Debin et al., 2013; Dórea et al., 2013; Hay et al., 2003a; Jafarpour et al., 2013; Pelecanos et al., 2010; Polanco et al., 2013)*. Our selection was based upon the principle types of definitions recommended in current outbreak guidelines *(Hutwagner et al., 2003; WHO, 2009)*, with proven uptake in selected countries (table 1) and some claims of consistent results *(Zhang et al., 2014)*. Additional definitions may identify more consistent outbreak characteristics, but their performance over wide-scale datasets have yet to be tested. We chose to evaluate outbreak definitions at the state scale as it is the most common level for strategic wide-scale outbreak response recommendations to be made *(Harrington et al., 2013)*. Given the general increase in heterogeneity at lower spatial scales and over shorter time periods, we would not expect existing outbreak definitions to be any more comparable at lower spatial scales or with more frequent reporting intervals. This is supported by the results of our further analysis presented in the Supplementary Information. It is possible that outbreak definitions in transmission-free settings (where any occurrence of cases typically triggers outbreak response *(Harrington et al., 2013)*) may be consistent and appropriate; this definition has not been tested in this analysis. Despite this, given the diverse nature of global DENV transmission *(Bhatt et al., 2013; Messina et al., 2014)*, of which Brazil represents a small subset, we would not expect outbreak definitions to be any more comparable in different countries or regions. We also confine our analysis

of disease outbreaks to dengue. While the four serotypes of dengue and their associated immune responses in humans mean patterns of DENV transmission are highly spatially and temporally heterogeneous, various intricacies of transmission in many other diseases *(Anderson and May, 1991; Reiner et al., 2013; Smith et al., 2014)* are likely to lead to similar complex reported case dynamics and equally unclear definitions of what comprises a disease outbreak. Similar analyses for other diseases should be conducted to test this hypothesis and to evaluate the usefulness of more established international guidelines for outbreak response.

For dengue, and many other diseases, information other than reported and suspected cases is available which may be more reliable for defining outbreaks due to the inherent temporal biases of reporting and diagnosis associated with simple reported case data *(Klaucke, 1994)*. Data on the percentage of positive diagnostic tests, entomological indices and environmental signals *(Harrington et al., 2013; Teklehaimanot et al., 2004b)* can be incorporated alongside reported cases in defining disease outbreaks or pre-emptive alert phases. Following an alert phase, an outbreak investigation is typically triggered which may involve forms of sentinel or enhanced passive surveillance. Despite these recommendations, what defines an outbreak following the results of these investigations remains unclear in many cases *(Harrington et al., 2013)*. Irrespective of their reliability, data additions are often logistically and financially costly to make timely and outbreak-relevant, making them counter-productive to the goal of internationally standardised outbreak response and less useful in resource-constrained settings. Furthermore, this does not represent outbreak identification in the majority of settings for dengue *(Badurdeen et al., 2013; Harrington et al., 2013)*. It is also likely that many of these additional data types are closely correlated with reported dengue cases, in which case the benefit of improved outbreak detection consistency needs to be weighed up against the cost of collecting the

additional data. If such additional data sources are found to be useful in defining a dengue outbreak, it should be emphasised that they should only be considered in addition to, and not at the expense of, routine passive surveillance of suspected and confirmed dengue cases. This basic measure has core value in identifying dengue seasonality *(Hay et al., 2013)* and detecting dengue outbreaks as it is the most direct measure of the number of people placing a burden on the healthcare system, in addition to being the most abundant data source for pattern recognition.

Given that dengue and likely many other diseases have frequent, varied and unpredictable deviations from seasonal mean case numbers, there is a rationale to reconsider what the terms "disease outbreak" and "outbreak response" refer to. In epidemiological terms, outbreaks occur at many different levels of intensity, duration and velocity in deviations from seasonal means, driven by a range of factors that determine transmission suitability *(Brady et al., 2013; Johansson et al., 2009; Wearing and Rohani, 2006)*. From a public health perspective, however, the term "outbreak" refers to a situation where routine surveillance, treatment and control capacities are exceeded and exceptional interventions are required. When we use epidemiological data to differentiate routine from excessive caseloads we assume that routinely deployed public health resources can be, and are, matched to the identified baseline transmission levels. For a number of logistical, financial and practical reasons this is unlikely to be the case, especially with some of the more variable outbreak definitions. Furthermore, many of these resources, such as patient beds in a hospital, are required for many different diseases and conditions and therefore have varying degrees of flexibility.

If the purpose of an outbreak definition is to identify periods of excess burden, a thorough assessment of baseline healthcare surveillance, control, and treatment capacities is needed. Measures such as the number of hospital beds, insecticide

stockpiles and the quantity of surveillance teams will all be important in defining the baseline capacity limits. Additional data on actions that are taken in outbreak times, such as staff surge capacities, resources for additional mass fogging, rapid sentinel surveillance and the speed of deployment of each of these, will then be useful for defining what numbers of cases per month should fall under the remit of routine activities and what number of cases should trigger exceptional measures. Given this data, modelling approaches can be used to optimally allocate resources between routine and exceptional responses given the logistical constraints of each resource. Such a model could be evaluated periodically and have a role in healthcare budget allocation as well as providing recommendations for specific actions to be undertaken in outbreak times. Any integration of early warning or early detection systems needs to be critically evaluated against investments in improvements of resource allocation or improved disease surveillance.

This alternative method of defining an outbreak may require unconventional and additional data sources, but has the potential to add much-needed clarity to the neglected issue of what is a practical definition of an outbreak. Only then is it feasible to undertake quantitative modelling approaches that aim to optimise prevention or amelioration activities that minimise the burden of dengue outbreaks. The scale and extent of Brazil's dengue surveillance system means it is well-placed to advance dengue outbreak research and could be a first adopter of a new generation of evidence-based dengue outbreak policy.

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Author contributions

OJB, DLS, TWS and SIH designed the experiment. OJB, wrote the manuscript and collected and analysed the data. All authors helped with data and results interpretation and were involved in drafting, revising and final approval of the manuscript.

Competing financial interests

The authors declared no financial interests.

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Chapter 5 – Modelling adult survival of the mosquito vectors of dengue

5.1. Cover page

The analyses presented in this thesis so far have only evaluated surveillance of human DENV infections. As illustrated in chapter 1, mosquito vectors have played a key role in the global expansion of dengue in the past and present and will likely do so in the future. Understanding the role mosquito vectors play, particularly for *Ae. aegypti* and *Ae. albopictus*, in the global spread of dengue is critical to forming policy that contains further expansion and lowers transmission in areas that have yet to become hyperendemic. While regular wide-scale surveillance of mosquito population dynamics is not currently reliable or feasible, it is possible to develop an understanding of their behaviours that are most important for transmission through models. These models can then be parameterised, using what limited data is available from experiments in particular locations. The uncertainty associated with extrapolating these predictions to novel locations can be quantified by cross-validation with other data sets.

The parameter describing adult female mosquito survival is of particular importance due to its effects on at least three rate-limiting steps in transmission. Adult survival affects the number of mosquitoes that will i) mature to human biting age, ii) survive long enough for the virus to complete its extrinsic incubation period (EIP) and iii) go on to deliver infectious bites on humans. Under certain conditions adult survival may also have additional effects on transmission potential through mosquito population dynamics *(Brady et al., 2014)*.

A key factor affecting adult mosquito survival and the length of the DENV EIP on a global scale is temperature, evidenced by the known global distribution of *Ae. aegypti*

and *Ae. albopictus* broadly aligning with winter minimum temperature isotherms *(Christophers, 1960; Nawrocki and Hawley, 1987)*. Understanding the role temperature plays in vector survival and virus development makes it possible to predict the global limits temperature places on DENV transmission. Refining the areas and times of the year at risk of introduction of disease vectors and/or future DENV spread will improve the targeting of limited mosquito surveillance and control capacities.

Chapters 5 and 6 explore the constraints temperature places on *Ae. aegypti* and *Ae. albopictus* population persistence and ability to transmit DENV. Chapter 5 is dedicating to a significant update in the way data from both the laboratory and the field is used to model survival of adult mosquitoes.

The manuscript presented here as chapter 5 was published in *Parasites and Vectors* in December 2013. The referred to supplementary information can be found in the Appendix section (page 199). The data collected in this analysis helped shape wider efforts to collate a universal database of mosquito mark-release-recapture studies to help inform other aspects of mosquito movement, behaviour and survival *(Guerra et al., 2014)*.

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Parasites & Vectors

RESEARCH Open Access

Modelling adult *Aedes aegypti* and *Aedes albopictus* survival at different temperatures in laboratory and field settings

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Abstract

Background: The survival of adult female *Aedes* mosquitoes is a critical component of their ability to transmit pathogens such as dengue viruses. One of the principal determinants of *Aedes* survival is temperature, which has been associated with seasonal changes in *Aedes* populations and limits their geographical distribution. The effects of temperature and other sources of mortality have been studied in the field, often via mark-release-recapture experiments, and under controlled conditions in the laboratory. Survival results differ and reconciling predictions between the two settings has been hindered by variable measurements from different experimental protocols, lack of precision in measuring survival of free-ranging mosquitoes, and uncertainty about the role of age-dependent mortality in the field.

Methods: Here we apply generalised additive models to data from 351 published adult *Ae. aegypti* and *Ae. albopictus* survival experiments in the laboratory to create survival models for each species across their range of viable temperatures. These models are then adjusted to estimate survival at different temperatures in the field using data from 59 *Ae. aegypti* and *Ae. albopictus* field survivorship experiments. The uncertainty at each stage of the modelling process is propagated through to provide confidence intervals around our predictions.

Results: Our results indicate that adult *Ae. albopictus* has higher survival than *Ae. aegypti* in the laboratory and field, however, *Ae. aegypti* can tolerate a wider range of temperatures. A full breakdown of survival by age and temperature is given for both species. The differences between laboratory and field models also give insight into the relative contributions to mortality from temperature, other environmental factors, and senescence and over what ranges these factors can be important.

Conclusions: Our results support the importance of producing site-specific mosquito survival estimates. By including fluctuating temperature regimes, our models provide insight into seasonal patterns of *Ae. aegypti* and *Ae. albopictus* population dynamics that may be relevant to seasonal changes in dengue virus transmission. Our models can be integrated with *Aedes* and dengue modelling efforts to guide and evaluate vector control, better map the distribution of disease and produce early warning systems for dengue epidemics.

Keywords: *Aedes*, Survival, Temperature, Mortality, Longevity, Modelling, Mark-release-recapture, Dengue, Transmission, Generalised additive models

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Background

Survival of arthropod vectors is one of the most important components of transmission of a vector-borne pathogen [1-3]. Increased survival allows the vector to produce more offspring, to increase the chances of them becoming infected, to disperse over greater distances, to survive long enough to become infectious, and then to deliver more infective bites during the remainder of its lifetime. As a result, small changes in survival rate cause large changes in the rate of pathogen transmission [3-7]. Furthermore, seasonality and the geographic distribution of vector borne diseases are often constrained by differences in vector survival [8,9].

Adult female *Aedes aegypti* (L.) are the principal vectors for dengue and urban yellow fever viruses, two globally important human arboviruses [10-12]. *Ae. albopictus* (Skuse) can also act as a secondary vector for dengue viruses (DENV) [13,14], along with 22 other arboviruses [13,15,16], some of which have an increasing public health burden [17]. There has, therefore, been considerable interest in investigating and quantifying factors affecting survival of adult females of both species and how this contributes to virus transmission [18,19]. Because *Aedes* mosquitoes are small-bodied poikilotherms it is logical that temperature is consistently observed as a principal factor affecting survival [20,21] and the current global range of both species align broadly with separate critical limits imposed by winter isotherms [9,22]. While other factors such as humidity and photoperiod are important, the effects of temperature have been most rigorously quantified and most frequently identified as limiting factors for survival [10,18].

Reconciling the expected difference between laboratory and field survival estimates has been complicated by the lack of precision of available field techniques and the limited temperature ranges over which field experiments have been undertaken. In mosquito cages in the laboratory, conditions can be controlled and the effects of temperature quantified accurately, but additional causes of mortality experienced in the field, such as predation or disease are absent. The most commonly used method for observing *Aedes* survival in the field is mark-release-recapture (MRR) where marked individuals of a known age are released and the number of recaptures observed over subsequent days [23]. While MRR experiments expose mosquitoes to all causes of mortality in the field, experiments with *Ae. aegypti* and *Ae. albopictus* typically have small sample sizes [24] and sensitivities of spatial mosquito sampling limit the level of survival detail that can be elucidated through the majority of MRR experiments [25]. Furthermore, MRR experiments are limited in number and cover only a narrow range of environmental temperatures (see Additional file 1). There is, therefore, considerable uncertainty over variation in *Aedes* survival in the field.

Using laboratory estimates of survival to predict field survival is complicated by the different mortality risks in the two settings, unknown importance of senescence in the field, and the transition from constant laboratory to fluctuating natural temperature regimes. It has long been assumed that the high rate of mortality in the field, due to external causes, ensures few mosquitoes live long enough to experience age-dependent mortality [26]. Observations from laboratory and field experiments on *Ae. aegypti*, however, have begun to challenge this assumption [23,27]. As a result, the conditions under which age-dependence or age-independence dominates mortality remain unresolved, yet are potentially of considerable epidemiological importance because older mosquitoes are more likely to have survived beyond the virus's extrinsic incubation period. Furthermore, controlled laboratory conditions are not expected to have the same effect as what is observed in the field. This is particularly apparent with observations at constant temperature in the laboratory in contrast to the fluctuating temperature experienced in the wild. At lower mean temperatures, fluctuation may increase survival because at least part of the day could permit significant increases in survival. The converse is true for optimal mean temperatures, where any positive or negative departure from the optimum could lead to decreased survival [28,29]. Both of these issues have widened the gap between field and laboratory observed survival in terms of the average and distribution of survival times.

Observations from temperature-controlled laboratory experiments have produced a range of candidate parametric functions that are suitable for modelling age-dependent mortality, such as the Gompertz and Logistic functions [27,30]. Early experiments were extended by Degallier *et al.* who observed age-dependent mortality in caged mosquitoes exposed to uncontrolled field temperature regimes [31]. Degallier *et al.* also extended the parametric analysis by using non-parametric Cox proportional hazard models, which allowed them to analyse the significance of both age and environmental conditions on adult mortality. The importance of seasonal changes in temperature was emphasised by Strickman *et al.* who conducted year-round field experiments on caged mosquitoes in Thailand [32]. Yang *et al.* attempted to explicitly model the relationship between temperature and survival, however, their model assumed age-independent mortality and relies on relatively limited data [21]. The paucity of data and the challenges of fitting robust statistical relationships to *Ae. albopictus* adult longevity data are discussed by Waldock *et al.* who highlight the disparity relative to the data available for development of the mosquito's immature stages at a range of different temperatures [33].

In this study we compiled published observations from a variety of studies and modelled the effect of temperature on survival of adult female *Ae. aegypti* and *Ae. albopictus*

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under laboratory and field conditions. Our models incorporated the effects of age-dependent mortality and predict survival under fluctuating temperature regimes. They provide a more detailed understanding of *Aedes* adult female mortality and present a statistical solution to investigating age-dependent survival. The resulting models may enhance current DENV transmission models [5,33-36], which could improve predictions that guide vector control, identify areas suitable for DENV transmission and contribute to outbreak early warning systems.

Methods

Data collection

Relevant publications were collected by searching the databases of PubMed, Google Scholar and the Armed Forces Pest Management Board Literature Retrieval System using the search terms *Aedes, aegypti, albopictus, survival, mortality, longevity*, *stegomyia* and *albopicta*. Resulting abstracts were examined for their likelihood to contain laboratory-based adult female survival experiments (closed cage with controlled or semi-controlled environmental and feeding regimes). A more detailed inspection of the manuscript allowed us to apply further inclusion criteria for the final database of laboratory survival experiments. Included experiments monitored mosquito survival of at least five individuals at least every two days and gave all mosquitoes access to nutrition (sugar or any form of blood feeding). In the field it appears that female *Ae. aegypti* feed almost exclusively on human blood [37,38], however, laboratory experiments use a range of diets, such as blood plus sugar, sugar only or blood only, artificial blood meals (blood drawn from a variety of different vertebrates presented to mosquitoes in different ways), and direct feeding on a variety of different vertebrates that can confer significant differences in survival [38-43]. As a result this information was collected and incorporated as a random effect in our analysis and all predictions were made for mosquitoes fed human blood. Due to factors other than mosquito diet, laboratory survival observations still showed a significant amount of variation (see Additional file 1), as a result we decided to include a random effect at the experiment level to control for the wide variety of experimental set ups. All experiments analysed used mosquitoes colonised in the laboratory for less than 2 years. Experiments where mosquitoes were chemically or genetically treated were excluded. Finally, *Ae. aegypti* used in the survival experiments were geographically distributed across five continents incorporating both tropical and temperate latitudes. There was a slight bias towards North American samples in the *Ae. albopictus* survival data, however, strains from tropical areas, such as Réunion and Malaysia, were also included.

Literature searches for MRR field experiments were completed independently under a parallel project to investigate mosquito movement (Guerra *et al.* unpublished observations). Briefly, existing literature was searched using a similar key word strategy, adult female mosquito MRR and ancillary geographic data were extracted from suitable articles. Using these references and ancillary data, daily recapture numbers were extracted from relevant articles. Of the *Ae. aegypti* MRR studies, 14% released field caught mosquitoes, 36% released laboratory reared mosquitoes from a recently caught strain and 50% released a colonised mosquito strain. For *Ae. albopictus*, the corresponding breakdown was 4%, 20% and 76%.

Where available, the initial number of mosquitoes (for laboratory experiments) and number of deaths/recaptures on each subsequent day of observation (for MRR experiments) were extracted. Where only graphical summaries were available, these observations were recreated using GetData Graph Digitizer [44]. For any article where observations could not be recorded or recreated the authors were contacted and primary data was requested. For laboratory data the mean temperature was recorded and for field data the maximum and minimum temperatures were recorded. Where unavailable, study site temperatures were estimated using the average climate for the particular location and time of year using WorldClim global climate data [45]. The resulting number of articles and data points included are summarised in Table 1. The full reference list and summary boxplots are available in Additional file 1.

Comparability of data and fitting parametric models to laboratory survival data

A range of parametric equations have been used to model the survival function of laboratory and field adult mosquitoes. For field data, the exponential function, adjusted for recapture number, was the most frequently used model [23,32,46-52]. For laboratory data, a range of functional forms were applied with the aim of detecting age-dependent mortality under conditions where overall mortality is lower. These have included the Gompertz function which can allow a non-symmetric mortality rate to increase or decrease exponentially with age [31,53,54], the Weibull function where the mortality rate is a power function of age [31,48,53,54] and the log-logistic function where the mortality rate is not restricted to a monotonic function [31,54,55]. Here we aimed to compare the fit of these different parametric models to laboratory survival data at each temperature regime across all of the studies to see if any one function offers a significantly improved fit and whether this changes with temperature. Using the laboratory data we re-examined the fit of the log-logistic, Gompertz, exponential and Weibull survival functions. Function fitting was performed in R version 2.14.2 [56] using the "flexsurv" library which fits a variety of parametric models using maximum likelihood. The Akaike Information

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Table 1 Summary of laboratory and field data

	Ae. albopictus laboratory data	*Ae. albopictus* MRR data	*Ae. aegypti* laboratory data	*Ae. aegypti* MRR data
Number of experiments	210	9	141	50
Mean temperature (°C) (±SD)	25.9 (22.3-29.5)	20.3 (17.0-23.7)	25.5 (21.9-29.1)	25.5 (22.9-28.3)
Minimum/Maximum temperature (°C)	15/35	16.7/26	10/35	20.7/30.1
Median number of mosquitoes observed/released (IQR)	29 (15–40)	552 (249–1007)	70 (25–382)	602 (493–798)
Dietary regime (%)	Blood: 7.1	-	Blood: 3.0	-
	Sugar: 1.0		Sugar: 38.2	
	Blood + Sugar: 91.9		Blood + Sugar: 58.8	
Age at release (Days) (±SD)	-	3.2 (0.6-5.8)	-	3.6 (1.3-5.9)

SD = Standard deviation, IQR = Interquartile range, MRR = Mark-release-recapture.

Criterion (AIC) was calculated for each model and used to calculate the relative likelihood (RL) [57] of each model to the model with the lowest AIC:

$$RL_j = e^{\frac{\left(AIC_{min} - AIC_j\right)}{2}}$$

The relative likelihood values for each model were averaged for experiments of equivalent temperature to give a balanced estimate of the each model's suitability at different temperatures.

Fitting non-parametric models to laboratory survival data

As the distribution of survival times may undergo complex changes across a range of temperatures we also chose to construct a non-parametric model that would not share the same restrictions as the aforementioned parametric models. For this we chose regression spline generalised additive models (GAMs) which apply a smoothing variable to the explanatory variables in order to model the response variable [58]. This method has the advantage of being able to model unknown and non-linear effects of covariates and thus elucidate the potentially complex effect of temperature on adult mosquito survival.

To evaluate the improvement of using GAMs over the parametric alternatives, we fitted parametric and GAM models to each laboratory experiment and calculated the difference in AIC between parametric and non-parametric models across all experiments.

A second GAM was then formulated to use the data from all experiments in one model to recreate the relationship between survival, time and temperature. The GAM was formulated as follows:

$$S_{ij} \sim Binomial\left(N_{ij}, p_{ij}\right)$$

$$logit\left(p_{ij}\right) = f(D_i, T_i) + \varepsilon_j + \varepsilon_d$$

$$\varepsilon_j \sim N\left(0, {\theta_j}^2\right)$$

$$\varepsilon_d \sim N\left(0, {\theta_d}^2\right)$$

S_{ij} = number of mosquitoes surviving at observation i in experiment j

N_{ij} = number of mosquitoes at start of time step at i, j

P_{ij} = survival probability for a mosquito at i, j

$f()$ = smooth term

D_i = day of observation i

T_i = temperature of observation i

ε_j = random error term for experiment j

ε_d = random error term for mosquito diet d

θ_j^2 = variance across experiments

θ_d^2 = variance across mosquito diets

Smoothing parameters were selected by restricted maximum likelihood with a data-driven basis dimension choice of $k_D = 8$ and 5 and $k_T = 5$ and 5 for *Ae. aegypti* and *Ae. albopictus* respectively [59]. Confidence intervals for the interquartile range of predictions were obtained by bootstrapping with 200 repeats, each the size of the original dataset. This model was fit using $D \geq 1$ to be consistent with the experimental observations that record mortality. Extrapolated model predictions for $0 \leq D < 1$ were scaled proportionally to ensure 100% survival at $D = 0$. All GAMs were implemented using the "mgcv" package in R [60].

For the model to fit biologically appropriate responses, additional data defining the limits of prediction were required. Observations from Christophers [18], suggest 4°C and 42-43°C as suitable minimum and maximum critical temperatures at which survival of *Ae. aegypti* is minimal (<24 hours). Similar observations for *Ae. albopictus* suggest values between -5°C and 40–40.6°C [61,62]. To constrain mortality in the model, all non right censored experimental observations were extended to 120 days at 0% survival and a maximum lifetime of 120 days was imposed at all temperatures, the maximum longevity observed in our dataset. Furthermore, to produce meaningful estimates of longevity, survival of less than 0.1% of the initial mosquito population was considered sufficient to indicate complete mortality.

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Reconciling laboratory survival predictions with survival measurements in the field

Because MRR data was too limited to fit models over a range of temperatures as we did for the laboratory mosquitoes, we used the laboratory-based models above to estimate temperature and age-matched expected mortality rates for mosquitoes in the field and compared those estimates to observed field mortality estimates from MRR data. To simulate the realistic effects of temperature in the field, in contrast to the constant temperatures of the laboratory, we recreated 59 separate daily fluctuating temperature regimes using the maximum and minimum temperatures of each MRR experiment and assumed sinusoidal progression in the day with a decreasing exponential curve at night [28,63]. These matching fluctuating temperatures were then applied to the laboratory survival model to estimate hourly survival for each of the MRR experiments. For each MRR experiment, we directly estimated the hourly mortality rate using the Buonaccorsi nonlinear regression method applied using the "stats" package in R [60]. This assumes that the mortality rate in the field is constant for each experiment and, therefore, our estimation of external mortality, due to causes such as predation or disease, also has this assumption. We then matched the laboratory-based predicted mortality with the directly estimated mortality and calculated the average difference, which we attribute to mortality due to external factors. We added this estimated hourly external mortality to the temperature-dependent laboratory model to produce the field survival model, which estimates mortality at all temperatures. Confidence intervals for the field survival model were derived by propagating the uncertainty of the laboratory model and combining this with 200 bootstrap samples of the MRR experiments to test the sensitivity of the calculated external mortality value. The overall schematic of the methods appears in Figure 1.

Results

Parametric models of laboratory data

Of the four parametric models tested, no one model was consistently most suitable across the range of temperatures tested (Figure 2). The log-logistic model was most suitable for *Ae. aegypti* with a relative likelihood of 0.441, whereas the exponential model was most suitable for *Ae. albopictus* with a relative likelihood of 0.397. The Weibull model was the least suitable for both species with relative likelihoods of 0.07 and 0.05 for *Ae. aegypti* and *Ae. albopictus*, respectively. While some models may be more suitable at specific temperatures, such as the exponential at 15°C for *Ae. aegypti*, they are not consistently more suitable at other temperatures, nor is there a clear trend suggesting some models are more suitable at higher or lower temperatures (Figure 2).

Non-parametric models of laboratory data

When non-parametric GAMs were used to fit the same data, both the overall model likelihood and the number of experiments for which it was optimal increased (Table 2). For both *Ae. aegypti* and *Ae. albopictus*, respectively, median difference in AIC between parametric and non-parametric models was 307.6 and 4.38. While still significant, this improvement was less pronounced in *Ae. albopictus* datasets due to a lower average number of mosquitoes under observation (Table 1), which limited the ability of some experiments to detect finer scale changes in survival. The number of experiments for which GAMs were the most suitable, however, (49.5%, Table 2) showed its improved fit over the entire dataset. The fits of both parametric and non-parametric models to two of the *Ae. aegypti* experiments are shown in Figure 3 as an example of the fit of each of the five models. Due to its more accurate quantification of mortality rate and its improved fit across a range of temperatures we chose a non-parametric GAM to construct our laboratory temperature survival model.

The non-parametric GAM model allowed us to quantify the effects of age and temperature on mortality whilst still taking into account the random effects at the experiment and mosquito diet level. Figures 4A and 4B show the model fit for each species using the laboratory data. Overall, *Ae. albopictus* had a greater longevity than *Ae. aegypti*, however, *Ae. aegypti* tolerated a wider range of temperatures (Figures 4A and 4B). The survival patterns also differ at the limits of survival, *Ae. aegypti* had optimal survival over only a narrow window around 21°C, while *Ae. albopictus* had optimal survival over a much wider range with only minor differences observed between 20-30°C. The mean and 95% survival limits for both species show that mortality was widely distributed across different ages with the majority of mosquitoes dying well before reaching maximum longevity. For *Ae. aegypti* the predicted median longevity was only 38 days even at optimal temperatures despite some individuals living over 100 days. This same pattern was observable in *Ae. albopictus* data, but to a lesser extent: median longevity at optimal temperatures was over 60 days despite some individuals living up to 120 days. Random effects for mosquito diet and study level were highly significant for both *Ae. aegypti* and *Ae. albopictus* models (Chi square, $p < 0.001$).

The greatest uncertainty around these predictions occurs around the 50% survival time (Figure 5A and 5B). For the majority of survival curves this is the point at which survival has the greatest rate of change; i.e. highest mortality rate, and it is, therefore, not surprising that uncertainty is higher around these points. Peaks in uncertainty can also be identified at low temperatures for *Ae. albopictus* (Figure 5B). Overall the uncertainty was higher for *Ae. albopictus* reflecting the limited data, particularly between 0 and 15°C.

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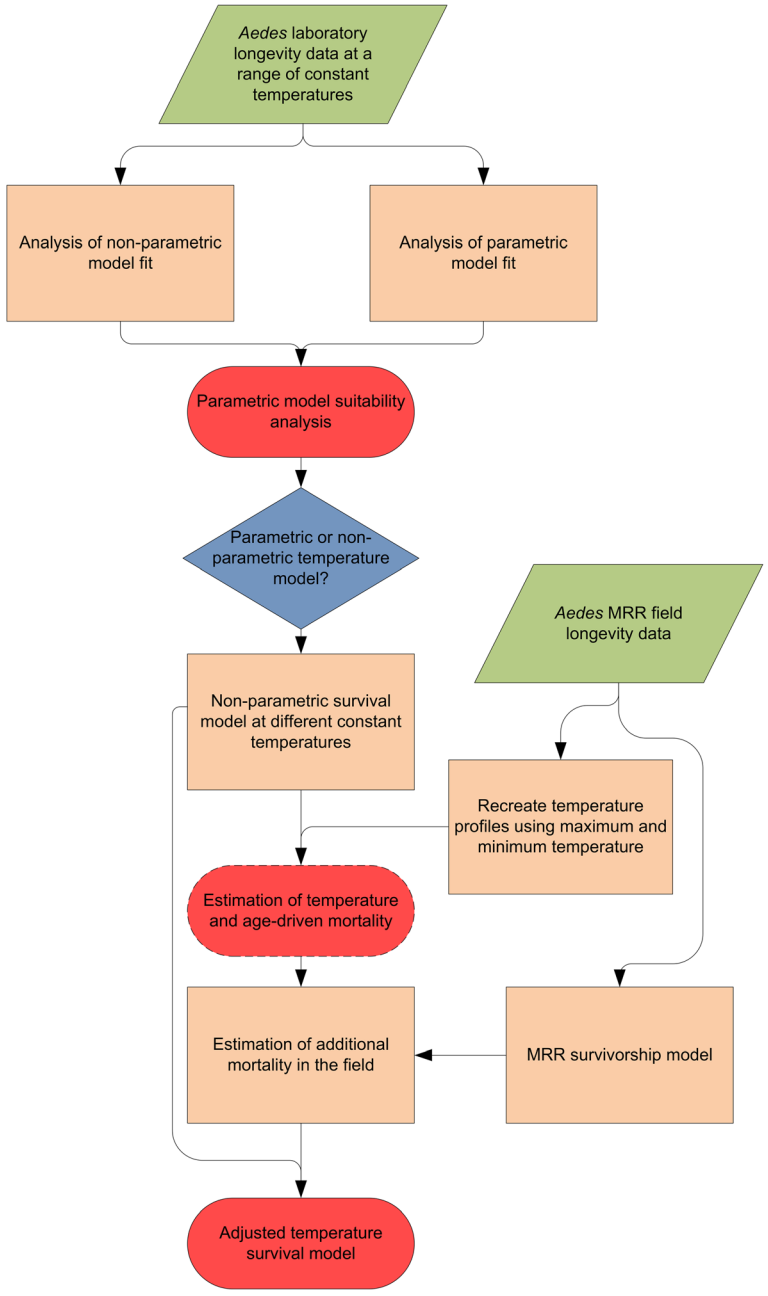

Figure 1 Schematic overview of the methods. Green parallelograms indicate input data, orange rectangles show processing or modelling steps, blue diamonds show decision steps and red boxes show output analysis and models (dotted shows intermediate, unbroken line shows final outputs). MRR = Mark-release-recapture.

Reconciliation with field data

To estimate longevity in the field, the daily mortality in the laboratory model was compared to field observations of mortality from MRR experiments where other, external factors may contribute to mortality. For *Ae. aegypti*, the laboratory model-predicted daily mortality rate varied between 0.05 and 0.082 (average 0.064) compared to an observed MRR mortality rate of between 0.033 and 0.595 (average 0.288). The average difference between these predictions for each experiment, and thus the calculated additional daily mortality, was 0.179 (standard deviation 0.012 – 0.346). For *Ae. albopictus* the external daily mortality was lower. Laboratory model predictions of daily mortality rate varied from 0.0024 to 0.289 (average 0.040)

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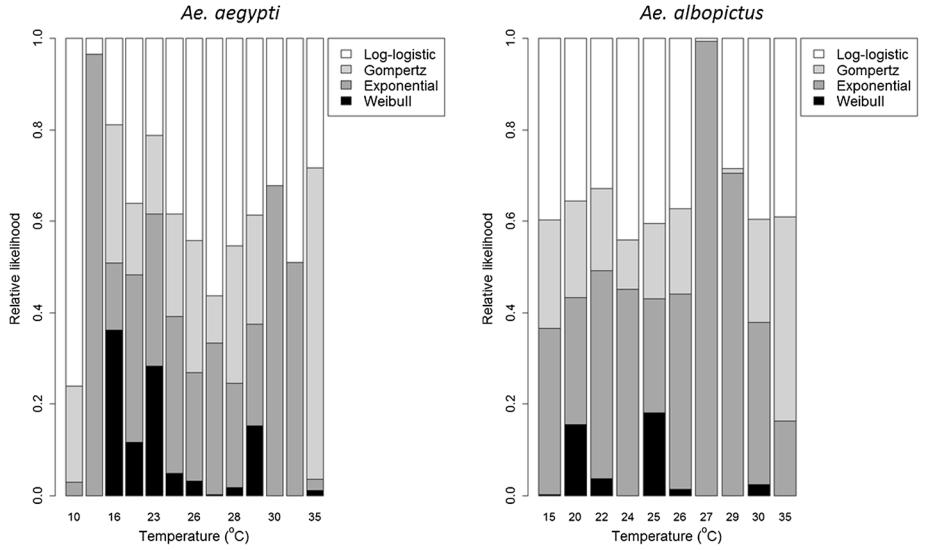

Figure 2 Relative likelihood of four different parametric models for *Aedes* adult female survival data over a range of constant temperatures. The models included are i) a two parameter Log-logistic model (shape and scale), ii) a two parameter Gompertz model (shape and rate), iii) a one parameter Exponential model (rate) and iv) a two parameter Weibull model (shape and scale).

compared to observed MRR mortality rate of between 0.031 and 0.231 (average 0.121) giving an average difference of 0.0621 (standard deviation −0.030 – 0.154). Due to the techniques used to measure external mortality, we had to assume it acted independently of mosquito age or temperature and so predicted survival in the field was calculated as the sum of laboratory model mortality and external mortality and is shown in Figure 4C and 4D.

Clear differences in survival can be observed between laboratory models (Figure 4A and 4B) and field models (Figure 4C and 4D). Survival is decreased for both species, but less so for *Ae. albopictus* due to the lower estimated external mortality. The effect of incorporating external mortality also reduces the relative influence of temperature and as a result less variation in survival is observed at mid-range temperatures (approximately 15-35°C). The most significant difference between laboratory and field models is the change in median longevity, which is reduced considerably in both species.

Table 2 Evaluation of parametric and non-parametric model fit to laboratory data

Model	Median increase in AIC relative to GAM (*Ae. aegypti*/*Ae. albopictus*)	Optimal model choice (%) (*Ae. aegypti*/*Ae. albopictus*)
Log-Logistic	320.40/22.620	4.4/1.5
Gompertz	304.83/0.005	2.2/21.9
Exponential	302.89/2.969	1.5/13.8
Weibull	302.90/0.023	0.7/13.3
GAM	-	91.1/49.5

AIC = Akaike information criterion, GAM = Generalised additive model.

When field data uncertainty was incorporated with the existing laboratory model uncertainty, overall uncertainty was reduced due to the higher value of external mortality and the lower uncertainty in estimating this parameter (Figure 5). Uncertainty was higher at extreme temperatures, particularly for *Ae. albopictus* at lower temperatures, however this uncertainty was confined to a limited range of temperatures and days, ensuring that overall prediction had low uncertainty. The predicted hourly survivorship from the models for *Ae. aegypti* and *Ae. albopictus* field survival and their associated uncertainty profiles are available for free download on the online data repository figshare (www.figshare.com) [65,66].

Discussion

In this study we used data from 410 *Ae. aegypti* and *Ae. albopictus* adult female survival experiments to construct a model of temperature and age-dependent survival. The best-fitting model was an age and temperature dependent GAM, which we combined with field-based mortality to estimate the distribution of longevity of mosquitoes in the field. These models should be interpreted in light of the limitations to the data used and the modelling methods. Of the data compiled, there were relatively few experiments conducted at more extreme temperatures, resulting in higher uncertainty in these temperature ranges. Considering the implications these extreme temperatures have for the geographic range of the mosquito, more data is necessary for better defining that range. Mosquito survival

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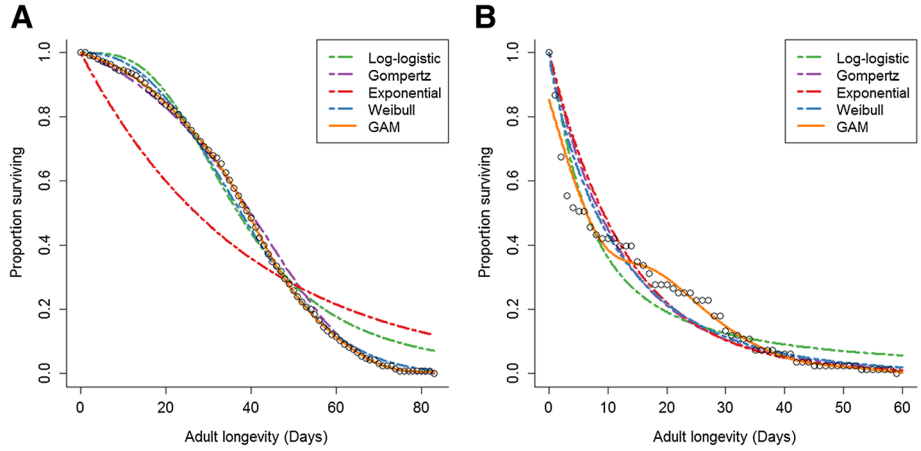

Figure 3 Examples of parametric and non-parametric model fit. Open circles show *Ae. aegypti* survival data under controlled laboratory conditions from Joy *et al.* [64] **(A)** and Yang *et al.* [21] **(B)**, two experiments that show contrasting survival curve shape. Parametric models are shown as dashed lines and the non-parametric GAM is shown as a solid orange line.

may also vary geographically due to variation in the genetic background of local mosquito populations. Our analysis incorporates experimental data from a wide variety of locations and conditions but, given the paucity of MRR data at climatic extremes, it is possible that estimates of field-based mortality may not be representative, particularly in locations where mosquitoes may be more tolerant of extreme climates. However, observations suggest that adult survival is one of the few bionomics of *Ae. aegypti* that remains consistent among geographically disparate populations [67,68]. North American samples of *Ae. albopictus* were over-represented in our laboratory data (91%) which may overestimate thermal tolerance at the lower temperature limits due to evidence of *Ae. albopictus* adaptation in temperate climates [53,69]. Perhaps the most important data limitation is that there are very few field mortality experiments upon which to estimate mortality outside of the laboratory, where arbovirus transmission actually occurs. More data would allow better estimation of field mortality and allow relaxation of

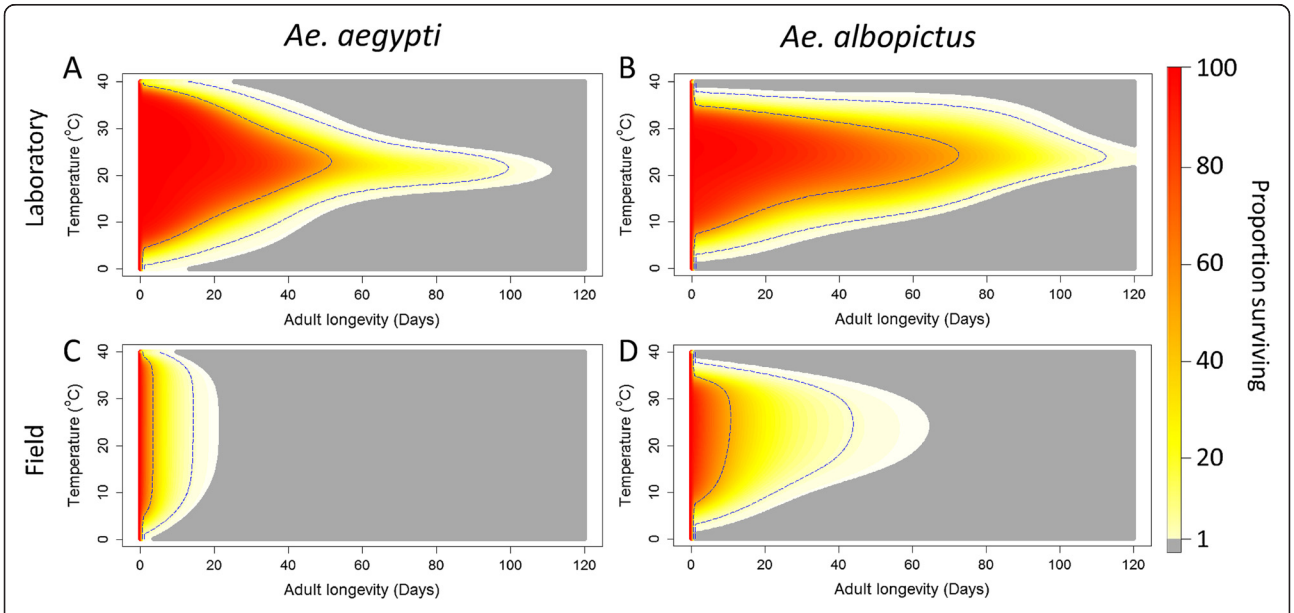

Figure 4 The distribution of adult female *Aedes aegypti* and *Aedes albopictus* survival across a range of temperatures under laboratory conditions (A and B) and field conditions (C and D). Colours from red to yellow show survival from 100% - 1% of the population remaining. Grey indicates <1% of the population remaining. Dotted blue lines show the limits for 50% and 95% of the original population remaining.

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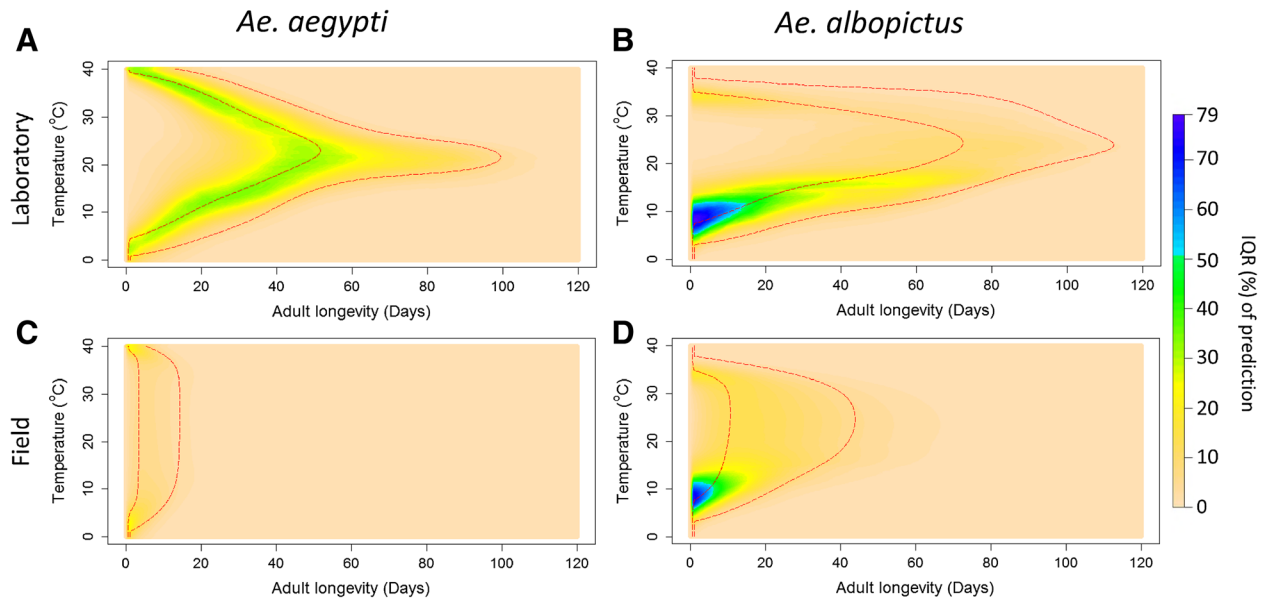

Figure 5 The distribution of uncertainty of the laboratory model prediction. Colours from blue to beige show the interquartile range (IQR) in predictions from 200 bootstrap runs of the laboratory model **(A and B)**. This uncertainty is then combined with the field data uncertainty quantified by 200 bootstrap runs of MRR data to give the IQR predictions for the field survival model **(C and D)**. Red dotted lines of the 50% and 95% of the population remaining are added for reference.

the assumption that external contributors to mortality are not age-related. An extension should include alternatives to MRR approaches that might be more sensitive for estimating survival of older potentially infectious mosquitoes, for example, using transcript levels of an age-associated gene [70] or measuring mosquito infection rates [71].

It is also worth considering the scope of the model we developed. While it may suggest that adult survival is possible even at low temperatures, factors limiting the development of the immature stages may preclude the establishment of an adult population. For adult *Aedes*, there may also be additional temperature-based limits to survival. Below 14-15°C, *Ae. aegypti* experiences reduced mobility and struggles to imbibe blood [18,21,72]. As observations (at tropical temperatures) suggest *Ae. aegypti* cannot survive longer than 2-3 days without a blood meal [73], extended periods of time below 14-15°C are likely to result in complete mortality. We extended our predictions below these temperatures because limited time spent at these colder temperatures, such as a few hours in the early morning, may not confer a lasting negative effect on survival, however, these effective mortality limits must be considered when integrating this into a wider *Aedes*/DENV transmission model.

Here we have shown that GAMs captured more of the variation in survival between different experiments than conventional, parametric models of mosquito mortality, including the Gompertz and logistic models which previously provided the best fit of the options examined [27,30,31]. In our analysis we did not consider frailty-based mortality models, however non-parametric approaches, such as GAM, offer more flexibility than parametric models, suitable predictive power and straightforward implementation in a variety of statistical software packages. We have also emphasised the importance of including random effects in analyses of this type as it has allowed us to comment on the effect of specific experimental treatments, such as feeding regime, and thus guide laboratory and field experimental design. Furthermore adding a random effect at the study level allowed us to comment on the generalisability of any one experiment to a wider context.

The relative fit of the GAM indicated the presence of age-dependent survival. This age-related effect may be related to senescence or other factors, but appears to be less important in the wild. On average we found that mortality due to external causes was significantly greater (4.5 times for *Ae. aegypti* and 3.0 times for *Ae. albopictus*) than mortality due to temperature and age at mid-ranging temperatures (20-30°C) indicating that few mosquitoes will be alive at older ages by the time senescence measurably impacts mortality. Results from our analysis indicate that across different temperature regimes the assumption of age-independent mortality for *Ae. aegypti* and *Ae. albopictus* in the field is likely to be appropriate. It may be possible that senescence acts at different ages in the

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laboratory than it does in the field, however, quantifying this would require unfeasibly large mosquito cohorts, or innovative new techniques to measure mosquito survival in the field. As a result, the statistical model presented here provides a novel approach for quantifying the effects of age-dependent survival among wild mosquitoes.

The greater tolerance of lower temperatures observed for *Ae. aegypti* compared to *Ae. albopictus* appears at odds with their observed geographic distribution. This finding can be explained, however, if we consider the adaptations of the egg stages of each mosquito. *Aedes albopictus* eggs are able to undergo diapause allowing the species to persist during cold winter temperatures that are unfavourable to adult survival [74,75]. *Aedes aegypti* shows only limited adaptation to egg stage survival in unfavourable periods [76,77] and there may, therefore, be greater selection pressure for thermal tolerance at the adult stage to resist diurnal and inter-seasonal variations in temperature.

The seasonal variation and geographic limits of *Aedes* transmitted viruses are intrinsically linked to the seasonality and geographic limits of mosquito populations required for their transmission. It is, therefore, important that the survival of adult *Aedes* is well estimated using appropriate data and considering the variety of conditions they may encounter in nature. Quantifying how changes in survival translate to dengue transmission potential is also important and requires integration with models of the DENV extrinsic incubation period, which is also sensitive to temperature [78]. Characterising the interaction between mortality and extrinsic incubation could be used further to explore entomological components of seasonal forcing among *Aedes*-borne viruses, which may help inform strategies for disease prevention and control.

Given that dengue is a disease that affects over half the world's population [12] with an estimated 390 million new infections per year [10], improvements in existing approaches and new strategies to control the disease are needed. Depending on the situation, both the type of vector control used and how it is implemented must be optimised to achieve the biggest reduction in dengue burden. Modelling and mapping are two important tools for addressing both of these issues, identifying risks and opportunities for control [34,79,80]. Considerable efforts have already been undertaken to re-examine many of the fundamental assumptions of early *Aedes* and dengue transmission models [6,78,81,82]. The next generation of models is likely to incorporate significant advances, including new frameworks for modelling survival of adult mosquitoes. The adult *Aedes* survival models developed here can be integrated into these new modelling approaches with the aim of refining the global distribution of *Aedes* vectors, guiding seasonal vector control efforts, modelling dengue transmission and developing early warning systems to prevent dengue epidemics [83].

Conclusions

We created an explicit model of *Ae. aegypti* and *Ae. albopictus* adult survival across a range of temperatures under both laboratory and field conditions. Commonly used parametric models did not capture the variation in survival to the same extent as our approach across a variety of temperatures and experiments. The use of non-parametric GAMs allowed the effects of senescence and temperature to be captured explicitly, revealing the importance of temperature and age-dependent mortality under laboratory conditions and fluctuating temperatures and external mortality in the field. The models developed here can be used for investigating seasonal variation in dengue transmission and will strengthen components to dengue transmission models that investigate various options for control.

Additional file

Additional file 1: Data bibliography and summary statistics.

Abbreviations

DENV: Dengue virus; MRR: Mark-release-recapture; AIC: Akaike's information criterion; RL: Relative likelihood; GAM: Generalised additive model.

Competing interests

Disclaimer: The findings and conclusions are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Authors' contributions

OJB designed the experiment, wrote the manuscript and collected and analysed the data. MAJ, DLS, TWS, PWG and SIH also helped conceive and design the experiments. MAJ, SB, NG, DMP, DLS and TWS helped with data analysis and interpretation. CAG, HD, MGG, PL, RM and LMS collected data. All authors were involved in drafting and revising the manuscript and all authors approved the final version.

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Chapter 6 – Evaluating the potential for dengue transmission in temperate regions

6.1. Cover page

Chapter 5 presented a model of the effects of temperature on adult female survival of two of the main vector species of DENV. This chapter integrates these outputs with further models of within-vector physiological processes to evaluate the principle rate limiting steps temperature places on vectorial capacity and vector persistence. These are illustrated in a series of maps that can be used to interpret relative risk in different areas at different times of the year for each vector species.

Over the past thirty years the invasion and spread of *Ae. albopictus*, and to a lesser extent *Ae. aegypti*, into continental Europe and the USA has led to limited outbreaks of dengue and other arboviruses *(Benedict et al., 2007; CDC, 2010; ECDC, 2014; Gjenero-Margan et al., 2011; Gould et al., 2010)*. Since then there have been considerable entomological surveillance and control efforts to intercept and limit mosquito spread and to minimise disease transmission risk in these well-resourced countries. By evaluating the regions and seasons in which temperature permits establishment of DENV transmission, surveillance efforts can be refined. Explicit spatio-temporal model-based predictions, such as those presented here are an important part of targeting surveillance efforts and can also inform which aspects of mosquito population surveillance might be most important to measure to understand true risk of DENV transmission. The analysis presented here was published in *Parasites and Vectors* in July 2014 *(Brady et al., 2014a)*. The referred to supplementary information can be found in the Appendix section (page 221).

The outputs of these models are also informative for wider disease and vector mapping applications. The temperature suitability metrics are more dengue transmission-relevant than measures of raw temperature variables themselves and thus offer improved predictive ability when integrated as covariates in disease models *(Bhatt et al., 2013; Brady et al., 2014b; Gething et al., 2011; Kraemer et al., 2015b)*. Alternatively, the limits predictions from the temperature suitability maps can be used to trim the predictions of other disease models that given uncertain predictions in temperate environments. By re-running these models on projected future global temperature changes it is possible to analyse how the limits to vector persistence or DENV transmission may change in the future. Wider efforts within the Spatial Ecology and Epidemiology Group are currently underway to analyse the future global distribution of *Ae. aegypti, Ae. albopictus* and DENV transmission by co-analysing the effects of temperature and a number of other variables *(Kraemer et al., 2015a; Messina et al., 2015)*.

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Parasites & Vectors

RESEARCH Open Access

Global temperature constraints on *Aedes aegypti* and *Ae. albopictus* persistence and competence for dengue virus transmission

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Abstract

Background: Dengue is a disease that has undergone significant expansion over the past hundred years. Understanding what factors limit the distribution of transmission can be used to predict current and future limits to further dengue expansion. While not the only factor, temperature plays an important role in defining these limits. Previous attempts to analyse the effect of temperature on the geographic distribution of dengue have not considered its dynamic intra-annual and diurnal change and its cumulative effects on mosquito and virus populations.

Methods: Here we expand an existing modelling framework with new temperature-based relationships to model an index proportional to the basic reproductive number of the dengue virus. This model framework is combined with high spatial and temporal resolution global temperature data to model the effects of temperature on *Aedes aegypti* and *Ae. albopictus* persistence and competence for dengue virus transmission.

Results: Our model predicted areas where temperature is not expected to permit transmission and/or *Aedes* persistence throughout the year. By reanalysing existing experimental data our analysis indicates that *Ae. albopictus*, often considered a minor vector of dengue, has comparable rates of virus dissemination to its primary vector, *Ae. aegypti*, and when the longer lifespan of *Ae. albopictus* is considered its competence for dengue virus transmission far exceeds that of *Ae. aegypti*.

Conclusions: These results can be used to analyse the effects of temperature and other contributing factors on the expansion of dengue or its *Aedes* vectors. Our finding that *Ae. albopictus* has a greater capacity for dengue transmission than *Ae. aegypti* is contrary to current explanations for the comparative rarity of dengue transmission in established *Ae. albopictus* populations. This suggests that the limited capacity of *Ae. albopictus* to transmit DENV is more dependent on its ecology than vector competence. The recommendations, which we explicitly outlined here, point to clear targets for entomological investigation.

Keywords: *Aedes*, *Aegypti*, *Albopictus*, Temperature, Distribution, Suitability, Competence, Disease model, Map

Background

Dengue is the most clinically important viral vector-borne disease with 96 million apparent infections per year among a population-at-risk of nearly four billion in 128 countries [1-3]. During the last century dengue virus (DENV) has rapidly expanded from its tropical origins to sub-tropical and temperate climates. Similarly, the geographic limits of transmission and endemic regions (with year round transmission) continue to expand [4-7]. While significant improvements have been made to our understanding of and our ability to map the current global distribution of dengue [1,2], we do not yet know which factors define the current and future geographic limits to the extent and endemicity of transmission of this still expanding viral infection. These limits are likely defined by a combination of different host (both mosquito and human) and virus factors in different environments. While the study of these factors in isolation is

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unlikely to define the full constraints of transmission, it nevertheless can identify areas where transmission cannot occur.

Of the many factors affecting dengue transmission, temperature has been most frequently investigated and rigorously quantified [8,9]. A variety of experiments have quantified how key vector and virus dynamics vary with temperature [10,11] and high spatial and temporal resolution meteorological data are available to apply these relationships on a global scale [12]. Using accumulated theoretical and experimental knowledge of the epidemiology of dengue, we use a dengue transmission model to identify where and when temperature is likely to be the most important factor limiting the rate of transmission. Among the limitations temperature can impose on DENV transmission, the most directly affected and most clearly understood mechanisms are its effects on adult female mosquito survivorship and DENV extrinsic incubation period (EIP). Transmission is only permitted when the longevity of the vector exceeds the EIP of the DENV. This is equivalent to the time between the mosquito imbibing an infected blood meal and becoming infectious (able to onwardly transmit the virus by bite). Similarly, a key limitation on vector persistence, the ability for mosquito population dynamics to sustain a population of adult mosquitoes, can be evaluated by determining the proportion of adult female mosquitoes that survive long enough to complete their first gonotrophic cycle (FGC), or the time between taking a blood meal and first oviposition [13]. Evaluating the effects of temperature on these two processes on a population level can determine when and where temperature causes breaks in transmission or vector population persistence. This can also give insights into the relative intensities of transmission and oviposition as if a greater proportion of mosquitoes survive the EIP and FGC they are thus more likely to deliver more infectious bites or oviposit a greater number of eggs respectively.

Evaluating the limits to global DENV transmission is complicated by differences in the global distributions of the DENV's primary vector, *Aedes aegypti*, and what is often considered a secondary vector, *Ae. albopictus* [14,15]. While *Ae. aegypti* appears to be confined to tropical and subtropical climates [8,16], *Ae. albopictus* eggs can undergo long periods of diapause that allow populations to persist in temperate environments despite winter temperatures that are unsuitable for adult survival. Survival of eggs for relatively long periods of time also allows them to be transported long distances using global travel and trade networks [17,18], resulting in multiple introductions outside their native range into tropical and some temperate environments, most well documented in Europe and North America [17]. The role of *Ae. albopictus* in DENV transmission remains unclear. In areas where it is the only DENV vector, outbreaks are rare and limited in incidence [15,19,20], whereas even recently introduced, isolated populations of *Ae. aegypti* have produced explosive and sustained outbreaks [21,22]. It has been suggested that this difference is primarily due to inter-species differences in the rate of DENV dissemination through the mosquito midgut to the salivary glands, leading to a longer EIP in *Ae. albopictus* [15]. The effect of this difference on transmission has not, however, been quantified taking into account the predicted longer lifespan of *Ae. albopictus* [10]. Alternative explanations involve differences in human blood feeding rates (*Ae. aegypti* has been known to deliver more frequent bites with a higher proportion of blood meals taken from humans than *Ae. albopictus* [23,24]) and possible differences in the proportion of mosquitoes per human resulting from their respective ecologies. *Ae. aegypti* typically inhabits more urban environments than *Ae. albopictus*, and even when both inhabit urban environments, *Ae. aegypti* typically lives in closer proximity to humans [25,26]. While both of these factors are also driven by temperature, they also have strong ecological influences that may in many cases be greater than the effect of temperature. We therefore chose to restrict our analysis to the effects of temperature on the duration of EIP and FGC.

Previous attempts to model the effects of temperature on *Ae. aegypti* and *Ae. albopictus* population persistence and ability to transmit DENV have either taken a statistical or mechanistic (biological or deterministic) modelling approach. Statistical or empirical approaches that attempt to find correlations between various measures of temperature and dengue or vector observations often find weak or only transient associations [27,28]. These approaches cannot be easily designed to include the complexities of the temperature response due to its highly non-linear effects and the dynamic time scale at which it acts [29]. Such limits are often simply defined by a constant mean or minimum temperature that fails to account for seasonal and daily temperature fluctuations throughout the year. Statistical approaches are further restricted in their ability to detect the limits of the distribution due to the inherent scarcity of reported data from environments where transmission or mosquito populations are marginal [30,31]. Conversely, previous mechanistic modelling approaches typically use temperature-based relationships defined by just one or two empirical studies and lack the geographic validation of the statistical approaches [9,32,33]. Furthermore, many studies using mechanistic models are not widely generalisable due to their reliance on locally-specific parameter estimates, particularly estimates of immature mosquito habitat and fluctuations in adult mosquito population sizes. This results in a high degree of uncertainty when trying to evaluate the effects of temperature on the persistence of vector

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populations and their ability to transmit DENV and this uncertainty is rarely quantified in the final prediction.

In this study we attempt to elucidate the thermal limits of *Ae. aegypti* and *Ae. albopictus* persistence and DENV transmission at a high spatial and temporal resolution using an empirically-parameterised mechanistic model that explicitly considers the interplay between temperature-dependent EIP and adult vector survival. We use existing temperature-based relationships [10,11,34] and define new ones by applying complex modelling techniques to the increasing breadth of data from many different laboratory and field conditions, also including measures of uncertainty in our estimates. These relationships are then combined in a dynamic modelling framework that evaluates the cumulative effects of changing temperature regimes on an index proportional to vectorial capacity. This approach reveals different distinct limits to persistence and virus transmission for each species and goes on to indicate that temperature can also limit levels of endemicity of DENV transmission. In addition to these findings, the predictive maps produced from these models can be used as more biologically-appropriate covariates than typical temperature averages in statistical modelling of dengue and as an informative subcomponent to link vector and human models of DENV transmission.

Methods

Relating temperature to transmission potential

Here we adapt a modelling framework from Gething *et al.* [35,36] that evaluates the principal effects of temperature on the basic reproductive number (R_0) of a pathogen. The concept of R_0, the number of secondary cases that will arise from a single infectious human in a totally susceptible population, is a natural framework for evaluating relative changes in transmission potential for a variety of vector borne diseases [37-40]. The entomological, and thus strongly temperature dependent, components of R_0 can be summarised by the equation for vectorial capacity (V):

$$V = \frac{ma^2}{g} e^{-gn}, \quad (1)$$

where m is the ratio of adult female mosquitoes to humans, a is the human blood feeding rate, g is the instantaneous per-capita death rate of adult female mosquitoes and n is the length of EIP for the pathogen. All of the parameters in vectorial capacity are likely to be affected to some extent by temperature, either directly or indirectly through interaction with the environment. In our generalised global approach, specific details of the environment are unknown, so we therefore chose to confine our analyses to mosquito mortality (g) and pathogen extrinsic incubation period (n) due to the direct effects of temperature on these parameters and subsequent potential virus transmission and because of the abundance of information available. If the human population size is stable, m can be replaced by two entomological parameters describing mosquito population dynamics: $m = \lambda / g$, where λ is the emergence rate of new adult mosquitoes. Though mosquito mortality already appears twice in Eq. 1, this substitution reveals a third order effect of mortality on transmission through effects on the adult population size [41] and allows us to define an index of temperature suitability Z as a function of temperature, T, which is linearly proportional to vectorial capacity over small changes in temperature:

$$Z(T) = \frac{e^{-g(T)n(T)}}{g(T)^2} \propto V(T) = \lambda a^2 \left(\frac{e^{-g(T)n(T)}}{g(T)^2} \right). \quad (2)$$

$Z(T)$ therefore effectively measures the relative number of infectious mosquitoes supported in an environment with temperature T, given a constant emergence rate λ and human blood feeding rate a. As estimates for λ and a are not readily available across the wide range of different environments that exist globally, $Z(T)$ provides a suitable metric for evaluating the effects of temperature on vectorial capacity, and therefore R_0. While $Z(T)$ can only measure relative rather than absolute change in vectorial capacity, a value of $Z(T)$ approximately equal to zero indicates that an environment does not permit onward transmission and can thus be used to predict the geographical limits of transmission.

While $Z(T)$ can be used to estimate relative suitability for transmission as a function of temperature, a modified version of the model can be used to estimate suitability for the persistence of mosquito populations also as a function of temperature. Rather than surviving long enough to transmit the virus, we need to evaluate whether the mosquito can survive long enough to complete one gonotrophic cycle (c), the minimum necessary for reproduction and thus persistence of a permanent mosquito population. A simple substitution of c for n in equation 2 enables the calculation of temperature suitability for oviposition. This allows us to evaluate the limits temperature places on the persistence of adult *Ae. aegypti* and *Ae. albopictus* populations, assuming the environment can support the juvenile stages of each species:

$$Z_{ovi}(T) = \frac{e^{-g(T)c(T)}}{g(T)^2}. \quad (3)$$

Temperature-based relationships

Calculation of temperature suitability for transmission and vector persistence requires evidence-based models for mosquito mortality, EIP and the length of the FGC.

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For the first two relationships we draw on two previously published studies investigating the effects of temperature on these parameters and incorporate both mean prediction and the uncertainty in these estimates. For FGC, we apply an existing model to newly available data from a literature review of entomological life cycle experiments.

Adult mosquito survivorship

Brady *et al.* [10] used generalised additive model regression to fit an empirical relationship between temperature and adult female survival in both *Ae. albopictus and Ae. aegypti* using over 350 laboratory and 50 field studies. An initial model of survival in a laboratory setting at a range of different temperatures was fitted, whilst accounting for potentially confounding factors. The laboratory survival model was then adapted to estimate survival under field conditions by calculating the additional hazard observed in the field as quantified by mark-release-recapture experiments. Laboratory and field models were derived separately for *Ae. aegypti* and *Ae. albopictus*, but in both mortality was modelled as a function of both temperature (T) and mosquito age (D):

$$logit\left(-\ln\left(g_{D,T}\right)\right) = f_s(D,T) + \gamma_s\,, \tag{4}$$

where f is a smooth term and y is the constant additional field mortality hazard for each mosquito species s.

DENV incubation period in Aedes aegypti

Chan and Johansson [11] used censored Bayesian time-to-event hazard models to parameterise a log-normal relationship between temperature and DENV EIP. This model was fitted to data from 140 mosquito transmission experiments where a cohort of mosquitoes was infected, incubated at constant temperature T, then transmission to human or primate hosts monitored at defined time intervals, accounting for inter-study differences in competence, to estimate the expected EIP duration (n).

$$n_T = e^{\beta X + \frac{1}{2\tau}} \tag{5}$$

$$\beta X = \beta_0 + \beta_T T \tag{6}$$

The vast majority of EIP experimental data was conducted using *Ae. aegypti* (96%), while only six experiments concerned EIP in *Ae. albopictus.* As these data were insufficient for detecting EIP differences between the two species, this model was fitted using only experiments that used *Ae. aegypti*, however, this yielded no difference in the fitted parameters than in the model that used data from both species. The final log-normal model was fitted with parameter estimates $\tau = 4.9$, $\beta_0 = 2.9$, and $\beta_T = -0.08$.

Inter-species differences in DENV incubation period

Few experiments have been conducted that have been designed to accurately measure DENV EIP in *Ae. albopictus.* To account for potential differences in EIPs for *Ae. albopictus*, we assessed data from vector competence assays in which cohorts of mosquitoes are experimentally infected and later assessed for disseminated DENV infection using immunofluorescent assays or similar techniques on cohorts extracted at one or more pre-defined time points.

To collate a comprehensive dataset with which to parameterise this model, databases of published literature (Pubmed and Google Scholar) were searched for the terms “aedes” AND “competence” and relevant data were extracted from the full text articles. Studies were included if DENV was ingested orally, DENV dissemination was measured in the head or salivary gland tissues and the mosquitoes had a laboratory colonisation history of five generations or fewer, as it has been found that long-term laboratory colonisation can increase vector competence [15]. The data extracted were: number infected at the beginning of the experiment, number sampled and number DENV positive at each time points and incubation temperature. We used this data to fit the following hierarchical model:

$$y_{t,T,\,i,j} \sim Bin\left(P_{t,T,\,i,j}\,,\; n_{t,\,T,\,i,j}\right)\,, \tag{7}$$

where y is the number of mosquitoes of species j in study i with DENV in their salivary glands at the assay time point t after incubation at temperature T, n is the number of mosquitoes infected at the beginning of the experiment and P is the probability of infection which is determined by:

$$P_{t,T,\,i,j} = \Phi\left(t_i \middle|\, \mu_{EIP_{T,i,j}},\; \sigma_{EIP}{}^2_{T,\,i,j}\right)\,,$$

in which Φ is the cumulative distribution function of the Gaussian distribution, t_i is the time in days between the initial infection and the assay, μ_{EIP} is the mean EIP and σ^2_{EIP} is the variance in EIP in each DENV assay experiment. We then used the assay data to calculate species-specific proportional differences (α_j) in the mean predicted EIP duration (from the EIP-temperature model, $\hat{\mu}_{EIP_T}$), taking into account inter-study variation due to different experimental designs, vector strains and virus types (ε_i):

$$\mu_{EIP_{T,i,j}} = \hat{\mu}_{EIP_T} + \alpha_j\,.\hat{\mu}_{EIP_T} + \varepsilon_i$$

$$\varepsilon_i \sim N\left(0,\; \sigma_{study}{}^2\right)$$

$$ln\left(\sigma_{EIP\,T,\,i,\,j}\right) \sim N\left(\mu_{assay},\; \sigma_{assay}{}^2\right)\,.$$

If viral dissemination and infectiousness are equivalent, we would expect $\alpha_{aegypti} = 0$, however due to various

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factors associated with sensitivities to DENV detection, we may expect detection of DENV dissemination to occur after the mosquito becomes infectious ($\alpha_{aegypti} > 0$). Alternatively, transmission experiments may produce a higher rate of false-negatives than dissemination assays [42] ($\alpha_{aegypti} < 0$). We therefore calculated species-specific values for α_j and took the relative differences in the means to calculate the efficiency adjustment for EIP duration in *Ae. albopictus* (ϕ):

$$\phi = \frac{\hat{\mu}_{EIP_T} + \alpha_{aegypti}.\hat{\mu}_{EIP_T}}{\hat{\mu}_{EIP_T} + \alpha_{albopictus}.\hat{\mu}_{EIP_T}} = \frac{1 + \alpha_{aegypti}}{1 + \alpha_{albopictus}}.$$

The above model was fitted as a hierarchical Bayesian model. A vague zero-mean Gaussian prior was specified for α_j. Twenty-eight assay experiments estimated the infected population at multiple time points by taking samples from the overall population. In order to incorporate this information, a cumulative normal distribution function was fitted to these experiments by least squares regression to generate empirical (though weakly informative) priors for μ_{assay} and σ^2_{assay}. Finally, we specified weakly informative priors for σ^2_{study} based on previous models of DENV EIP [11]. See Additional file 1 for the specific priors used. The model was parameterised by MCMC using WinBUGS [43]. Three Markov chains were run for 10000 iterations each with the first 2000 iterations discarded. Convergence was assessed based on visualisations and the Gelman-Rubin statistic.

Length of first gonotrophic cycle

To calculate the relationship between temperature and length of the first gonotrophic cycle we fitted an enzyme kinetics model first suggested by Focks *et al.* [9] to an updated database. The database was updated by searching literature databases spanning 1960-2014 for the terms "aedes" and "oviposition" or "gonotrophic". Experiments that monitored time of emergence, temperature and time of first oviposition were included as long as the mosquitoes had access to a food source and oviposition substrate. Due to the limited detail with which the data were presented, we were only able to extract mean time to oviposition, thus limiting the scope of our modelling options. The four parameters of the following equation was then optimised by minimising mean-squared error using the "L-BFGS-B" method with no upper or lower bounds in R version 3.0.1 [44]:

$$c(T_k) = \frac{\rho(T_k/298)e^{[(H_A/1.987)((1/298)-(1/T_k))]}}{1 + e^{[(H_H/1.987)((1/T_{0.5})-(1/T_k))]}},$$

where T_k is the temperature in °K, and ρ, H_A, H_H and $T_{0.5}$ are parameters to fit. The model was run 500 times with different starting values to ensure global and not local minima were found. Uncertainty in these parameters was estimated using the inverse Hessian of the likelihood surface.

Calculating temperature suitability at high temporal and spatial resolution

Long term average (1950-2000) interpolated weather station temperature data was obtained from WorldClim in the form of global 5 km × 5 km global rasters containing monthly maximum and minimum temperatures [12]. These were aligned back to back into three repeated years then cubic spline interpolated using the stats package in R to give annual weekly maximum and minimum temperature values pixel-by-pixel. Finally, to simulate the effects of diurnal temperature changes, weekly maximum and minimum temperatures were used to fit a sinusoidal day and exponential night temperature profile for each day of the week [32,45-49]. This resulted in global rasters giving interpolated temperature values at two hour time steps throughout an average year. We limited our predictions of temperature suitability to areas with a maximum monthly temperature exceeding 13°C for *Ae. albopictus* and 14°C for *Ae. aegypti*. These thresholds represent the observed temperatures below which biting and movement behaviours are impaired [8,24,46,49]. This impairment affects survival, blood feeding and larval development in ways that are outside the scope of the mortality models included in this analysis. As such, we do not predict below these temperature limits.

Vectorial capacity, and thus $Z(T)$, is a measure of transmission potential indexed to the time point when a mosquito becomes infected after blood feeding on an infectious human. To calculate the number of infectious mosquito days that arise from this one event we therefore need to consider: i) mosquito mortality in the time preceding mosquito infection, ii) mosquito mortality and virus EIP proceeding infection but preceding mosquito infectiousness and iii) mosquito mortality after they become infectious. In an environment with constantly changing temperatures it is important to incorporate the sequence and magnitude of temperature changes over time and their resultant effect on $Z(T)$. In a temporal sense, therefore, $Z(T)_i$ measures the relative number of infectious mosquito days that will arise in the future following infection on day i.

To capture changes in $Z(T)_i$ over time, we used a simulation-based discrete time step approach at a high temporal resolution to estimate the integral of the $Z(T)$ time series, where T is a vector of different temperatures. This modelled adult mosquito population size in three compartments: i) uninfected, ii) infected but not yet infectious and iii) infectious, each made up of cohorts that emerge or are infected at different times. Temperature- and age-dependent death rates were applied to each compartment up until an imposed maximum lifetime of

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22 days for *Ae. aegypti* and 65 days for *Ae. albopictus*, by which time best-case survival is below 1% in the mosquito survival model [10]. At each two hour time point a population of 100 adult mosquitoes (note that the choice of emerging population size is arbitrary as it has no effect on the relative measure $Z(T)$) was simulated to emerge into the uninfected compartment and all mosquitoes in all compartments were exposed to the mortality rate for that time point. Two days after emergence, when *Aedes* mosquitoes are first capable of blood feeding [50], all surviving mosquitoes in the uninfected compartment become infected. For infected mosquitoes, temperature dependent EIP completion proportion was tracked using equations 5 and 6 for each cohort in the infected compartment. Upon completion of the EIP, mosquitoes were transferred from the infected to infectious compartments then the number of infectious mosquito-days was summed up until the maximum lifetime.

By summing the number of infectious mosquito-days arising from cohorts of different ages all infected at time point i, the contribution of time step i to future transmission, or $Z(T)_i$, can be estimated. From the surveillance perspective, however, it would be useful to know the proportion of vectors infectious X_i at time step i given the environmental conditions that have occurred in the time leading up to that point. We therefore also calculate this number as the proportion of mosquitoes from all existing cohorts at time step i that are infectious. Each model was initiated with a burn in period exceeding the maximum lifetime of the vector, after which $Z(T)_i$ and $X(T)_i$ was calculated at two hour time steps from 1st January to 31st December. Equivalent time series for $Z_{ovi}(T)_i$ and $X_{ovi}(T)_i$ were also evaluated using the same approach with three compartments: i) newly emerged, ii) blood-fed but not ovipositing and iii) ovipositing.

Summarising annual time series

The four time series outputs from the temperature suitability simulation ($Z(T)$, $X(T)$, $Z_{ovi}(T)$ and $X_{ovi}(T)$) represent subtly different measures of the effect of temperature on dengue transmission and *Aedes* persistence. Over the course of an average year, if we take the sum of all the $Z(T)$ and $X(T)$ values for each time step $\sum Z(T) = \sum X(T)$, and $\sum Z_{ovi}(T) = \sum X_{ovi}(T)$, however, the timing of peaks and troughs will differ. $Z(T)_i$ and $Z_{ovi}(T)_i$ summarise the contribution of a single event at time i to future transmission and oviposition respectively. This is equivalent to the introduction of one infectious human or one emerging population of *Aedes* mosquitoes. By contrast $X(T)_i$ and $X_{ovi}(T)_i$ measure the contribution of all past events to suitability at time i, which is equivalent to multiple infectious events or emerging mosquito populations having occurred in the past that determine current suitability. Each of these measures has different implications depending on DENV endemicity and *Aedes* persistence status in the environments they summarise. The global distribution of dengue transmission varies by season, however, the global distribution of *Ae. aegypti* and *Ae. albopictus* shows little intra-annual variation mainly due to a limited flight range [8,51]. It follows that DENV transmission can exist in many stable states between sporadic transmission ($Z(T)$) and hyperendemicity ($X(T)$), however, *Aedes* populations are either absent with a risk of introduction ($Z_{ovi}(T)$), or persist year-round with varying degrees of suitability ($X_{ovi}(T)$). The measures for oviposition therefore reflect more absolute limits, while the measures of transmission document two ends of the spectrum of the many possible transmission intensities.

Vector temperature suitability

To determine the thermal limits of *Ae. aegypti* and *Ae. albopictus* persistence, we compared our measures of persistence suitability ($X_{ovi}(T)$) to known geographic occurrences of the vectors. Both *Ae. aegypti* and *Ae. albopictus* populations are robust to brief to medium term periods of environmental unsuitability due to transient behavioural and physiological adaptations at various life-cycle stages [52,53]. To determine the maximum unsuitable time period for each species the number of days in the year where $X_{ovi}(T)_i = 0$ was calculated for 4053 known geographical occurrences of *Ae. aegypti* and 1459 of *Ae. albopictus*. This database of known *Ae. aegypti* and *Ae. albopictus* occurrence points is composed of precise geographic locations where each species has been identified and reported in peer-reviewed literature sources. Full details of the database sources and extraction are available in Additional file 2. As the majority of these occurrence points only detect presence at one point in time, the database may contain identifications of temporary novel introductions as opposed to confirmed year round persistence of mosquito populations. As these records are likely to exist in the minority given their relative rarity, we chose a threshold of the number of suitable days ($X_{ovi}(T)_i > 0$) in an average year that incorporated 95% of these known occurrences. Separate thresholds were chosen for each species then the $X_{ovi}(T)$ index, normalised between 0 and 1, was displayed inside these limits, to show cumulative temperature suitability from established populations. In subsequent maps of temperature suitability for dengue transmission, the limits to vector establishment were used as a constraint, as significant dengue outbreaks can only be sustained in established vector populations.

Exploring spatial and temporal variations in suitability

To compare intra-annual variation in suitability across the globe two separate maps were created summarising the $Z(T)$ and $X(T)$ time series. The number of days in a

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year where $Z(T)_i > 0$ or $X(T)_i > 0$ were calculated and mapped on a continuous scale.

Finally, to further distinguish areas that have year-round temperature suitability for oviposition or transmission we summed the total values of $Z(T)$ or $Z_{ovi}(T)$ for each time step and each vector species. These annual totals were then divided by the maximum pixel value for each species to give a relative index between 0 and 1 that can be compared across geographic areas, but not between species. To compare temperature suitability between species total values were normalised relative to the maximum value in either species.

Weekly average animations were also created to display intra-annual variation in temperature suitability. Animations were created for constrained $X_{ovi}(T)$, $Z(T)$ and $X(T)$ indices and are available in Additional files 3, 4, 5, 6, 7 and 8.

Quantifying uncertainty

To quantify uncertainty in our temperature suitability predictions, uncertainty in the individual temperature-based relationships parameterised from empirical data was propagated through the model using a Monte-Carlo method. Global pixels were divided into 10000 bins using k-means clustering based on their annual temperature profiles then, for each bin the pixel closest to the mean temperature regime of all pixels within the bin was selected as representative. The simulation model was re-run 100 times on the 10000 representative pixels but each time using a different set of parameters to define the temperature-based relationships. For each model run the parameters from one of the 200 bootstrap models used to fit the data [10] (selected at random) was used to form the temperature-vector survival relationship. For the temperature-EIP relationship for *Ae. aegypti* we took one sample at random from the posterior distribution of the parameters directly from the MCMC chain which gave us one set of model parameters. For the relative EIP differences between *Ae. aegypti* and *Ae. albopictus*, one sample from the posterior distribution of ϕ was selected at random from the MCMC chains. Parameter estimates for the temperature-time to oviposition relationship were obtained by randomly sampling from a multivariate normal distribution with mean vector given by the optimal parameters and covariance matrix given by the negative inverse Hessian matrix estimated during optimisation. The model for each species was run 100 times with these randomly selected parameters and uncertainty was quantified as the interquartile range of the predictions. The uncertainty analysis was performed at 20 km × 20 km resolution rather than the full 5 km × 5 km resolution of the final model due to computational constraints. Note that the model is entirely based on temperature and so is not scale-dependent.

Results

Temperature-based relationships

In this analysis we considered three separate temperature-dependent relationships for each of *Ae. aegypti* and *Ae. albopictus*. The relationship between temperature and adult female survival of the two species is available elsewhere [10,54,55]. Similarly, temperature-dependent EIP in *Ae. aegypti* has been modelled previously using data from natural transmission experiments [11], however the corresponding relationship for *Ae. albopictus* was calculated for the first time using assay data collected in this analysis. In total we found 29 references detailing 498 assay time-point measurements that met our inclusion criteria (373 for *Ae. aegypti*, 125 for *Ae. albopictus*). Of the 29 different studies, ten tested the competence of both *Ae. aegypti* and *Ae. albopictus*. Fitting the previously described Bayesian hierarchical model we found a significant difference in mean EIP as determined by transmission experiments and mean EIP as determined by assay experiments for *Ae. aegypti* ($\alpha_{aegypti} = 1.10$, standard deviation 0.97 – 1.23), suggesting that mosquitoes become infectious before the DENV is detectable in the head tissues through standard immunofluorescent assays. The corresponding difference between mean EIP for *Ae. aegypti*, as determined by transmission experiments, and mean EIP for *Ae. albopictus*, as determined by assay experiments, was slightly higher ($\alpha_{albopictus} = 1.17$, sd 1.03 – 1.30). Taking the difference between the two kinds of experiment into account, mean EIP for *Ae. albopictus* was found to only be 1.03 times longer than for *Ae. aegypti*. This equates to a very similar relative efficiency (ϕ) of 0.968 (0.95-0.98), which was used to rescale the temperature-EIP relationship, as determined by transmission experiments, to predict EIP for *Ae. albopictus*. We found the variance for the rate of EIP accumulation to be slightly higher for *Ae. aegypti*. This would mean infectious mosquitoes would be seen earlier, but complete infection of the cohort would be observed later, with the mean time to infection very similar to that of *Ae. albopictus* (Figure 1). The study effect in all analyses was found to be significant, indicating the high variability in measurement of mean EIP when using different experimental designs and different mosquito and viral strains.

The relationship between temperature and FGC length was defined by optimising an existing enzyme kinetics equation to new data. A total of 13 references contained 54 experiments that timed the mean length of the FGC (divided equally between *Ae. aegypti* and *Ae. albopictus*). The optimised enzyme kinetics equation for each species is shown in Figure 2. The model predicts broadly similar FGC length for the two species with *Ae. albopictus* having a slightly longer FGC length as well as higher uncertainty in its estimation.

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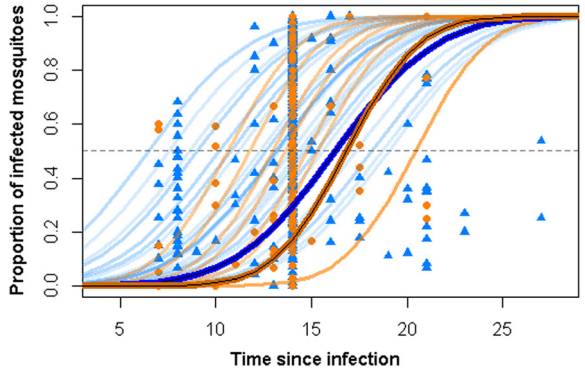

Figure 1 Rate of incubation in *Ae. aegypti* (blue) and *Ae. albopictus* (orange). The data from each individual assay are shown by blue triangles for *Ae. aegypti* (n = 373) and orange circles for *Ae. albopictus* (n = 125). The fitted lines for rate of EIP completion for each experiment are shown in faded blue lines for *Ae. aegypti* and faded orange lines for *Ae. alboipictus*. The mean fit for each species is shown in thick solid blue (*Ae. aegypti*) and orange/brown (*Ae. albopictus*) lines. The black dotted line indicates the point at which 50% of the population will have completed incubation, which is equivilent to the mean EIP.

Temperature suitability for oviposition

The thermal limits of persistence for each species were defined by comparing the predicted number of days in a year where $X_{ovi}(T)_i > 0$ at the occurrence points where each species has been reported. For *Ae. aegypti,* 95% of occurrences had 219 or more days in an average year suitable for oviposition. The equivalent value for persistence of *Ae. albopictus* was 365 days, suggesting that temperature does not limit the persistence of *Ae. Albopictus* through inability of the adults to complete oviposition. The limits defined by these thresholds were used to constrain the geographic extent of all the predictions in Figures 3 and 4.

Within these limits, temperature suitability for oviposition (relative values of $X_{ovi}(T)$) varies considerably, both geographically and between species. For *Ae. aegypti* oviposition, temperature suitability peaks in the warmest tropical environments in the northern Amazon, sub-Saharan Africa and South East Asia but drops by up to 100 fold as conditions become more temperate. The distribution of oviposition suitability for *Ae. albopictus* is similar, but the hottest regions are predicted to be less suitable due to the vector's reduced survival at higher temperatures relative to *Ae. aegypti*. The geographic extent of *Ae. albopictus* is also increased relative to that of *Ae. aegypti*, although often with low suitability, such as in the USA. The temperature suitability index also predicts high suitability in many arid areas where neither species has been observed, such as the Sahara desert and the interior of Australia. In these areas, factors other than temperature are clearly more likely to be the limiting factors defining the absolute extent of oviposition suitability.

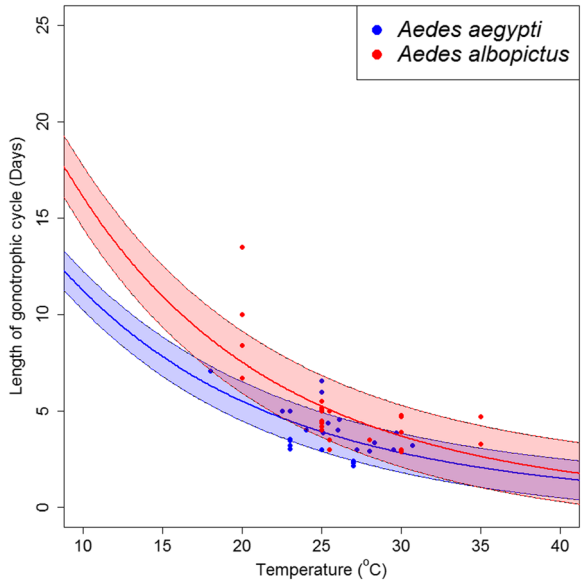

Figure 2 Relationship between temperature and length of the first gonotrophic cycle for *Ae. aegypti* and *Ae. albopictus*. The bold line shows the predicted mean first gonotrophic cycle length and the shaded areas show the prediction standard deviation for *Ae. aegypti* (blue) and *Ae. albopictus* (red).

Seasonal profile of temperature suitability

To summarise global intra-annual variation in temperature suitability two measures are presented in Figures 3B-C and 4B-C. The first summarises the number of days in an average year where $Z(T)_i > 0$. This summarises the number of days in a year where if an infectious human was introduced temperature would permit subsequent transmission, hereafter called introduction suitability, shown in Figures 3B and 4B. The latter of these indices summarises the number of days in which $X(T)_i > 0$, or if an infectious human population is omnipresent, the number of days in the year where temperature would permit an infectious mosquito population would be present, hereafter called persistence suitability, shown in Figures 3C and 4C. The former measure is more representative of a transmission free environment, while the latter is more representative of an endemic environment.

For both species, there are considerable differences between the introduction suitability and persistence suitability maps, indicating that temperature plays an important role in limiting not only the absolute geographic limits, but also in enabling different levels of endemicity. Surprisingly few environments were receptive to DENV introduction on every day of the year, which is consistent with the seasonal nature of reported dengue illness in many regions, such as southern Brazil, Mexico and China (Figures 3B-C and 4B-C). The map of introduction suitability does, however, highlight the prolonged periods of

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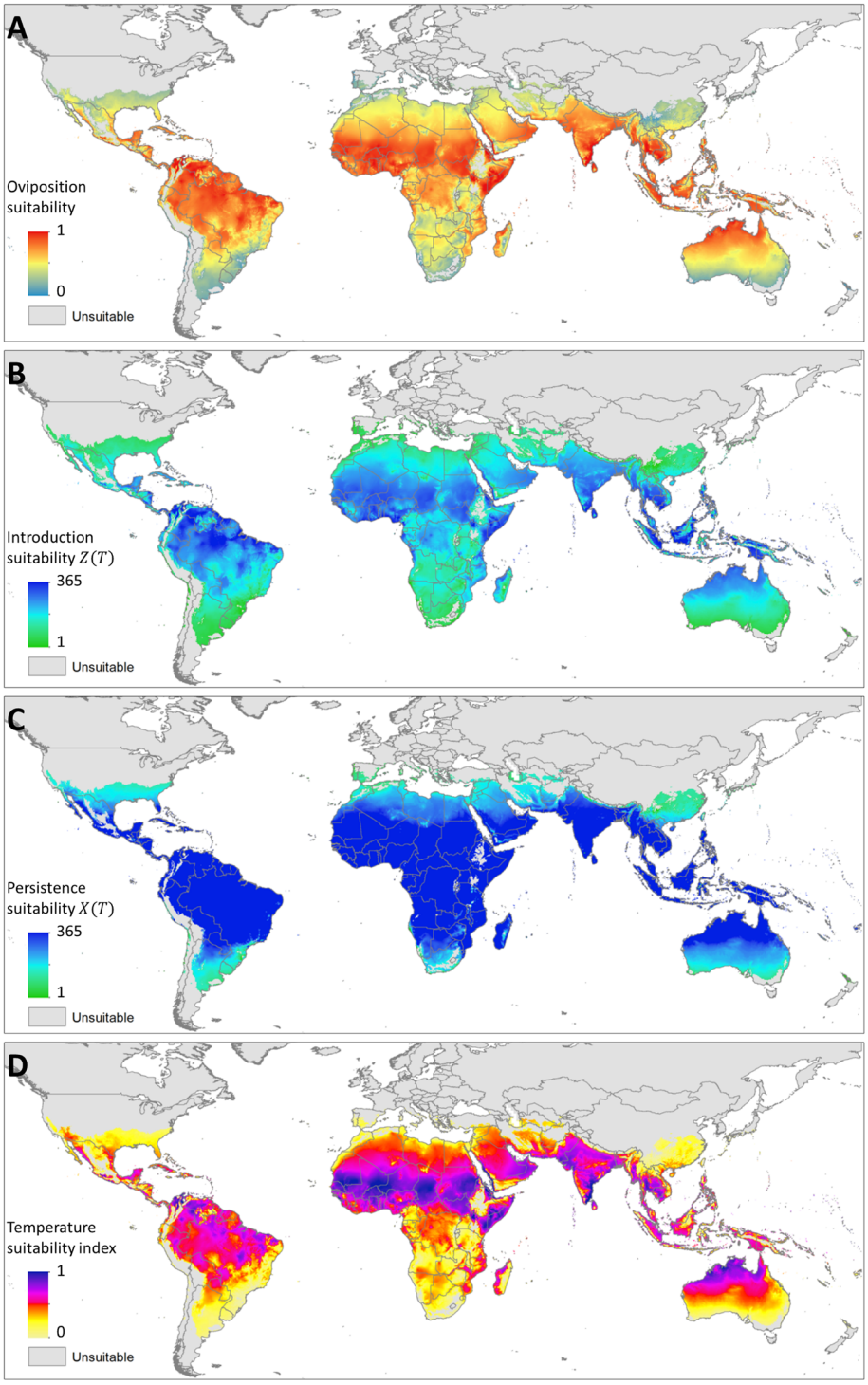

Figure 3 *Ae. aegypti* temperature suitability for persistence and DENV transmission. (A) The annualised summary of temperature suitability for oviposition ($X_{ovi}(T)$) on a normalised scale. **(B)** Introduction suitability; the number of days in a year where introduction of a DENV infected human would lead to ongoing transmission ($Z(T)_i > 0$). **(C)** Persistence suitability; the number of days in the year where onward DENV transmission could occur if a constant source of infectious humans were available ($X(T)_i > 0$). **(D)** The annualised summary of temperature suitability ($X(T)$) on a normalised scale. Predictions in all above maps are constrained to areas that permit oviposition ($X_{ovi}(T)_i > 0$) on 219 or more days in the year, as determined by comparison with known occurrences of *Ae.aegypti*.

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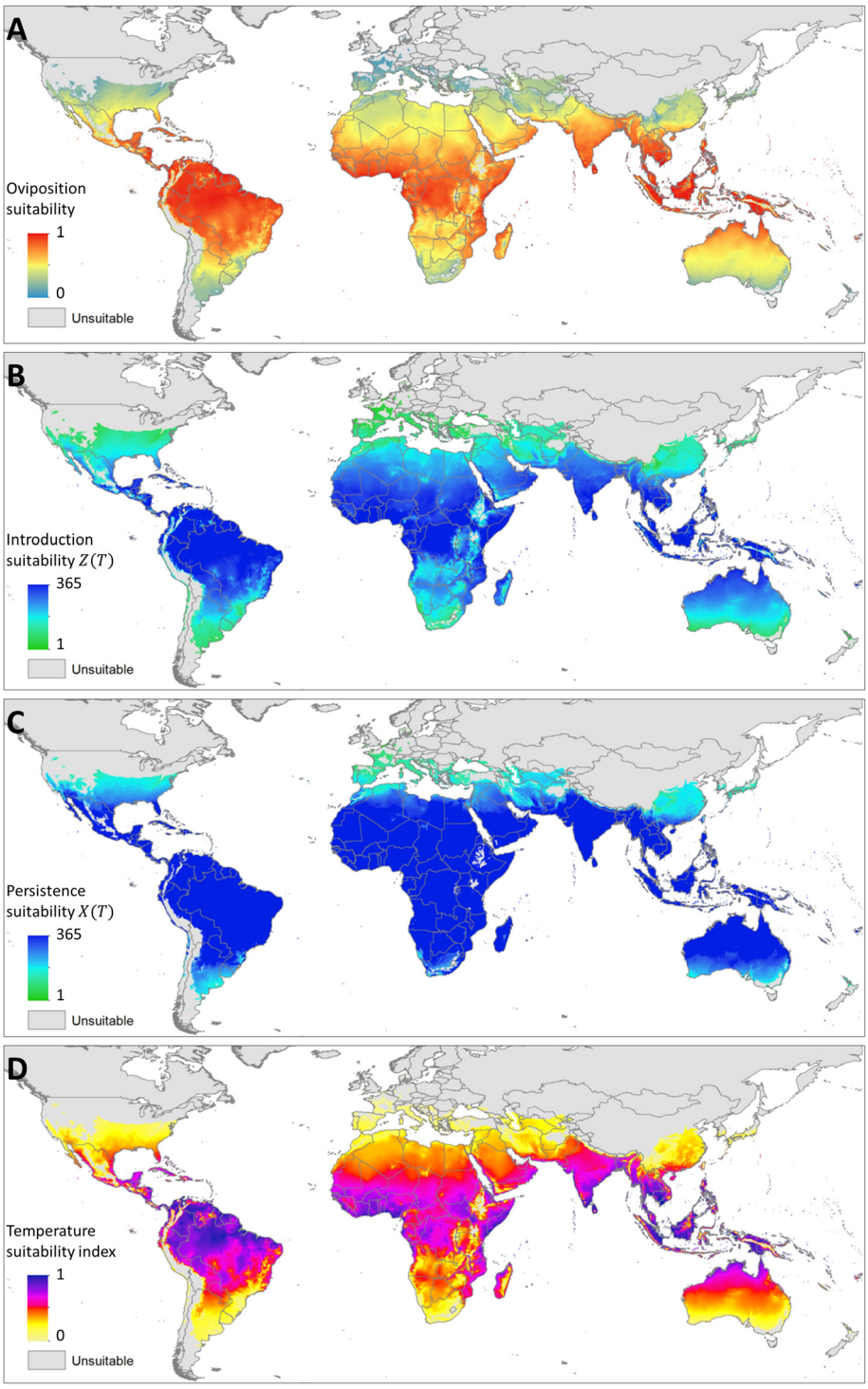

Figure 4 *Ae. albopictus* temperature suitability for persistence and DENV transmission. Panels correspond directly to those described for *Ae. aegypti* in Figure 3, but constraints are expanded to areas that permit oviposition for 365 days in the year.

suitability in areas where dengue is presently underreported, such as sub-Saharan Africa [2,22,56], or has yet to be reported, such as the eastern Arabian peninsula and Australia's Northern Territory. In such areas, if suitable mosquito breeding habitat and overlapping susceptible human populations were present, our results indicate that the potential for DENV introduction would be high (Figures 3D and 4D) and distributed throughout the year

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(Figures 3B and 4B). By contrast, many temperate environments have a limited window of transmission, particularly in many areas of North America and Europe. The predicted distribution of persistence suitability is far less heterogeneous with large portions of the world where onward transmission would be enabled on every day of the year given sufficient reservoirs of infectious humans. At the fringes of the distribution, although the window of transmission is expected to be longer than that of introduction suitability, the transmission season is still predicted to be significantly restricted. This indicates that even if mosquitoes in these areas were frequently exposed to infectious humans through either autochthonous or imported cases, we expect temperature would prevent year-round transmission and thus prevent the area from becoming endemic. The limits temperature places on endemicity can be identified by areas that allow transmission for 365 days minus the incubation period in humans and the duration of human infectiousness (typically 3-10 days [11] and 2-5 days [57] respectively).

The maps of both introduction and persistence suitability for *Ae. aegypti* and *Ae. albopictus* show a similar distribution. This is consistent with DENV EIP being a main limiting factor to the seasonal extent of DENV transmission. Although, DENV EIP is slightly longer in *Ae. albopictus*, adult females are predicted to live longer leading to similar seasonal profiles. The only place in which the two predictions differ is at the fringes of transmission where the wider predicted distribution of *Ae. albopictus* could allow a very limited transmission season at higher latitudes.

Total suitability throughout the year

To summarise total suitability across the year $Z(T)$ values for each time step were summed then normalised relative to the maximum global pixel value. This index provides discrimination of the effects of suitability between environments that are suitable throughout the year and also highlights the contribution of environments that have shorter, but much more pronounced periods of suitability. Temperature suitability for *Ae. aegypti* peaks in the warmest regions of the world, particularly in the sub-Saharan region and southern India. By contrast, *Ae. albopictus* temperature suitability peaks in northern South America where the diurnal temperature fluctuation is less pronounced and thus more favourable to *Ae. albopictus* survival at extreme temperatures. For both species, temperature suitability in the most suitable regions (typically tropical regions) is 100-1000 times the value in the least suitable regions (typically temperate regions). Alternatively, given equivalent conditions temperate regions would need 100-1000 times the number of mosquitoes to sustain equivalent levels of transmission. Predicted weekly changes in the temperature and oviposition suitability indices throughout an average year are also shown in animations in Additional files 3, 4, 5, 6, 7 and 8.

Prediction uncertainty

Additional files 9 and 10 show the interquartile range in predictions for the temperature suitability index and oviposition suitability for both species. Uncertainty predictions of the temperature suitability index for *Ae. aegypti* (Additional file 9A) scales approximately linearly with the initial prediction (Figure 3D), while in the corresponding measure for *Ae. albopictus* (Additional file 10A) uncertainty values level off at high and low temperature suitability index extremes (Figure 4D). Uncertainty in predicting oviposition suitability for *Ae. aegypti* (Additional file 9B) is high in all but low suitability regions (Figure 3A), while uncertainty is highest in the mid oviposition suitability (Additional file 10B, Figure 4A) regions in the equivalent measure for *Ae. albopictus*. Average uncertainty in predicting the temperature suitability index for both species is similar, however uncertainty for oviposition suitability is lower for *Ae. albopictus* likely due to the longer lifespan reducing the overall effect of uncertainty in FGC length.

Relative role of *Ae. aegypti* and *Ae. albopictus*

Figure 5 shows the temperature suitability index displayed in Figures 3D and 4D, but with pixel values normalised to the maximum value of the temperature suitability index for *Ae. albopictus* and re-plotted on a log-scale. On average the temperature suitability index for *Ae. albopictus* is around 42 times higher than for *Ae. aegypti*, meaning that suitability is predicted to be far higher for *Ae. albopictus*. Even the most suitable regions for *Ae. aegypti* DENV transmission have equivalent temperature suitability values as relatively marginal areas for *Ae. albopictus* DENV transmission, for example temperature suitability for *Ae. aegypti* in the tropical regions of Brazil is equivalent to temperature suitability for *Ae. albopictus* in northern France (Figure 5). This difference is due to the much longer survival times of adult female *Ae. albopictus* which far offsets the minor differences in vector competence. The relative values of the temperature suitability for oviposition index also show the same degree of disparity between the two species with *Ae. albopictus* able to produce on average 33 times the population of parous females.

Discussion

In this study we used a range of biologically relevant temperature-based relationships in a dynamic model framework to evaluate the effects of temperature on DENV transmission on a global scale. For the first time we examined the global limits temperature places on DENV transmission taking into account seasonal and diurnal variations in temperature and their cumulative effects on

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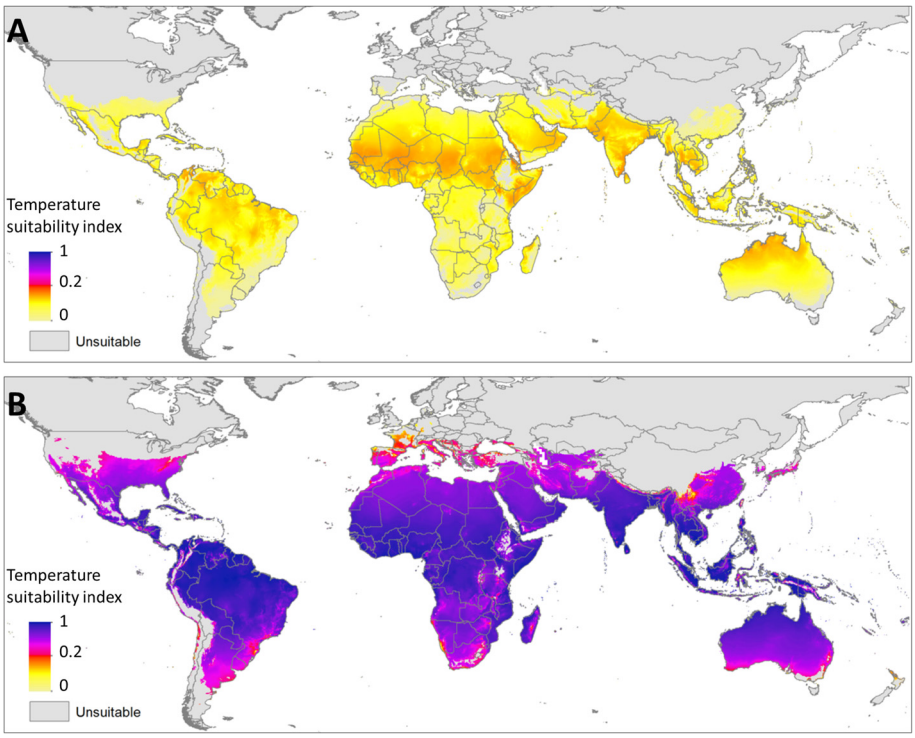

Figure 5 Comparative temperature suitability of *Ae. aegypti* (A) and *Ae. albopictus* (B). The annualised temperature suitability index ($X(T)$) normalised relative to the maximum value of both species and plotted on a logarithmic scale.

mosquito cohorts and present explicit high spatial and temporal resolution predictions. We also show that temperature has the potential to limit different levels of DENV endemicity and map the predicted temperature constraints in both transmission free environments and highly endemic environments and suggest that the true limits for other endemicity settings are likely to lie between the two. Finally, we conclude that when considering the currently available data from existing competence experiments, our analysis of temperature and its effects on DENV development and mosquito survival indicates that DENV transmission in *Ae. albopictus* is only marginally less efficient than in *Ae. aegypti* and when considering the increased longevity of the former, we predict that *Ae. albopictus* has the potential to be a more efficient DENV vector, even if this potential is not fully realised in its current niche.

The prediction that *Ae. albopictus* has a higher potential for DENV transmission than *Ae. aegypti* is at odds with the current understanding of the global epidemiology of DENV where sustained high incidence and explosive outbreaks are rarely observed in locations where *Ae. albopictus* is the sole vector. This was previously thought to be at least partly due to reduced competence of *Ae. albopictus* (reduced rates of virus dissemination from the midgut to the salivary glands) [15,58]. Our results indicate, however, that by incorporating the full breadth of data from previous experiments over a wide range of mosquito and virus strains and experimental designs, competence of *Ae. albopictus* was similar to that of *Ae. aegypti*. This small difference becomes even less meaningful when incorporated into a DENV transmission model due to the longer lifespan, and thus longer time to become and remain infectious, of *Ae. albopictus*. By assuming a normally distributed probability of EIP completion we assumed that given enough time, every infected mosquito would progress to becoming infectious and the inter-species difference is associated with the efficiency with which DENV can cross the mid-gut barrier and disseminate to the salivary glands. Although the existence of species-specific immune responses [59], protective gut microbiota [59,60], protective extreme temperatures [32] or *Wolbachia* [61] may block transmission, this would be challenging to demonstrate experimentally and full recovery from infection before onward transmission seems an unlikely outcome. Irrespective of whether this phenomenon occurs, the modelling approach effectively accounts for recovered mosquitoes in the data by predicting an EIP that exceeds their lifespan, therefore modelling the progress of DENV infection in the mosquito through EIP has the ability to incorporate a variety of species-specific aspects of competence.

Assuming EIP and adult survival are modelled correctly, species-specific differences in vectorial capacity can be accounted for by differences in i) the ratio of mosquitoes to humans, which seems unlikely to be driven by inter-species differences in mosquito population size given similar survey rates [62-64], but may be affected by shifts in habitat preferences that increase the degree of mosquito-human population overlap, ii) features of vector competence that are not accounted for in current study designs [65-67] or iii) differences in the human blood feeding rate. Differences in blood feeding behaviour and the degree of mosquito-human population overlap are perhaps the most plausible explanations, given that *Ae. albopictus* typically either inhabits more rural environments (or in urban environments often in much less close human association than the highly peridomestic *Ae. aegypti* [26,64]) and that *Ae. aegypti* has a well documented tendency to feed frequently on human hosts [68,69]. This has the effect of reducing the mosquito-human ratio, but also affects the source of potential blood meals, with *Ae. albopictus* known to feed opportunistically on a variety of non-human hosts [24]. It has been shown, however, that *Ae. albopictus* is capable of both inhabiting high density urban environments and will sometimes feed frequently and almost exclusively on human blood [23,70,71]. Although specifically designed comparative studies between *Ae. aegypti* and *Ae. albopictus* that control for the underlying mechanisms are needed to further clarify differences in their DENV vector competence [65-67], our results would suggest that the capacity of *Ae. albopictus* to transmit DENV is primarily driven by some aspect of its ecology that affects how closely *Ae. albopictus* habitats align with human population densities and how frequently they feed on human blood.

Further experiments need to be conducted on temporal and environmental variation in the ratio of mosquitoes to humans and human blood feeding habits of *Ae. albopictus* to explore these hypotheses further. Given the second order effect of human blood feeding rate on vectorial capacity, and given a conservative crude estimate that the mosquito-human proportion is halved for *Ae. albopictus,* we would expect an *Ae. albopictus* human blood feeding rate of less than $\frac{2}{\sqrt{42}} = 0.31$ times that of *Ae. aegypti*. This has been observed in some settings [23,72]. We do not mean to assert that *Ae. albopictus* is an unrecognised and underemphasised vector of DENV, but rather suggest that its involvement in transmission may be locally dependent and that further entomological investigation into human blood feeding rate and the overlap between mosquito and human populations needs to be undertaken to better understand its relative role in DENV transmission.

The results presented here apply generalised relationships to long-term average temperature data to predict the constraint temperature places on DENV transmission. While our predictions are likely to be representative of the majority of global environments most of the time, local adaptations and abnormal weather patterns may explain temporary deviations from predicted temperature constraints. It has been suggested that *Ae. albopictus* has adapted its immature stages to local environments to allow it to persist long inhospitable winters in Europe and the USA [73,74], however, when considering the temperature constraints solely on the adult forms, we found no difference between the temperature suitability for oviposition values attributed to *Ae. albopictus* occurrence points in its native Asian range and those in its newer invasive range. It has also been hypothesised that vector competence in *Ae. aegypti* varies geographically [75,76] and may even be influenced by co-evolution of local vector and virus populations [65,66,77]. Although we found the study effect to be highly significant in both EIP models, we were unable to distinguish the effects of experimental design from the effects of mosquito or viral strains. Previous work did not detect significant differences in EIP between DENV serotypes [11] and wide-scale differences in competence between different strains of *Ae. aegypti* are difficult to demonstrate considering the degree of variability in vector competence studies (Figure 1). Although there is some evidence of fine scale variation in competence in both mosquito [78-80] and virus [81,82] populations, more research is needed to determine if such structure exists at a wider scale considering human assisted rapid movement of vector and viruses may minimise opportunities for evolutionary interactions [83,84]. Until wider spatial scale structuring of mosquito-virus interaction can be demonstrated, a generalised prediction using vector and virus strains from a variety of different conditions is most likely to be representative of the majority of transmission environments. It is possible that behavioural adaptations to the micro-climates of the places where they rest or avoid extremes may allow adult mosquitoes to minimise the effects of harsh temperatures. While this may be possible in some local settings [52], protection is likely to be limited and environmental stochasticity is likely to minimise opportunities for permanent population establishment and subsequent DENV transmission. As such, we find no strong evidence that any local adaptations of the adult forms are directly temperature dependent and as a result, the predictions made here are likely to be indicative of the long-term environmental average.

The predictions of the limits temperature places on persistence and transmission broadly align with the known distribution of each species and the global distribution of DENV transmission (see Additional file 2, [1,2]). Discontinuities between the two most commonly occur in extremely arid environments, such as Australia and the Arabian Peninsula, where limited immature development

habitat (through either low precipitation or minimal open human water storage) prevents the establishment of permanent vector populations and where humidity may be an important additional limiting factor [85]. We also predict brief periods of low risk in some temperate environments that have yet to experience DENV transmission. This approach predicts the absolute limits temperature places on DENV transmission, however, in some low temperature suitability environments, the mosquito population size required to reach a certain threshold vectorial capacity may be biologically implausibly high, thus further limiting the constraints temperature places on global DENV transmission. Alternatively, DENV transmission may have yet to expand to these new areas to realise its full niche, or additional factors other than temperature, particularly humidity, may be providing additional limits to transmission. To fully delineate the temperature-permitted niche, the effects of temperature on aspects of immature mosquito population dynamics and other aspects of transmission would need to be predicted.

In this manuscript we predict the absolute limits temperature places on global expansion of DENV, but we do not evaluate what is driving its expansion. Further analyses need to be conducted to identify how temperature interacts with other environmental and genetic factors to limit mosquito vector and virus distributions. This kind of analysis could be done by studying areas that have undergone recent DENV expansion and test whether this coincides with peak periods of risk as determined by temperature suitability along with relevant intra-annual changes in other candidate covariates. This would bring about a greater quantitative understanding of global DENV expansion and allow preventative measures to be put in place in areas and times of peak or elevated risk.

By comparison with the global distribution of dengue we have revealed that while temperature provides key constraints on global DENV transmission it is not the only factor limiting DENV transmission on a global scale. Despite this, many attempts to model the current and future global distribution of dengue and its vectors have predominantly or exclusively relied on temperature as a predictor [86-91]. Through a detailed biological consideration of the way in which temperature acts, it is possible to better inform statistical approaches in two key ways: i) constraining predictions to eliminate areas where temperature does not permit transmission and ii) including this information as a more biologically relevant covariate than standard temperature measures, but always in addition to other covariates to explain the complex epidemiology of DENV transmission. Combining mechanistic and statistical approaches will allow us to utilise the wealth of theoretical and experimental knowledge of the epidemiology of DENV transmission in generalised analyses [1,40,92].

While in this manuscript the temperature suitability index has been summarised in a broadly spatial analysis, the same index could be used in models that analyse time series data of vectors or dengue cases using a similar method. Such biological consideration is essential if we are to extrapolate past or current conditions to make predictions about the future, such as in projections of the future distribution of dengue or in dengue early warning systems. Temperature suitability is just one example of this and consideration of further biological limitations, such as geographic spread of dengue's vectors, the role of other limiting factors such as humidity and the rate of dengue expansion need to be considered if projections of the future distribution of dengue are to be more reliable and useful.

The predictions from this analysis suggest that current day temperature regimes permit both vector establishment and DENV transmission beyond their current ranges. While factors other than temperature are likely to further limit the distribution of dengue and its vectors, if expansion is to occur, temperature suitability can elucidate the regions and suitable seasons most at risk. For *Ae. aegypti* and/or *Ae. albopictus* free areas, the introduction suitability ($Z_{ovi}(T)$) maps and annual profiles can be used to direct surveillance to the regions and times of the year where a single mosquito introduction would carry the highest risk of population persistence. For areas with year-round populations of *Ae. aegypti*, but without persistent DENV transmission, the DENV introduction ($Z(T)$) maps and annual profiles can be used to guide vector control to limit periods and areas of peak risk. For areas with persistent dengue transmission (endemic) the annual profiles of persistence suitability ($X(T)$) can be used to direct control towards the time of year that is most likely to break the on-going transmission cycle. Finally, in areas with established *Ae. albopictus* populations, their potential for DENV transmission can be evaluated through a combination of $Z(T)$ and $X(T)$ indices that elucidate areas and times of peak risk which can then guide further entomological investigation regarding human blood feeding rate and mosquito-human population overlap, thereby further improving estimates of DENV vectorial capacity of this species. This combination of high spatial and temporal resolutions of risk estimates coupled with guided local entomological investigation is likely to give a more complete understanding of future DENV expansion risk than a consideration of the risk imposed by extrapolated niche approaches alone [86,87,89-91].

Conclusions

Here we used a range of entomological and virological experimental data to parameterise temperature-based relationships in a dynamic transmission model to assess the limits that changing temperature regimes place on

Ae. aegypti and *Ae. albopictus* persistence as well as the global distribution of DENV transmission. The model predictions were used to map the absolute and seasonal temperature limits to vector persistence and DENV transmission on a global scale. These maps reveal that as well as constraining the absolute extent of transmission, temperature may also limit levels of DENV endemicity. A comparison of temperature suitability for DENV transmission by *Ae. aegypti* vs. *Ae. albopictus* revealed that given similar population sizes and human blood feeding rates, *Ae. albopictus* has a higher vectorial capacity. This finding contradicts existing explanations and suggests that ecological factors, such as blood feeding patterns and the degree of mosquito-human population overlap, may explain why areas with established *Ae. albopictus* populations do not experience sustained dengue outbreaks. The resulting models can inform statistical approaches to model DENV transmission and persistence of its vectors by replacing raw temperature variables to include known biological understanding of transmission. The risk of dengue expansion into novel areas and at novel times of the year can be estimated by using the seasonal profiles presented here to target entomological investigation of human biting rate and the degree of mosquito-human population overlap. As well as being informative for surveillance purposes, this further investigation will help identify and understand additional factors limiting DENV transmission and will inform and support new ways to limit the expansion of the geographic extent and range of endemicity of dengue.

Additional files

Additional file 1: Priors used for Bayesian Inter-species EIP comparison model.

Additional file 2: Assembly of the supplementary *Ae. aegypti* and *Ae. albopictus* database.

Additional file 3: Animation of *Ae. aegypti* temperature suitability for oviposition ($X_{ovi}(T)$) throughout an average year.

Additonal file 4: Animation of *Ae. albopictus* temperature suitability for oviposition ($X_{ovi}(T)$) throughout an average year.

Additional file 5: Animation of *Ae. aegypti* temperature suitability for introduction of dengue virus transmission ($Z(T)$) throughout an average year.

Additional file 6: Animation of *Ae. albopictus* temperature suitability for introduction of dengue virus transmission ($Z(T)$) throughout an average year.

Additional file 7: Animation of *Ae. aegypti* temperature suitability for persistence of dengue virus transmission ($X(T)$) throughout an average year.

Additional file 8: Animation of *Ae. albopictus* temperature suitability for persistence of dengue virus transmission ($X(T)$) throughout an average year.

Additional file 9: *Ae. aegypti* model prediction uncertainty for the temperature suitability index (A) and oviposition suitability (B). The output shows the interquartile range in predictions presented in maps 3A and 3D.

Additional file 10: *Ae. albopictus* model prediction uncertainty for the temperature suitability index (A) and oviposition suitability (B). The output shows the interquartile range in predictions presented in maps 4A and 4D.

Abbreviations
EIP: Extrinsic incubation period; DENV: Dengue virus; FGC: First gonotrophic cycle.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
OJB, DLS, PWG and SIH designed the experiment. OJB, wrote the manuscript and collected and analysed the data. MUK collected additional data. NG, DLS and MUK helped with data analysis. All authors helped with data and results interpretation and were involved in drafting, revising and approving the final version of the manuscript.

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Chapter 7 – Discussion

The primary aims of this thesis were to assess current gaps in global surveillance of dengue and its vectors. A range of modelling techniques were applied to identify specific surveillance targets in specific locations. In doing so, this thesis has updated the contemporary knowledge of the global distribution and burden of dengue and the relative role different mosquito species play in constraining where transmission is possible. The models and maps presented here provide a useful evidence base with which to inform the next generation of dengue surveillance policy.

This discussion section begins with a recapitulation of the main results of each chapter followed by a dissection of the strengths, limitations and main conclusions of the methods used in this thesis. Evaluating contemporary global dengue surveillance presented many challenges involving the use of disparate or incomplete data in different flexible modelling frameworks. While this gave novel insights and conclusions, these results must be interpreted taking into account uncertainty in the model predictions. In light of this, this discussion section will go on to suggest future surveillance priorities with reference to specific surveillance gaps that remain challenging to address for a number of reasons. In particular it will discuss the issues of surveillance, burden estimation and development of dengue policy in low endemicity environments, with specific reference to dengue in Africa. The discussion section will also explore some of the issues faced in modelling the mosquito vectors of dengue, their role in transmission and how this information can be effectively used to guide public health policy.

7.1. Chapter summary

The first three research chapters (chapters 2-4) of this thesis focussed on assessing surveillance of human dengue virus (DENV) infections. Chapter 2 used maps to summarise the quality and contemporariness of dengue data that is reported by each country using a metric termed evidence consensus. This showed that despite the rise of dengue as a global public health threat, there are still many countries that lack sufficient evidence to determine disease presence or absence with certainty, particularly in Africa where the most accurate diagnostic methods are not in use and reports are often out of date. The maps also identified specific data needs in certain countries that, if met, would significantly improve the estimates of the contemporary global distribution of dengue presented in this thesis.

In chapter 3 the evidence consensus map from chapter 2 was used to generate fine-scale risk maps, which were then used to derive the first evidence-based estimate of global dengue case burden. At 390 million (95% credible interval 284-528) infections per year, this figure was over three times higher than the WHO's estimate at the time *(WHO, 2009)*. Much of this burden is predicted to be concentrated in the Indian subcontinent and China, where regular dengue reporting is geographically sparse and temporally inconsistent. A systematic literature review showed that a large proportion of these infections (up to 91%) are unlikely to ever be detected by even the most efficient passive surveillance systems. This emphasised the importance of sentinel active serology-based surveillance and, in particular, its role in burden estimation and in setting policy goals for interventions.

Chapter 4 looked at how dengue burden is distributed through time, with a particular focus on high-burden dengue outbreaks that overwhelm healthcare facilities, yet are potentially preventable. This chapter showed that outbreak identification presents a

major hurdle to effective outbreak response, as currently implemented outbreak definitions do not identify outbreaks with any consistent characteristics. This may be causing sub-optimal outbreak response at different times, or in different geographic regions. Given this, a new framework for outbreak definition was recommended based upon healthcare capacity restrictions which would be more relevant for measuring the excess burden and how best to prevent it during outbreaks.

Chapters 5 and 6 addressed the role mosquito vectors play in allowing or constraining the global distribution of DENV transmission in temperate environments. In these regions temperature plays an important role in determining where and when mosquito populations can support DENV transmission. Chapter 5 updated and refined models of adult *Ae. aegypti* and *Ae. albopictus* survival. This involved developing a framework to integrate data from laboratory and mark-release-recapture field studies that often give conflicting results about the importance of senescence. Using these datasets, a model of adult mosquito survival was developed which incorporated the effects of both temperature and age. This model showed that *Ae. albopictus* adults are less tolerant of extreme temperatures than *Ae. aegypti*, thus suggesting that the former's ability to persist in temperate environments is more likely due to diapausing adaptations of the juvenile stages than robustness of the adult forms. At tropical temperatures *Ae. albopictus* adults live significantly longer than *Ae. aegypti* adults, a factor that has numerous implications for the species' population persistence and ability to transmit DENV.

In chapter 6 this adult survival analysis was integrated into a wider model based on the formula for vectorial capacity. This combined model was then used to predict the effects of dynamic temperature changes on the ability of *Ae. aegypti* and *Ae. albopictus* populations to persist and transmit DENV. Using interpolated temperature data it was possible to make weekly global predictions for temperature suitability throughout an

average year. This resulted in explicit predictions of where and when there is a risk of DENV transmission or vector establishment in different temperate environments. Surprisingly the models showed that adult *Ae. albopictus*, which is rarely associated with sustained DENV transmission, is physiologically more capable of sustained transmission than adult *Ae. aegypti*, due to the former's longer life-span. This suggests that surveillance of behavioural traits of *Ae. albopictus* may be more informative about transmission risk than traditional abundance measurements currently collected in temperate regions.

7.2. Methodological discussion

Throughout this thesis a range of different modelling methods was used, each of which maximised data availability by incorporating data of different types and from different sources. It is important that this is done in a systematic way that incorporates and propagates the associated uncertainty. This section discusses the strengths and limitations of each of these modelling approaches in their different applications. The modelling approach and data included in each chapter are summarised in Table 7.1. with full details available in each chapter.

	Evidence consensus map	Burden estimates	Outbreak identification	*Aedes* survival model	*Aedes* temperature suitability
Input data types	International health organisations, peer-reviewed evidence, MoH case data, web-pages, questionnaires	Incidence cohort studies with and without active fever surveillance	Reported clinical dengue cases to the Brazilian MoH	Observations of mosquito population mortality at different time points by direct observation in the laboratory or MRR in the field	Integration of four models related to temperature-driven virus and mosquito physiological processes
Total data points	505 sources	95 apparent or inapparent incidence estimates	4,212 province-months of observation	22,239 mosquito population mortality measurements	For each model: 22,239 / 54 / 140 / 686
Modelling framework	Semi-quantitative scoring system	Hierarchical Bayesian linear model	Evaluation of a range statistical trend prediction models	Regression spline GAMs	GAMs, censored Bayesian hazard models and optimised functions nested in a simulation
Measuring uncertainty	Sensitivity analysis of scoring weights	Posterior distribution from processes and parameter models	Sensitivity analysis of model parameters and evaluation at different spatial and temporal scales	Bootstrap samples of field and laboratory data	Propagation of each model's uncertainty using a Monte-Carlo method
Outputs	Map of certainty of dengue presence or absence	Relationship between probability of occurrence and incidence of dengue	Evaluation of consistency of outbreak definitions	Models of *Ae. aegypti* and *Ae. albopictus* survival as a function of temperature	Maps of temperature suitability for DENV transmission and mosquito population persistence

Table 7.1. Summary of data types and modelling approaches taken in each chapter. MoH = ministry of Health, MRR = mark-release-recapture, GAM = generalised additive model, DENV = Dengue virus

7.2.1. Modelling approach strengths

For the global analyses described in this thesis there was a need to aggregate data from different geographic regions, sources and experimental designs (Table 7.1.). For

the evidence-consensus maps in chapter 1 incorporating a wide variety of evidence types in a semi-quantitative framework allowed a more accurate differentiation of certainty on dengue presence for a given area. This helped address key weaknesses in previous dengue distribution maps, which neither down-weighted older or less reliable data nor allowed for documentation of spread to new areas due to their omission of rapid reports from online reporting systems *(CDC, 2012; WHO, 2011)*. Similarly, use of model co-inference in several chapters of this thesis allowed incomplete datasets to be enhanced by drawing on information from closely related complementary, or more complete datasets. In the dengue burden model apparent infection numbers were only available for 73% of the cohort studies, but it was possible to impute the remaining values by comparing the parameters of the posterior distribution of inapparent infection data. Similarly, data on *Ae. albopictus* extrinsic incubation period (EIP) is comparatively incomplete relative to the data available for *Ae. aegypti* making hierarchical Bayesian modelling essential for assessing the relative transmission potential of each species in chapter 6. In chapter 5, extensive comparisons were also made between laboratory and field-based *Ae. aegypti* and *Ae. albopictus* survival data. In the laboratory, the full effect of temperature changes can be analysed, while in the field, temperature cannot be controlled but the effect of other external causes of mortality can be measured. In each of these cases the modelling approaches used allowed the maximum number of data points to be included and improved the generalisability of the outputs (Table 7.1.).

While including data from many different sources improves model inference, under some circumstances it can also increase uncertainty. Quantification of this uncertainty was an essential component to all model outputs in this thesis. In many cases prediction uncertainty arose due to uncertain parameter value specification. Sensitivity of model outputs was evaluated either with sensitivity analyses of each parameter

under a range of value combinations (chapters 2, 4 and 5) or in a Bayesian framework with evidence-based estimates for weakly informative priors (chapters 3 and 6). Data uncertainty was also important in several of the approaches and extensive bootstrap analyses in chapters 5 and 6 combined with additional statistical approaches to account for variation in different study designs allowed the optimal fit of generalisable models. In addition to quantifying uncertainty, propagating uncertainty estimates from both model fitting and model prediction was of paramount importance, especially when the models were used to make global predictions, such as in chapters 3 and 6. The advantages to quantifying this uncertainty are that: i) it allows a more rigorous assessment of the predictions and conclusions reached, ii) it allows recommendation of explicit future data needs for model prediction improvement and iii) it provides a more informative output for downstream analyses that can propagate this uncertainty through to their own outputs.

This thesis combines statistical and mechanistic modelling approaches to improve both predictions and understanding of the processes they represent. The former type allows trends between inputs and outputs of a particular process to be quantified and replicated, but does not enable clear interpretation of the underlying processes. By contrast, mechanistic modelling defines how a particular process is known to work and can inform why particular trends occur, but may be less accurate in consistently replicating trends between inputs and outputs as they may not have considered all the particular processes involved and their interaction. This combination was most apparent in the temperature suitability model in chapter 6 where a number of statistically derived temperature-based relationships were applied to a deterministic model of the transmission process. Complex, multi-causal, environmental phenomena, such as adult mosquito survival, are better represented by statistical models. These types of models can provide useful input parameters for deterministic models of known

processes, such as vectorial capacity, as implemented in chapter 6 with complex environmental factors affecting adult mosquito survival. While not explicitly combining two types of different models, biological model theory was applied to interpretation of the statistical model results in chapter 3. For the statistical dengue burden model specified in chapter 3, relationships that were biologically implausible could be specifically excluded due to theoretical knowledge about immunity to DENV infection. As dengue confers type-specific sterilizing immunity each individual can only receive a maximum of four infections, and thus incidence must level off to a maximum value even if transmission intensity keeps increasing *(Simmons, 2012)*.

7.2.2. Modelling approach limitations

The analyses presented here must be interpreted within the context of certain limitations on the data they are based upon, the methods employed and on how the results are presented.

As with all secondary data analyses, all chapters of this thesis were reliant on reported data. As outlined in the introduction, a number of factors complicate the reporting of dengue, and inequity in availability of diagnostic resources and reporting capacity means that each dataset contained an element of spatial, and in some cases, temporal reporting bias. Reporting bias affects the generalisability of the predictions and therefore must be explicitly considered in global approaches such as those presented here. The global burden predictions in chapter 3 are dependent on a relatively small number of cohort studies, often conducted in highly endemic areas and are poorly representative of the African continent. Similarly, when estimating *Ae. aegypti* and *Ae. albopictus* adult survival (chapter 5) there is a deficit in data from extreme temperatures where survival is more marginal and harder to measure. There is also a tendency for under-diagnosis of dengue at times of low incidence but over-diagnosis of dengue in outbreak times *(Teutsch and Churchill, 2000)*. The relative balance of this

may also change over time as healthcare systems become more familiar with dengue diagnosis. All of these specific temporal changes in passive reporting must be taken into account when distinguishing baseline transmission levels from outbreaks and how this distinction might change over time (chapter 4).

This heavy reliance on reported data adds additional emphasis on approaches that try to identify where new data are most urgently needed, including evidence-consensus type approaches and directly interpreting model uncertainty outputs. By doing this, maps and models can be iteratively improved by targeted data collection.

Each of the models used in this thesis make certain assumptions about the DENV transmission process, or the population dynamics of its vectors. Due to data paucities in both the dengue cohort studies and global demographic data, the burden model in chapter 3 had to assume that disease severity was independent of age. This assumption allowed data from cohorts of different ages to be pooled, increasing the total number of data points available for model fitting. There is some evidence that dengue disease severity is dependent on age *(Garcia-Rivera and Rigau-Perez, 2003; Hammond et al., 2005)*, but this is complicated by differing exposure histories and may be more relevant for refining predictions of severe dengue (SD), than for apparent infections, as predicted in chapter 3. The approach also assumes that there are no significant human genetic traits that affect DENV infection susceptibility or apparent infection risk. Recent human genome-wide association studies have independently identified various population-level traits that are associated with dengue shock syndrome (DSS); the most severe clinical manifestation of dengue *(Beatriz, 2014; Dang et al., 2014; Khor et al., 2011; Whitehorn et al., 2013)*. Understanding how these traits relate to less severe disease may be particularly important in producing more accurate dengue burden predictions in certain global regions, in particular Africa where

dengue is comparatively rarely reported despite large regions of high environmental suitability *(Jaenisch et al., 2014)*.

The models in chapters 5 and 6 also make a number of assumptions about the role of mosquitoes in DENV transmission, which are detailed in each chapter. Particularly important, however, is the assumption that mosquito behaviour and its effect on biting, movement, survival and oviposition are not affected by temperature, either directly or through local adaptation. There are, of course, many examples of local *Ae. aegypti* and *Ae. albopictus* populations adapting to micro-climates and micro-environments to increase their ability to persist or transmit DENV, but it is unclear if these adaptations are widespread enough to affect DENV transmission dynamics at a city-wide or regional scale *(Hayden et al., 2010; Kessler and Guerin, 2008; Lounibos et al., 2010; Lumsden, 1947)*. These transmission models also assume that adult mosquitoes can only transmit DENV if they survive long enough to i) mature to biting age, ii) complete viral incubation (EIP) and then iii) survive long enough to deliver infectious bites. Recent findings may put into question the first part of this process as some laboratory and field studies have documented vertical transmission of DENV between mosquito generations *(Buckner et al., 2013; Espinosa et al., 2014; Martínez et al., 2014)*. Despite this being a physiological possibility, the relative proportions of DENV-infected eggs to DENV-infected adults would suggest that this transmission route would have only a minor role relative to more conventional vector-host routes.

One of the biggest limitations to interpretation of global analyses such as those presented here is the issue of scale. Dengue, like many other vector-borne infectious diseases, exhibits complex heterogeneous transmission dynamics that vary both spatially and temporally *(Honorio et al., 2009; Johansson et al., 2009; Reiner et al., 2014)*. As data from multiple years or over a wide area are aggregated, however, these local variations are averaged out and more generalised patterns can be observed

(Nigmatulin, 1979). Most of the analyses presented here are designed to give larger spatial-scale predictions with the global maps presented at a subnational or 5km x 5km pixel scale and often using or representing data from long-term averages. When these models are trained, it is assumed that they have adequately captured enough variation from local studies to reliably estimate a generalisable mean value with a suitable confidence interval. Numerous cross-validation techniques were implemented for the analyses where data were limited to account for any potential biases (Table 7.1.). Despite this, the specific issue of sampling at different spatial and temporal scales was only explicitly addressed with regards to the reliability of outbreak definitions in chapter 4. As well as being important in fitting models, issues of scale are equally important in the interpretation of the results and validation of the predictions. In any particular year or smaller area, DENV transmission may differ substantially to the long-term average 5km x 5km pixel predictions. It is thus important to match these predictions to surveillance and control activities that are also implemented over long time scales and wide spatial scales. Additional modelling efforts may be required to enact further responses to more temporary and finer-scale variations in DENV transmission.

7.3. Surveillance of human dengue infections

Quantifying the distribution and intensity of DENV transmission in areas where it continues to expand has important practical implications for surveillance, diagnosis and control, particularly at a time when many of these countries are beginning to see improvements in other aspects of health *(Lozano et al., 2011; Murray et al., 2013)*. As outlined in the introductory chapter, the emergence of dengue as a public health problem can occur over a relatively short time period. Accurate, up-to-date surveillance of dengue and its vectors is the best approach for advocating for timely decision-

making that can control further increases in burden. This situation is currently most acute in the Indian sub-continent, southern China and mainland continental Africa, which all face differing dengue surveillance challenges. Surveillance in these regions will play a pivotal role in determining and controlling the global distribution and burden of dengue.

Chapter 2 showed that the spatial distribution of dengue is most uncertain in Africa. The volume, quality and timeliness of dengue data reported from this continent are all significantly lower than elsewhere despite wide areas of environmental suitability, widespread proliferation of *Ae. aegypti* and a predicted 16% of the global burden of DENV infections *(Bhatt et al., 2013; Brady et al., 2012; Kraemer et al., 2015)*. Occasional reports strongly suggest that urban dengue fever does occur in Africa, including severe cases that are suggestive of repeated infection *(Brady et al., 2012; CDC, 2013; Faye et al., 2014)*. These isolated reports do not, however, confirm endemic or widespread circulation of DENV, and the regular passive surveillance for dengue which would be needed to confirm this is not currently conducted by any African nation *(WHO, 2014)*. Even if dengue were to become a notifiable disease in these regions, clinical diagnosis of dengue would be extremely challenging due to shared symptoms with up to 12 other febrile illnesses *(Simmons, 2012)*, many of which are at high prevalence in Africa, and resources for serological confirmation are stretched thinly across the continent (Figure 7.1.) *(Jaenisch et al., 2014)*. Perhaps a more feasible first step would be to conduct age-stratified population seroprevalence surveys targeted using high-resolution risk maps, such as those presented in chapter 3 *(Bhatt et al., 2013; Phoutrides et al., 2011; Vairo et al., 2011)*. While cheaper enzyme-linked immunosorbent assay (ELISA) IgG tests sometimes cross-neutralise other flavivirus antibodies, and thus are less specific when multiple flaviviruses are circulating, their reliability can be improved by performing more specific plaque

reduction neutralization tests (PRNTs) on sub-samples *(Koraka et al., 2002; Vaughn et al., 1999)*. Seroprevalence surveys will give a more accurate measure of the total number of DENV infections and, if age-stratified, a measure of how transmission has changed over the past 60-80 years. In areas of high DENV seroprevalence, acute febrile illness (AFI) cohorts at local hospitals with reference laboratory testing support could better characterise the clinical presentation of dengue in Africa and thereby lead to the improvement of the initial estimates of dengue burden presented in chapter 3. This approach would help estimate the contribution of dengue to the high number of undiagnosed AFI cases in many African regions, and thus justify its inclusion in routine public health surveillance.

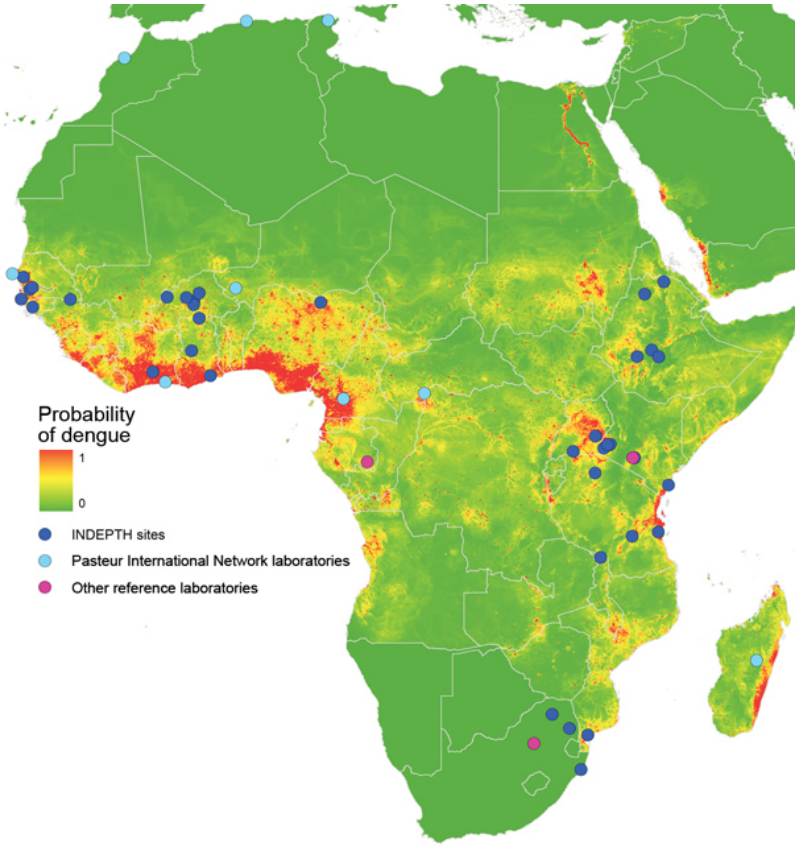

Figure 7.1. Predicted dengue risk and laboratory capacity in Africa. The background map shows the probability of dengue occurrence map described in chapter 3 with overlaying dots showing major reference laboratories for viral disease diagnosis. The blue dots show candidate sites for future dengue investigations that could be integrated with the International Network for the Demographic Evaluation of

Populations and Their Health (INDEPTH) site network. Figure reproduced from Jaenisch *et al.* (2014).

The Indian subcontinent faces very different dengue surveillance challenges. The region is home to over 1.7 billion people who are nearly all at risk of dengue infection. Recording a variety of relevant and useful epidemiological data (age, sex, household location) in addition to providing treatment for an estimated 41 million infections per year presents a vast logistical challenge for the ministry of health (MoH). Here the focus must be on quantifying the scale of the problem and building a relevant evidence base to tackle specific problems. Age-stratified seroprevalence data suggests that the force of infection in urban areas is very high *(Padhi et al., 2014; Sharma et al., 2012)*, yet some parts of India report few, if any, dengue cases and parts of Pakistan and Nepal have variable incidence year-to-year *(Khan et al., 2010; Sharma, 2010)*. These results may suggest that the burden estimates presented in chapter 3 may be an overestimate and that, even in a hyperendemic situation, dengue still has considerable spatio-temporal heterogeneity in infection, even if the environment is perpetually highly suitable for transmission.

The next step to refine these predictions for hyperendemic areas is to take into account the age distribution of infections. Age-stratified IgG seroprevalence studies conducted across a wide geographic area, e.g. national surveys, give estimates of prevalence and the force of infection, which could be used to improve the probability of occurrence metric used in chapter 3. While this data is unlikely to become available at a global scale, a small number of surveys across the Indian sub-continent would considerably improve estimates of incidence in hyperendemic settings in addition to improving estimates of the age-distribution of cases. In November 2014 Sanofi Pasteur released the results of its dengue vaccine phase 3b trial which suggested that the vaccine gave

the highest levels of protection against severe disease if given to those who have already had a natural primary (often asymptomatic) DENV infection *(Capeding et al., 2014)*. Determining the optimal age of vaccination, vaccine coverage and the degree of vector control all require more refined estimates of force of infection and the age distribution of dengue cases, therefore making this a research priority in hyperendemic settings.

With the global distribution of dengue expanding, increasing endemicity in Southern China presents the greatest threat for rising global burden. With a hot and humid summer season and a large number of dense urban cities that are well connected to hyperendemic South East Asia, the potential for widespread transmission is very high. Chapter 6 showed that temperature is not a limiting factor to year-round vector establishment nor to DENV transmission, making the area highly suitable for endemic transmission. Despite this high environmental suitability and clear documentation of dengue emergence in surrounding countries (e.g. northern Vietnam *(Cuong et al., 2011)*), there remains considerable debate as to whether the consistent caseloads in big cities are indicative of endemic transmission or repeated introduction *(Guo et al., 2014; Luo et al., 2013; Luo et al., 2012)*. In these environments, determining seasonal incidence patterns and deviations that might be indicative of increasing endemicity is of paramount importance. The results of chapter 4 would suggest that simple historical mean statistical methods for outbreak detection are of limited use, particularly given the complexity of seasonal transmission in a setting where dengue endemicity is increasing.

Integration of mechanistic transmission models in addition to more detailed data collection would improve predictions of outbreak risk in Southern China. For example, more rigorous recording of case histories is an important first step that will allow differentiation between imported and autochthonous cases and the quantification of

dengue introduction rates and local effective reproductive numbers (R_C) *(Churcher et al., 2014)*. This data could be strengthened by the addition of more frequent sequencing of DENV isolates to confirm lineage comparability and likely source of origin *(Luo et al., 2012)*. Integration of this new data into a transmission model would allow an assessment of the relative importance of internal versus external dynamics for triggering dengue outbreaks in Southern China and thus provide an evidence base for the distribution of surveillance efforts. If imported infections are an important contributor, human movement models will also be important for optimising the deployment of screening resources to the highest-risk importation routes *(Bogoch et al., 2014; Huang et al., 2012; Khan et al., 2014)*. The current dengue situation in China is a microcosm of global efforts to combat dengue; it is a test of whether modern surveillance and control methods applied in a rapidly economically developing nation can demonstrate for the first time that geographic expansion of dengue can be halted and potentially reversed.

7.4. Surveillance of the vectors of DENV

Research from this thesis has shown that many temperate areas are warm enough to support DENV transmission for several months of the year. Despite this, dengue outbreaks are mostly rare, brief and sporadic *(CDC, 2010; La Ruche et al., 2010; ProMED, 2014)*. Understanding what links suitability for transmission to outbreaks of disease will require better defining the role of factors other than temperature in determining suitability for transmission and further investigating the different roles played by different vector species.

Other meteorological factors will also likely play an important role in limiting transmission in certain environments. For example, precipitation affects the availability

of mosquito juvenile habitats and humidity affects adult survival and behaviour, making these two factors particularly likely to be important. While it would be possible to model these by considering their effects on vectorial capacity, as with temperature in chapter 6, this would have to make considerable assumptions about the environment the mosquitoes inhabit (e.g., number of containers and household structure *(Focks et al., 1995)*). Instead, a statistical niche model applied to the occurrence point data from chapter 6 may be more appropriate and, although it would be less directly interpretable and would not give intra-annual variation, it would allow a refinement of the spatial extent suitable for transmission *(Hales et al., 2002; Sinka et al., 2011; Sinka et al., 2010a; Sinka et al., 2010b)*.

Clearly an area can only be suitable for transmission if vector populations are present, so producing maps of the global distribution of *Ae. aegypti* and *Ae. albopictus* is also a priority. Producing an evidence consensus map of each species would approximately delineate areas of known current risk and identify surveillance gaps. Comparison between this evidence consensus map and measures of suitability for mosquito population persistence (such as those presented in chapter 6 and additional niche modelling outputs *(Kraemer et al., 2015)*) may identify areas at risk of future mosquito introduction. Combining such predictions with data on international trade and transport networks could enable prediction of which destinations and routes are at highest risk of introduction and preventative measures prioritised accordingly *(Tatem et al., 2006)*. In particular, the task of mapping the current and future distribution of *Ae. aegypti* in temperate environments has become a higher priority following events in Madeira which saw the arrival of the mosquito species in 2005 followed by the largest outbreak in any European nation in 2012 with over 1000 cases *(ECDC, 2014)*.

Vector and disease surveillance in temperate regions could also be improved by refining the temporal predictions of suitability. Comparing the annual profiles of

temperature suitability from chapter 6 with dengue case data from areas in different endemicity settings would allow an analysis of the relative influence of meteorological factors and human immunological factors in defining the timing of the seasonal cycles of dengue. This kind of analysis would be increasingly important if further major outbreaks similar to that in Madeira were to occur in temperate regions, as the exposure histories of populations to the four different DENV serotypes would largely determine levels of risk for severe disease *(Cuong et al., 2011)*.

While further delineating spatio-temporal transmission and disease risk patterns is important, understanding risk in temperate environments will not be complete without a full understanding of whether *Ae. albopictus* can support significant dengue outbreaks, and if it can, where and when it can support them. Answering these questions requires further investigation of the ecological and behavioural differences between *Ae. aegypti* and *Ae. albopictus* mosquitoes.

Human biting rate, a product of mosquito biting rate and human biting propensity, is currently poorly enumerated for *Ae. aegypti* and *Ae. albopictus*. Better measurements of this rate will improve our understanding of why *Ae. aegypti* is the more effective vector, or indeed if certain populations of some vectors are more or less suitable for supporting transmission *(Harrington et al., 2001)*. While human landing catches still remain problematic, several technological advances mean it is possible to improve estimates of human biting rate for both species and how they may differ between sites or times of the year. New approaches to mosquito trap design that either prevent biting contact or use alternative attractants could be adapted for more frequent *Aedes spp.* biting rate measurements given appropriate validation *(Bond et al., 2013; Silver, 2007)*. New PCR-based approaches also offer opportunities to investigate mosquito blood-feeding on an individual level by comparing DNA samples in blood meals at the species, and sometimes even individual level to determine which individuals are being

bitten *(De Benedicts et al., 2003; Harrington et al., 2014)*. Comparatively little is known about how much human biting rate varies between mosquito populations, environments and times of the year. Understanding why some populations of *Ae. albopictus* are more anthrophillic than others in particular seasons and how this relates to outbreaks of dengue (and other arboviruses), will also be important *(Gould et al., 2010; Panning et al., 2008)*. Routine larval household surveys also have a role to play in determining the degree of overlap between human and mosquito populations *(Ministerio da Saude Brasil, 2012)*. This data could be better used to characterise micro-niches of each species and how they relate to human biting preference and the effective density of both human and mosquito populations given the movement of hosts and vectors between different habitats *(Guerra et al., 2014; Smith et al., 2014)*.

In addition to targeted surveillance to improve model parameter estimates there is also a need to use surveillance data to test certain assumptions about regularly used models of DENV transmission. Much of what is known about the contribution of the mosquito to pathogen transmission is based on the formula for vectorial capacity originally proposed by George Macdonald in 1952 *(Macdonald, 1952)*. This model has since been developed in many different ways, but these advancements retain many of the assumptions of vectorial capacity as it was initially proposed *(Reiner et al., 2013)*. In chapter 6 the theory of vectorial capacity was expanded to address one of these core assumptions: that mosquito populations and transmission exist in a state of equilibrium. The models from chapter 6 can now be used to investigate changes in vectorial capacity in dynamic environments which is important for understanding seasonality, response to control and increasing hyperendemicity. Another important assumption to test is that of homogeneous biting. Work on *Anopheles spp.* has repeatedly shown that some people are bitten more frequently than others – in general 20% of individuals receive 80% of the bites *(Dye and Hasibeder, 1986)*. This creates

important non-linear properties for the establishment and saturation of transmission dynamics and needs to be investigated in the context of DENV transmission considering its unique properties afforded by four serotypes with complicated human immune responses and vectors with varying degrees of anthropophily.

Understanding the contribution of human movement and how it affects serotype spread is also important in challenging the assumption of heterogeneous mixing of vectors and hosts *(Nunes et al., 2014)*. The study of human movement in disease-endemic regions is a rapidly expanding area and several novel investigations have elucidated the importance of different types of human movement for pathogen transmission at different spatial scales *(Liebman et al., 2012; Stoddard et al., 2009; Wesolowski et al., 2012)*. Movement of adult mosquitoes, which is limited in the case of *Ae. aegypti*, may also be important for DENV transmission dynamics which may exhibit more spatial structuring due to the lack of mosquito movement *(Guerra et al., 2014)*.

The final, and perhaps most difficult to test, assumption is that of uniformity across spatial scales. Each of the assumptions above may be more or less realistic at different spatial scales, for example the assumption of homogeneous mosquito movement is unlikely to influence national level predictions, but may be important for making predictions at a village level. Determining the pertinent constituent processes of transmission at different spatial scales is a major challenge that modelling theory of a number of vector borne pathogens must rise to meet in the coming decade if predictions are to take on a wider relevance in policy making.

7.5. Conclusions

The maps and models presented here are by no means final and perhaps their biggest strength is in their ability to identify gaps both in the evidence base used and in our understanding of DENV transmission. The maps do, however, represent the best currently available estimates of many metrics of disease that were previously outdated or absent all together. These global assessments of the distribution and burden of dengue have outlined the contemporary public health challenge the disease presents and advocated for renewed surveillance efforts to understand suspected underreporting in Africa and the Indian subcontinent. Quantifying the limiting effects of temperature on DENV transmission has also posed new questions about how mosquito population-level effects interact to determine outbreak susceptibility in temperate environments. Answering these questions and addressing these surveillance gaps will require substantial and sustained interdisciplinary efforts. These will iteratively improve model estimates and data collection strategies that will provide the strongest possible evidence base for dengue control policy.

The beginning of this thesis introduced dengue as a disease that has undergone a formidable expansion over the past century, far outpacing our efforts to control or even monitor it. The results of the research presented here help address some of the key knowledge gaps in estimating important global measures of the disease. These serve as a contemporary evidence base with which to advocate for dengue as a global public health priority, and to improve specific surveillance and control efforts. It is hoped that this thesis has demonstrated the value of combining maps and models for evaluating and improving contemporary disease surveillance. Moving forward, these maps can be used to guide future efforts to iteratively improve the epidemiological and entomological evidence base that is so sorely required at a time when novel interventions offer new prospects for dengue control.

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APPENDIX

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Appendix to chapter 2 - *Refining the known global distribution of dengue*

Protocol S1. Assembly of the supplementary dengue database

S1.1 Overview

The dengue database comprises occurrence data linked to point or polygon locations, derived from peer-reviewed literature. Data sources are described in full here. To collate the peer-reviewed database, literature searches were undertaken using the major search engines and the resulting articles were manually reviewed. Geo-spatial information for 7,419 occurrences of dengue was extracted using site descriptions within 2,838 of the articles and recorded as specific coordinates or by a polygon definition when this was the only information available. All data entries from both data sources were manually checked by the authors and then underwent a series of routine quality control procedures to ensure correct geo-positioning.

S1.2 Peer-reviewed Literature Search

PubMed (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi) 1920 to 2009 was searched using term "dengue". The MESH term technology used in the PubMed citation archive ensured all pseudonyms were automatically included (http://www.nlm.nih.gov/mesh/2008/MBrowser.html) in the searches. The same process was repeated for ISI Web of Science (http://wok.mimas.ac.uk) and ProMED (http://www.promedmail.org). The searches were last updated on 8th February 2012. No language restrictions were placed on these searches, however, only those citations with a full title and abstract were downloaded into Endnote version 10 (Thomson ResearchSoft, USA, http://www.endnote.com). A total of 5,876 references were retrieved and 2,883 unique articles were identified as potentially containing useable location data. The full articles were obtained for 2,838 or 98.4% and the references are listed in S1.6.

In-house language skills allowed processing of all English, French, Portuguese and Spanish articles. Russian articles with data were translated and included in the

database. We were unable to extract information from a small number of Turkish, Polish, Hebrew, Italian, German and Chinese articles.

S1.3 Disease data and imported cases

Confirmed occurrences of dengue virus transmission, dengue haemorrhagic fever or a specific dengue serotype found within the peer reviewed literature were recorded as a dengue occurrence datapoint. A report of multiple cases (i.e. an outbreak) was reported as a single occurrence datapoint, however, later outbreaks at the same location were recorded as new occurrence datapoints. Reports of autochthonous (locally transmitted) cases or outbreaks were entered as an occurrence within the country. If imported cases were reported with information on the site of contagion, they were recorded as an occurrence from the country of contagion within the database. If imported cases were reported with no information about the site of contagion they were not entered into the database. If an imported case led to an outbreak/local transmission within the recipient country and location information was available for the site of initial contagion and the site of the outbreak, this was recorded as two occurrences; one in the country of contagion and one in the country where the outbreak occurred.

Data from both sources was entered into a bespoke PostgreSQL database that links disease data to spatial data. A link to the PDF library was also included in the database.

S1.4 Geo-positioning occurrence points

All available location information in the peer-reviewed literature was extracted from each article. The site name was used together with all contextual information provided about the study site position, for example the approximate distance from the nearest city or the district within which the site was located. The site name and contextual information were used together to locate the study site using Microsoft Encarta (Microsoft Corporation, Redmond, WA) and Google Earth 5.1. Place names are often duplicated within a country so the contextual information was used to ensure the right site was selected. Where the site name was not found, the contextual information was used to scan sites in the approximate area to check for names that had been transliterated in Microsoft Encarta/Google Earth in a different way to the

published article (e.g. Imichli and Imishly). If the site was not located using Microsoft Encarta or Google Earth, a range of online georeferencing resources were tried including Getty Thesaurus (http://www.getty.edu/research/conducting_research/vocabularies/tgn/index.html), Alexandria (http://middleware.alexandria.ucsb.edu/client/gaz/adl/index.jsp) and Falling Rain (http://www.fallingrain.com/world/). If the study site could be georeferenced to a specific place, it was recorded as a point location. In total, 3,286 point locations from the peer-reviewed literature were included in the database at this stage. If the study site could only be identified at an administrative area level (e.g. province or district, etc), it was recorded as a polygon and the centroid (mean centre) was derived to give a latitude and longitude for the site. A total of 5,234 polygon locations from the peer-reviewed literature were recorded in the database. All point locations and polygon centroids were converted to decimal degrees before entering the quality control process described below.

S1.5 Automatic Validation and Quality control

All datapoints were crosschecked and validated using a standardised validation procedure. First a multi-polygon shapefile defining land/water boundaries was created as a template to validate geopositioned points (see Protocol S2: Environmental Covariates). This land/water shape file was rasterised to spatially overlapping raster pixels (the polygon boundaries must cover the centre of the raster pixel) at a 5 x 5 km resolution and was used to ensure all disease occurrence points were positioned on a valid land pixel.

Any points that met the following criteria were excluded from the database:

1. Points found further than 10km from a land boundary. Points located less than 10km from a land boundary were repositioned to the nearest land raster cell.
2. Points representing the centroid of an administrative division having an area greater than 111km^2 (1 degree at the equator).

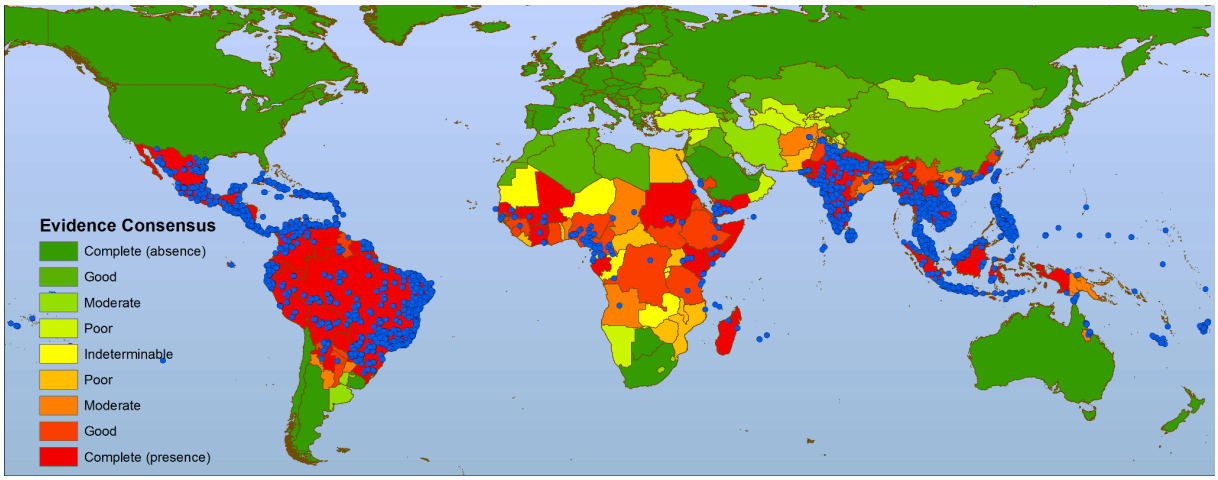
Once the quality control procedure was complete, the final database contained 7,419 occurrence datapoints (including 3,237 point locations and 4,182 small polygon

centroids) covering a period from 1956 to 2012. Maps displaying the 7,419 locations are provided in Figures S1-5, and the number of occurrence locations per sampling period are shown in Figure S6.

S1.6 Bibliography of final endnote library

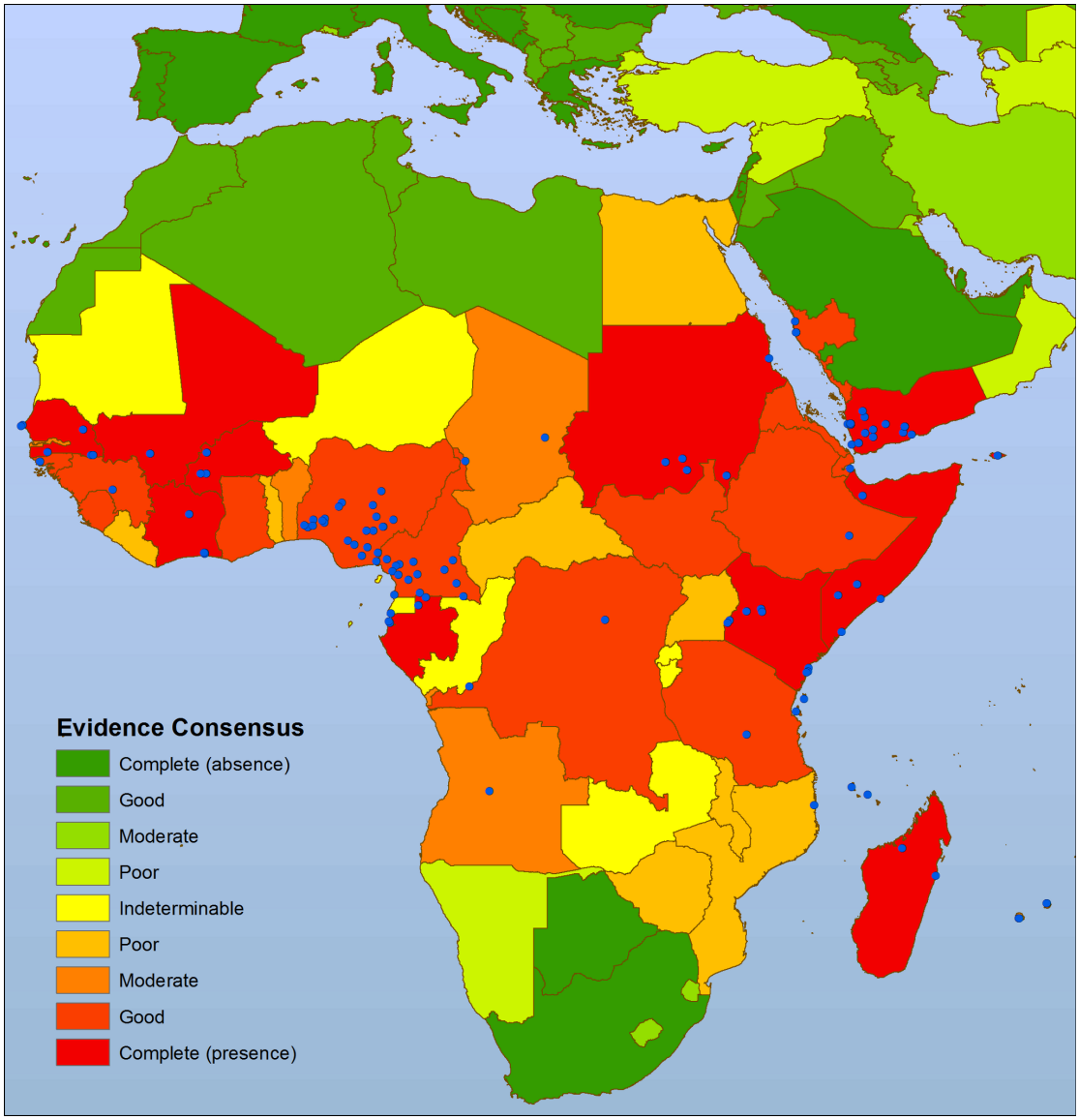
N.B. this section has been omitted due to excessive length, but is available for download here:
http://journals.plos.org/plosntds/article/asset?unique&id=info:doi/10.1371/journal.pntd.0001760.s008

Figure S1. Geographic locations of occurrence data globally



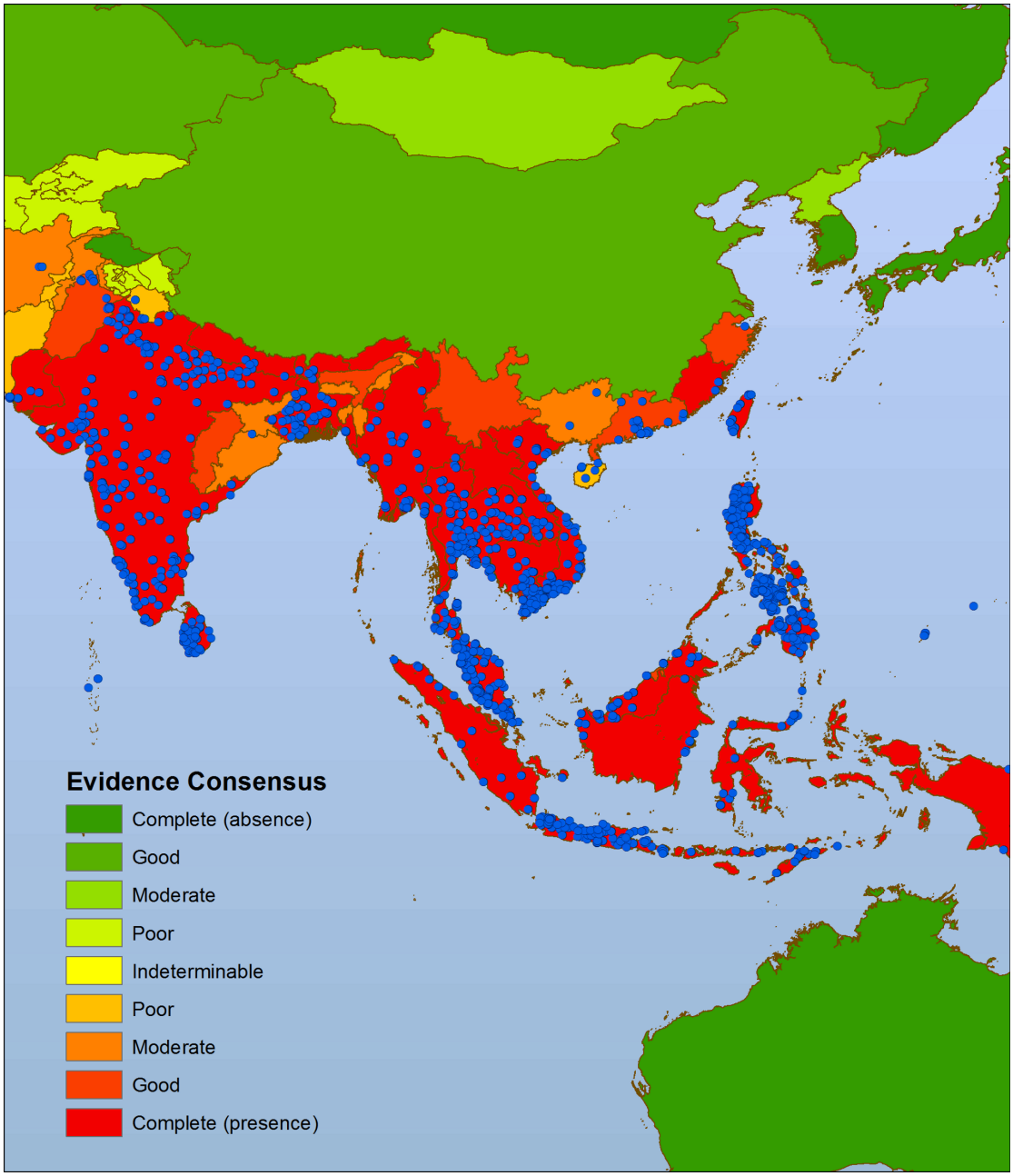
Country colouring is based on evidence based consensus (see main manuscript) with green representing a complete consensus on dengue absence and red a complete consensus on dengue presence.

Figure S2. Geographic locations of occurrence data in Africa+



Country colouring is based on evidence based consensus (see main manuscript) with green representing a complete consensus on dengue absence and red a complete consensus on dengue presence.

Figure S3. Geographic locations of occurrence data in Asia

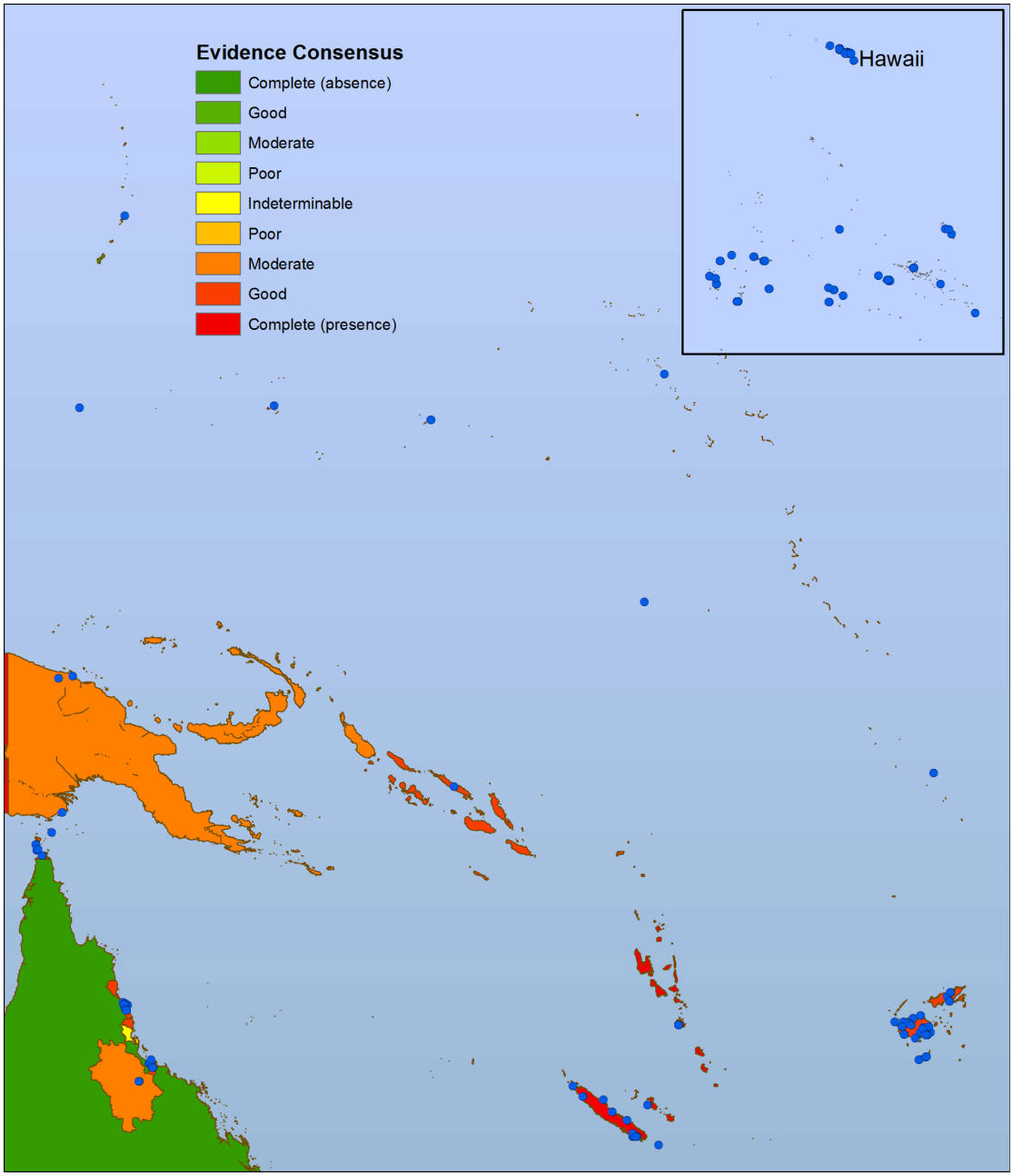


Country colouring is based on evidence based consensus (see main manuscript) with green representing a complete consensus on dengue absence and red a complete consensus on dengue presence.

Figure S4. Geographic locations of occurrence data in the Americas

Country colouring is based on evidence based consensus (see main manuscript) with green representing a complete consensus on dengue absence and red a complete consensus on dengue presence.

Figure S5. Geographic locations of occurrence data in Australia



Country colouring is based on evidence based consensus (see main manuscript) with green representing a complete consensus on dengue absence and red a complete consensus on dengue presence.

Figure S6. Number of occurrence samples per year globally (a) and for Africa+ (b), Asia (c), Australasia (d) and the Americas (e)

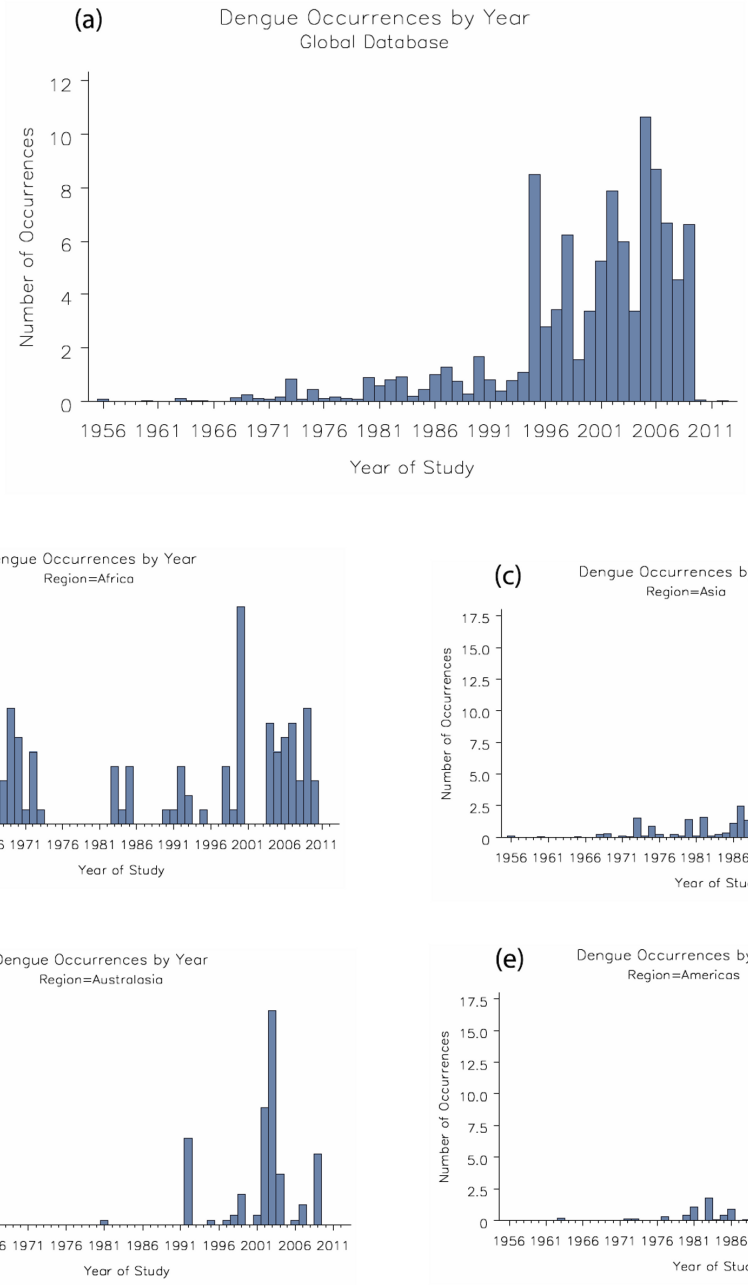


Figure S7. Map of evidence types used for each national and subnational area.

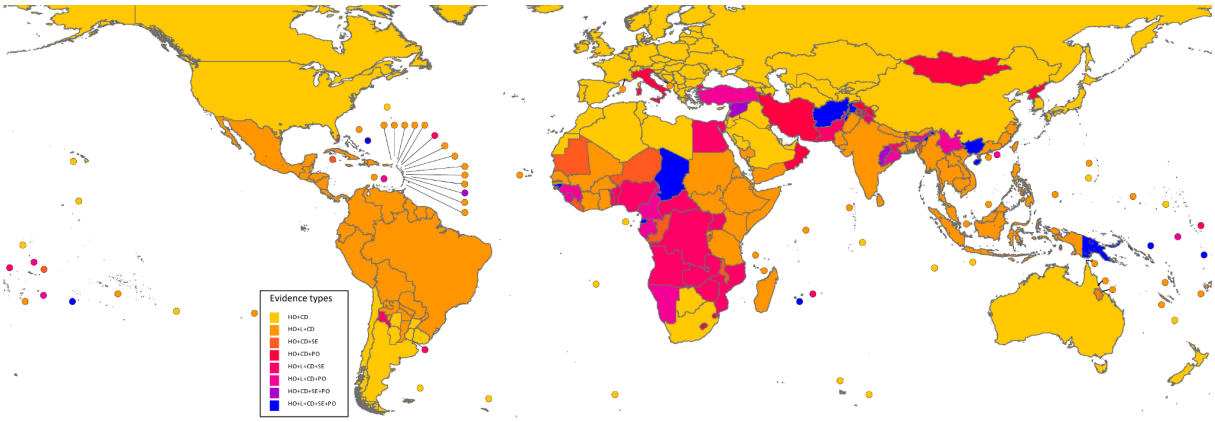


Figure S7 shows the different evidence categories used in assessing evidence consensus for each country and Admin1/2 area. HO = health organisation status, L = literary evidence, CD = case data, SE = supplementary evidence, PO = professional opinion.

Table S1. The collection of evidence used to assess evidence consensus for each country and Admin1 and Admin2 areas

Due to space constrains this table is not included in this appendix, but is available here:

http://journals.plos.org/plosntds/article/asset?unique&id=info:doi/10.1371/journal.pntd.0001760.s009

Appendix to chapter 3 – *Estimating the global burden of dengue*

3.1. Bhatt *et al.,* The global distribution of dengue, main manuscript

3.2. Bhatt *et al.,* The global distribution of dengue, supplementary information

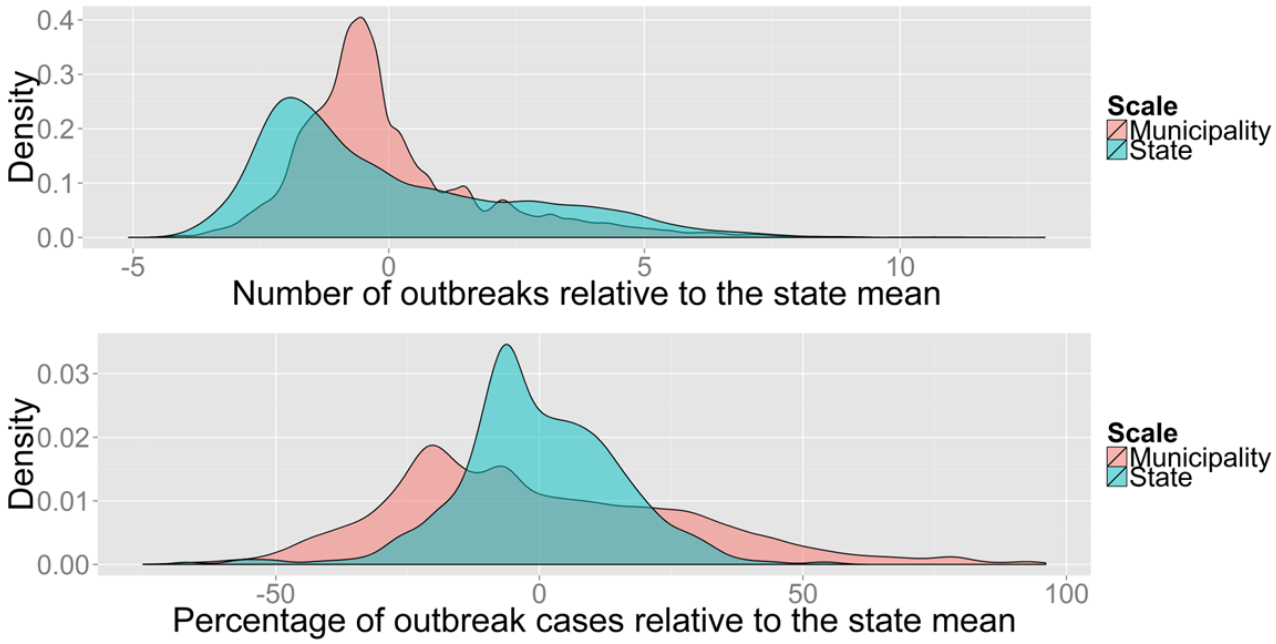
Due to space constrains this supplementary information is not included in this appendix, but is available here:

http://www.nature.com/nature/journal/v496/n7446/extref/nature12060-s1.pdf

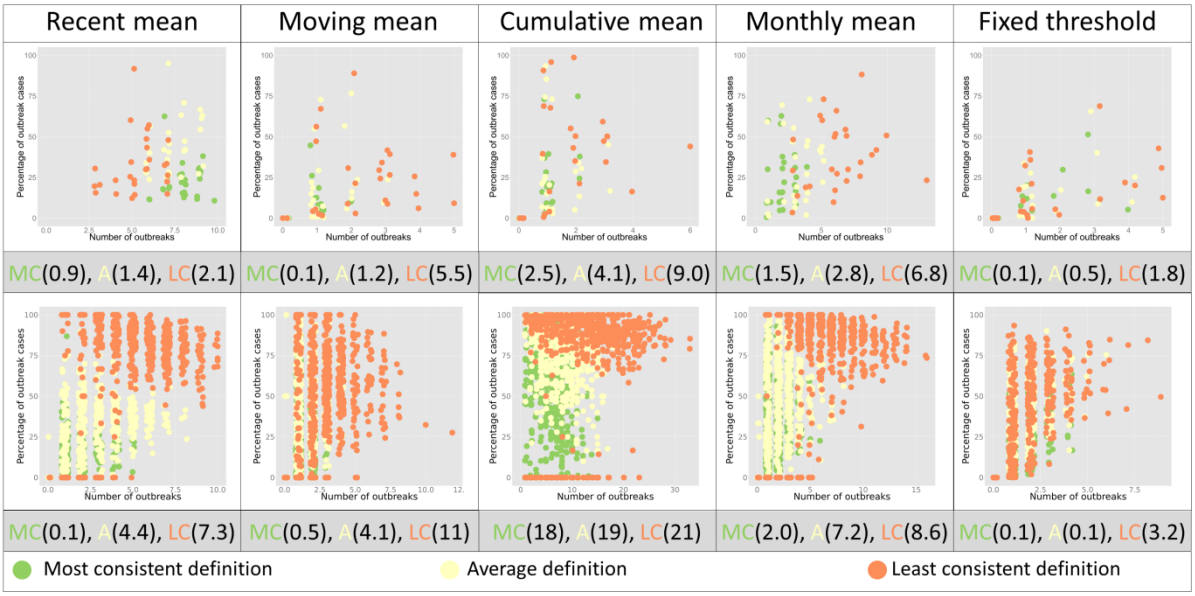
Supplementary Information parts D and E make up chapter 3.

Appendix to chapter 4 – *Assessing the applicability of dengue outbreak response guidelines*

4.1. Supplementary figures



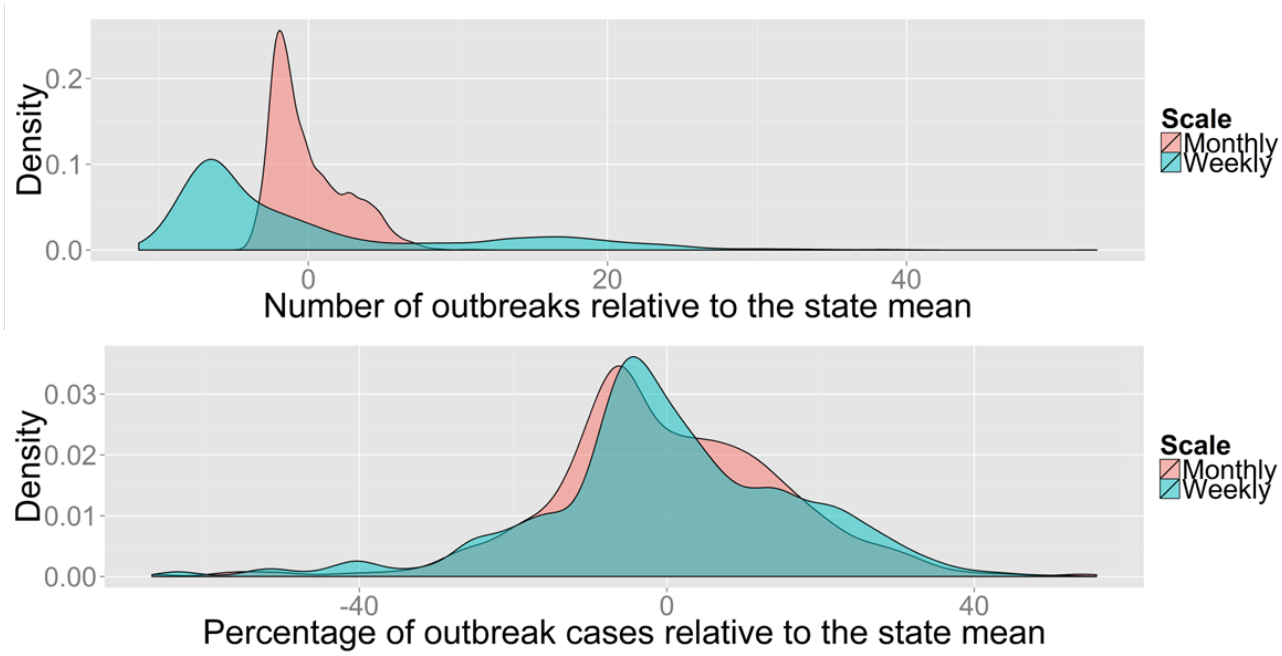
Supplementary figure S1. Variability between state and municipality level outbreak characteristics among all outbreak definitions. This figure summarises the variation in the number of outbreaks identified (upper panel) and the percentage of cases classified as an outbreak (lower panel) across all outbreak definition variations when applied to the 602 municipalities of São Paulo (red) or the 27 states of Brazil (blue). This figure is analogous to figure 2d in the main manuscript.



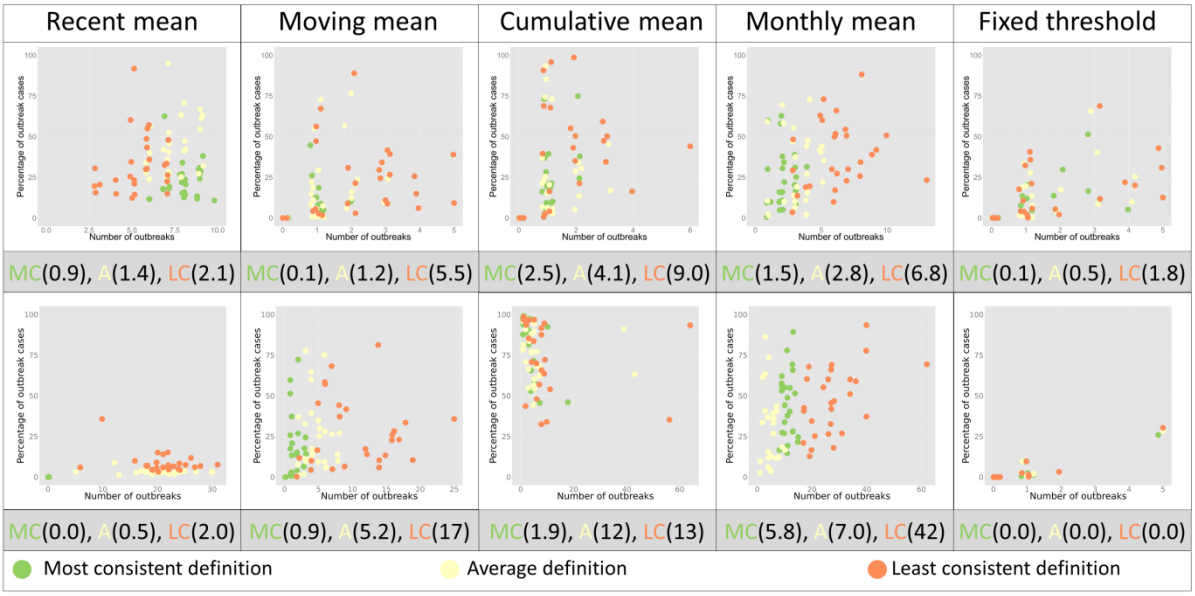
Supplementary figure S2. Variability between state and municipality level outbreak characteristics when just one outbreak definition is applied. This figure shows the variation in outbreak characteristics when just a single definition is applied to the 27 states (upper panel) of Brazil or the 602 municipalities (lower panel) of São Paulo. The most consistent definition (equally weighted between the number of outbreaks and percentage of outbreak cases identified) across the different geographic regions is shown in green, while the average definition is shown in beige and the least consistent in orange. These are divided by different endemic channel types. This figure is analogous to figure 3 in the main manuscript.

Endemic channel	Parameterisation	Years of baseline data	Number of consecutive observations above threshold	Outbreak years included	Standard deviations above mean	Relative consistency (D)
Recent mean	Most consistent	5	3	No	2	0.1
	Average	5	2	Yes	1	4.4
	Least consistent	All	1	Yes	1	7.3
Moving mean	Most consistent	5	3	Yes	2	0.5
	Average	All	2	No	2	4.1
	Least consistent	All	1	Yes	1	11
Cumulative mean	Most consistent	5	3	Yes	2	18
	Average	5	2	No	2	19
	Least consistent	5	1	No	1	21
Monthly mean	Most consistent	5	3	Yes	2	2.0
	Average	All	2	Yes	2	7.2
	Least consistent	All	1	No	1	8.6
Fixed threshold	Most consistent	-	3	-	0.01 fixed	0.1
	Average	-	2	-	0.01 fixed	0.1
	Least consistent	-	1	-	0.01 fixed	3.2

Supplementary table S1. Parameter details of the most and least consistent definitions shown in Supplementary figure S2. Parameters are explained in the methods section of the main manuscript. Fixed threshold methods use fixed values of incidence (100 or 300 cases per 10,000) instead of standard deviations above the mean.



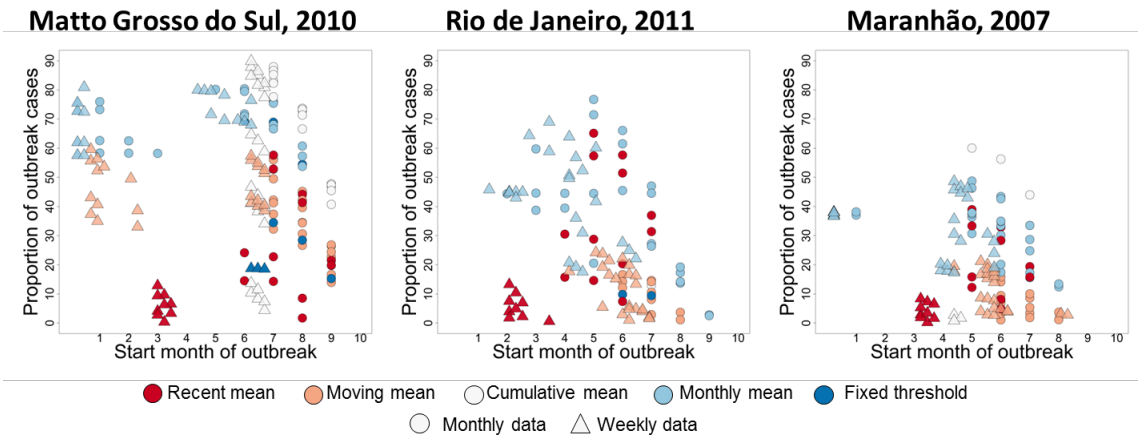
Supplementary figure S3. Variability between outbreak characteristics among all outbreak definitions when applied to monthly or weekly case data. This figure summarises the variation in the number of outbreaks identified (upper panel) and the percentage of cases classified as an outbreak (lower panel) across all outbreak definition variations when applied to the monthly dengue case data (red) or simulated weekly case data (blue). This figure is analogous to figure 2d in the main manuscript.



Supplementary figure S4. Variability between outbreak characteristics when just one outbreak definition is applied to monthly or weekly case data. This figure shows the variation in outbreak characteristics when just a single definition is applied to monthly dengue case data (upper panel) or simulated weekly case data (lower panel). The most consistent definition (in terms of both number of outbreaks and percentage of outbreak cases identified) across the different geographic regions is shown in green, while the average definition is shown in beige and the least consistent in orange. These are divided by different endemic channel types. This figure is analogous to figure 3 in the main manuscript.

Endemic channel	Parameterisation	Years of baseline data	Number of consecutive observations above threshold	Outbreak years included	Standard deviations above mean	Relative consistency (D)
Recent mean	Most consistent	5	3	No	2	0.0
	Average	All	3	Yes	2	0.5
	Least consistent	All	3	Yes	1	2.0
Moving mean	Most consistent	All	3	No	2	0.9
	Average	5	3	Yes	1	5.2
	Least consistent	All	1	Yes	1	17
Cumulative mean	Most consistent	5	3	No	1	1.9
	Average	5	1	Yes	1	12
	Least consistent	5	1	No	2	13
Monthly mean	Most consistent	5	2	No	1	5.8
	Average	5	3	No	2	7.0
	Least consistent	All	1	No	1	42
Fixed threshold	Most consistent	-	3	-	0.01 fixed	0.0
	Average	-	2	-	0.01 fixed	0.0
	Least consistent	-	1	-	0.01 fixed	0.0

Supplementary table S2. Parameter details of the most and least consistent definitions shown in Supplementary figure S4. Parameters are explained in the methods section of the main manuscript. Fixed threshold methods use fixed values of incidence (100 or 300 cases per 10,000) instead of standard deviations above the mean.



Supplementary figure S5. The difference in time of onset and overall outbreak size for three example outbreaks when all definitions are applied to weekly or monthly case data. The results of definitions applied to monthly dengue case data are shown by circles, while those based on simulated weekly case data are shown as triangles. Different colour coding shows the division of these results by the different endemic channels.

4.2. Supplementary Results

4.2.1. Comparison of outbreak characteristics when standard outbreak definitions are applied to municipality instead of state level dengue case data

The variation in outbreak characteristics is comparable whether standard outbreak definitions are applied to the 27 states of Brazil, or the 602 municipalities of São Paulo. The number of outbreaks identified in the two datasets is highly comparable with a slightly higher consistency when the definitions are applied to the municipality dataset (figure S1, upper panel). Despite this slightly higher consistency on the number of outbreaks, the proportion of cases that are identified as outbreak cases is considerably more variable when definitions are applied to the municipality dataset rather than the state-level dataset (figure S1, lower panel). Considering both measures, definitions applied at state level show greater consistency ($D = 11.8$) than definitions applied at the municipality level ($D = 20.4$).

Additionally, when just a single definition is applied across all states or municipalities the variation in the outbreak characteristics identified is greater amongst the municipality dataset than the state-level dataset (figure S2, $\widehat{D} = 7.11$ and $\widehat{D} = 2.69$ respectively). The difference between the most consistent, average and least consistent outbreak definition is also smaller when applied to states (figure S2, upper panel) as opposed to municipalities (figure S2, lower panel).

Both of these results could be exacerbated by the fact that there are a greater number of areas under examination in the municipality-level dataset (27 vs 602). This is, however, one practical component that needs to be considered when moving to a higher spatial scale, thus emphasising the increased problem of standardisation and comparability inherent in evaluating dengue outbreaks at this level.

4.2.2. Comparison of outbreak characteristics when standard outbreak definitions are applied to weekly instead of monthly dengue case data

When the whole range of standard definitions are applied to simulated weekly data, instead of reported monthly data, a similar proportion of cases are identified as outbreak cases (figure S3, lower panel), but the number of outbreaks differs (figure S3, upper panel). On average, outbreak definitions applied to weekly case data identify fewer outbreaks, however there are a smaller proportion of definitions that identify a very large number of outbreaks (figure S3, upper panel). This ensures that overall variability in outbreak number is higher when outbreak definitions are applied to weekly rather than monthly data ($\widehat{D} = 48.0$ and $\widehat{D} = 11.8$ respectively).

When just a single definition is applied to the weekly or monthly datasets from 27 different states, very similar levels of variation are seen (figure S4). Where a greater degree of consistency among a particular definition exists, it is usually found when definitions are applied to weekly data, but only identify a very small proportion of cases

as an outbreak and are thus of questionable use as operationally relevant outbreak definitions (figure S4, lower panel, far right).

Finally, using weekly data does appear to lead to earlier detection of outbreak onset compared to using monthly data for the three outbreaks tested (figure S5). This difference is more pronounced in some outbreaks (Matto Grosso do Sul, Rio de Janeiro) than others (Marãnhao). This difference is also more pronounced for some endemic channel definitions, for example the recent mean method is improved in consistency and timing of response by using weekly rather than monthly data despite having a more limited use as an outbreak definition as it only detects a much smaller proportion of outbreak cases (figure S5). Despite these improvements in detection time and some specific examples of improved consistency, if all definitions are considered, using weekly as opposed to monthly datasets offers no improvement in outbreak definition consistency in terms of both proportion of outbreak cases or the month of outbreak onset.

4.3. Supplementary Methods

4.3.1. Assembly of municipality level data for the state of São Paulo

While the main focus of our analysis was to test the utility of standard outbreak definitions at wide-scale, state-level spatial resolutions, it is also likely that many dengue control programme coordinators at more local levels may also be using these standard outbreak definitions for decision making. Here we test the hypothesis that the amount of heterogeneity in outbreak characteristics identified by these standard definitions will only increase when applied to geographic units smaller than state-level. This hypothesis is based on the assumption that in areas with a lower population number, and therefore a lower probability of observing at least one case, the seasonal trend that the standard outbreak definitions attempt to capture will be more difficult to separate from stochastic variations in case infection and reporting and thus the characteristics of the outbreaks identified will be more inconsistent.

The state of São Paulo (B9) was chosen for the comparison between state-level and municipality level datasets due to its range of endemicity settings and its high population density [1], which increases the chance of seasonal patterns being detectable over background stochastic cases. São Paulo is, therefore, likely to be one of the more suitable candidates for outbreak evaluation at the municipality-level. Monthly total dengue cases were extracted from January 2001 to December 2013 from Brazilian Ministry of Health surveillance system SINAN [2,3]. A total of 602 municipalities reported at least one case of dengue over the time period. Missing data values were assigned a zero value. All definitions were applied to the new municipality-level data as previously stated in the Methods section of the main manuscript.

4.3.2. Simulation of comparable weekly data from monthly datasets

In a similar manner as above, the focus of our analysis was on wide-scale, state-level analyses where delays in data processing and assimilation may mean that dengue case data are unavailable at a higher temporal resolution than monthly records for evaluation in real-time. Under some circumstances, however, highly efficient electronic surveillance systems may have access to weekly data in real-time for decision making. This higher-resolution data may make elucidating seasonal trends more accurate, and in particular improve consistency among definitions about the start date of the onset of an outbreak. We hypothesise that, as above, the increased resolution will increase heterogeneity meaning that there is little or no improvement in consistency among standard outbreak definitions when using higher temporal resolution data.

Weekly reported dengue case data over comparable time periods and geographic areas is not publicly available, although it is available for selected weeks in 2013-2014 [4]. To characterise the variability in weekly case counts that make up the aggregated monthly values we evaluated the deviation of each weekly observation from the mean weekly value across a moving 4-week window period in the 2013-2014 weekly

datasets. These deviations were then summarised by a normal distribution with mean 7.9×10^{-3} and standard deviation 4.5×10^{-2}. This estimation method was then validated using bootstrap procedure where 70% of the data were used to predict against the holdout 30% of the data. The model gave good predictive performance with an $R^2$of the logged predictions of 0.88.

Monthly data from the main dataset was then converted to weekly values by sampling three values from this distribution scaled by the expected mean monthly values. A fourth weekly value was then computed based on the monthly value minus the sum of the previous three simulated values. Every fourth month contained five weeks ensuring an annual total of 52 weeks of observation. Standard outbreak definitions were applied to this weekly data using the same approach as with the monthly data, but with the week being the focus unit of analysis instead of the month.

4.3.3. Supplementary Methods Bibliography

1 Instituto Brasileiro de Geografia e Estatística. *IBGE: 2010 Population census results*, <http://www.ibge.gov.br/english/estatistica/populacao/censo2010/default.shtm> (

2 Ministério da Saúde. *Dengue: Notificações registradas no Sistema de Informação de Agravos de Notificação – SINAN, Brazil, 2001-2006*, <http://dtr2004.saude.gov.br/sinanweb/tabnet/dh?sinan/dengue/bases/denguebr.def> (

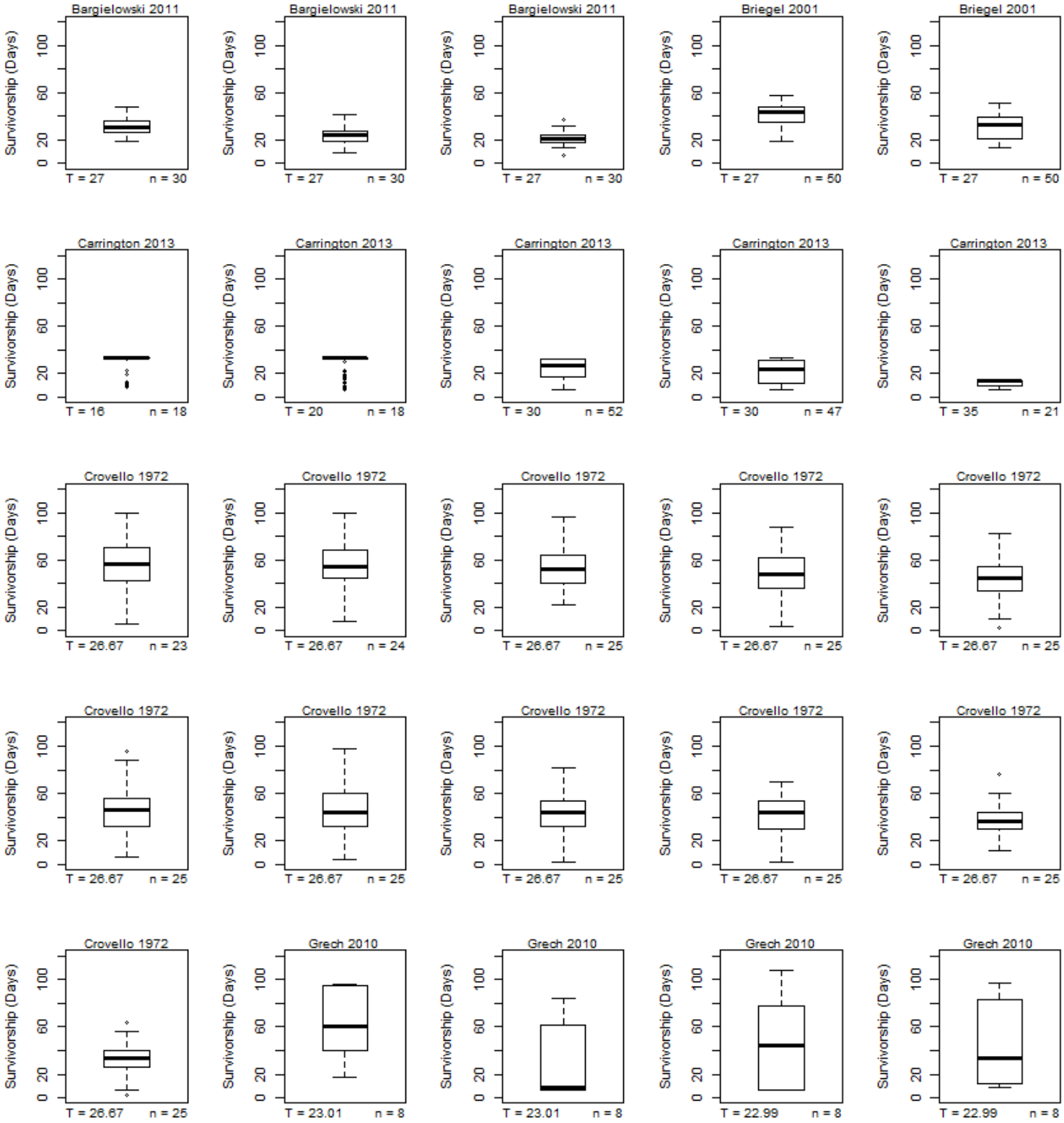
3 Ministério da Saúde. *Dengue: Notificações registradas no Sistema de Informação de Agravos de Notificação – SINAN, Brazil, 2007-2012*, <http://dtr2004.saude.gov.br/sinanweb/tabnet/dh?sinannet/dengue/bases/denguebrnet.def> (

4 Brasil Ministerio da Saude. *Boletim Epidemiológico - Volume 45*, <http://portalsaude.saude.gov.br/index.php/situacao-epidemiologica-dados-dengue> (2014).

Appendix to chapter 5 – *Modelling adult survival of the mosquito vectors of dengue*

Additional file 1: data bibliography and summary statistics

Figure SI1. Boxplots of the *Ae. aegypti* survival under controlled conditions in the laboratory for each experiment used in this study. T = the temperature at which the experiment took place, n = the total number of mosquitoes under observation.


Bargielowski 2011
Survivorship (Days)
0 20 60 100
T = 27 n = 30
Bargielowski 2011
Survivorship (Days)
0 20 60 100
T = 27 n = 30
Bargielowski 2011
Survivorship (Days)
0 20 60 100
T = 27 n = 30
Briegel 2001
Survivorship (Days)
0 20 60 100
T = 27 n = 50
Briegel 2001
Survivorship (Days)
0 20 60 100
T = 27 n = 50
Carrington 2013
Survivorship (Days)
0 20 60 100
T = 16 n = 18
Carrington 2013
Survivorship (Days)
0 20 60 100
T = 20 n = 18
Carrington 2013
Survivorship (Days)
0 20 60 100
T = 30 n = 52
Carrington 2013
Survivorship (Days)
0 20 60 100
T = 30 n = 47
Carrington 2013
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Survivorship (Days)
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Crovello 1972
Survivorship (Days)
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Crovello 1972
Survivorship (Days)
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Crovello 1972
Survivorship (Days)
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Grech 2010
Survivorship (Days)
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Grech 2010
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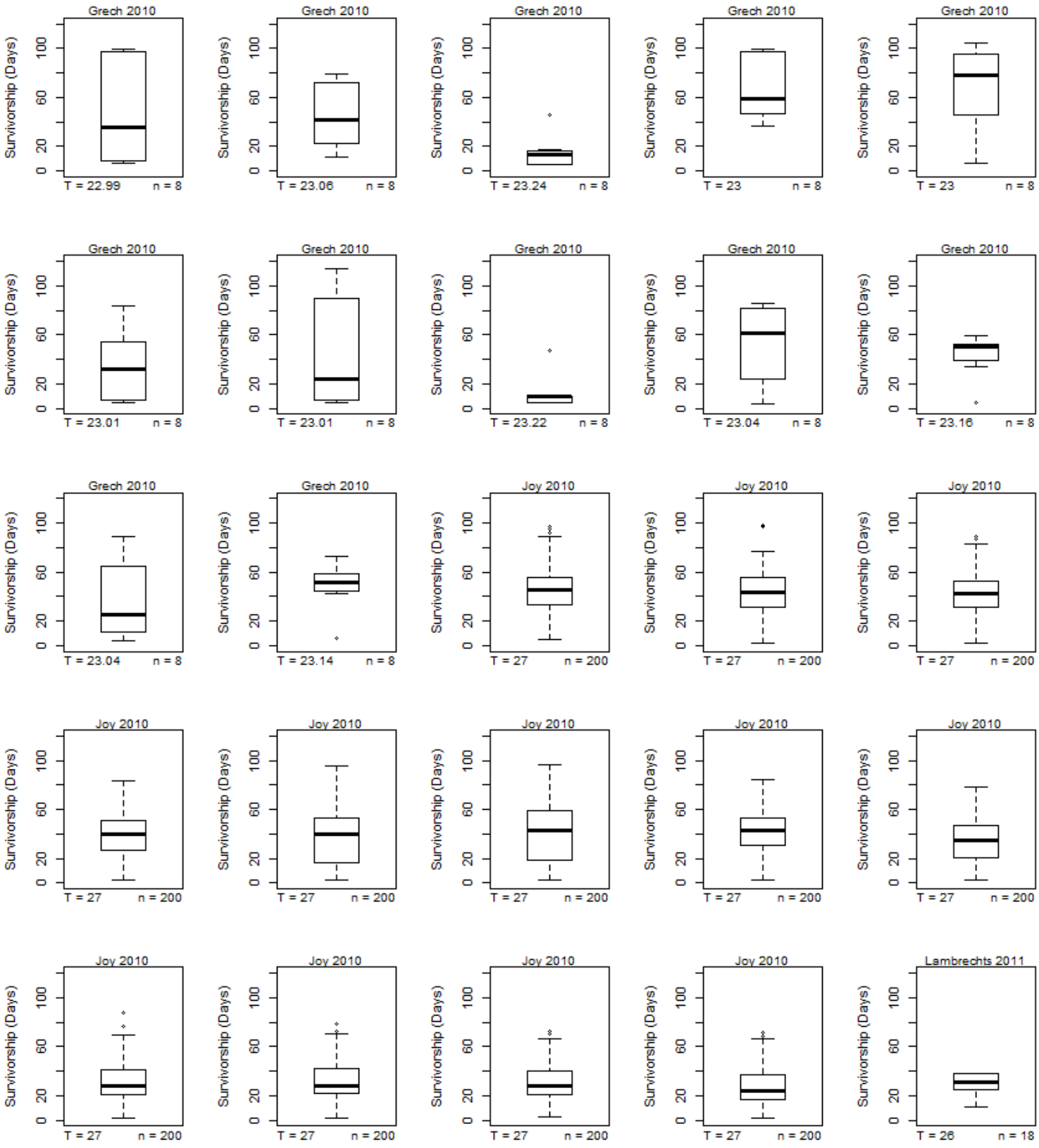
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Joy 2010
Survivorship (Days)
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Joy 2010
Survivorship (Days)
0 20 60 100
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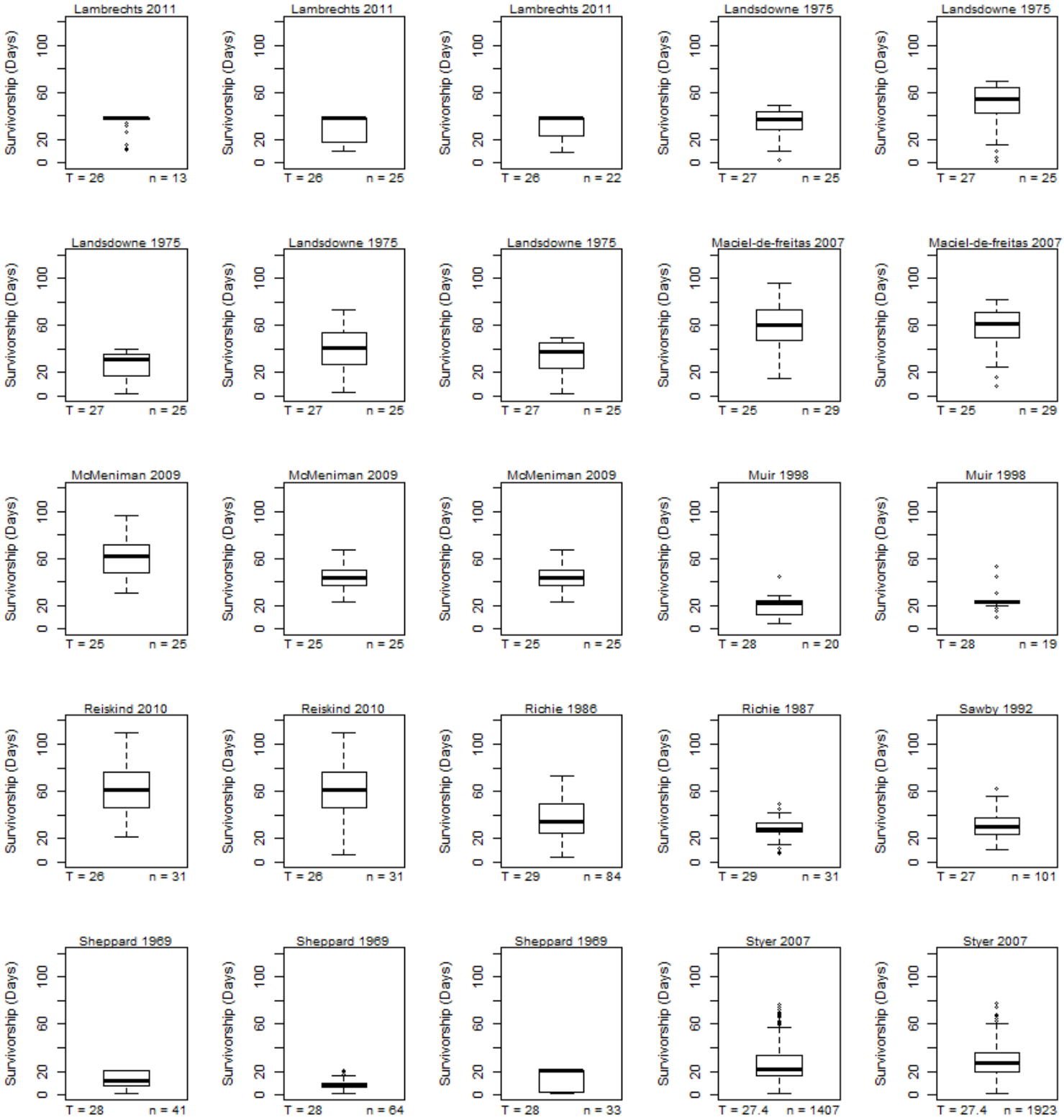
T = 26 n = 18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T = 26 n = 22
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Survivorship (Days)
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Landsdowne 1975
Survivorship (Days)
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Landsdowne 1975
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Muir 1998
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Sheppard 1969
Survivorship (Days)
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Sheppard 1969
Survivorship (Days)
0 20 60 100
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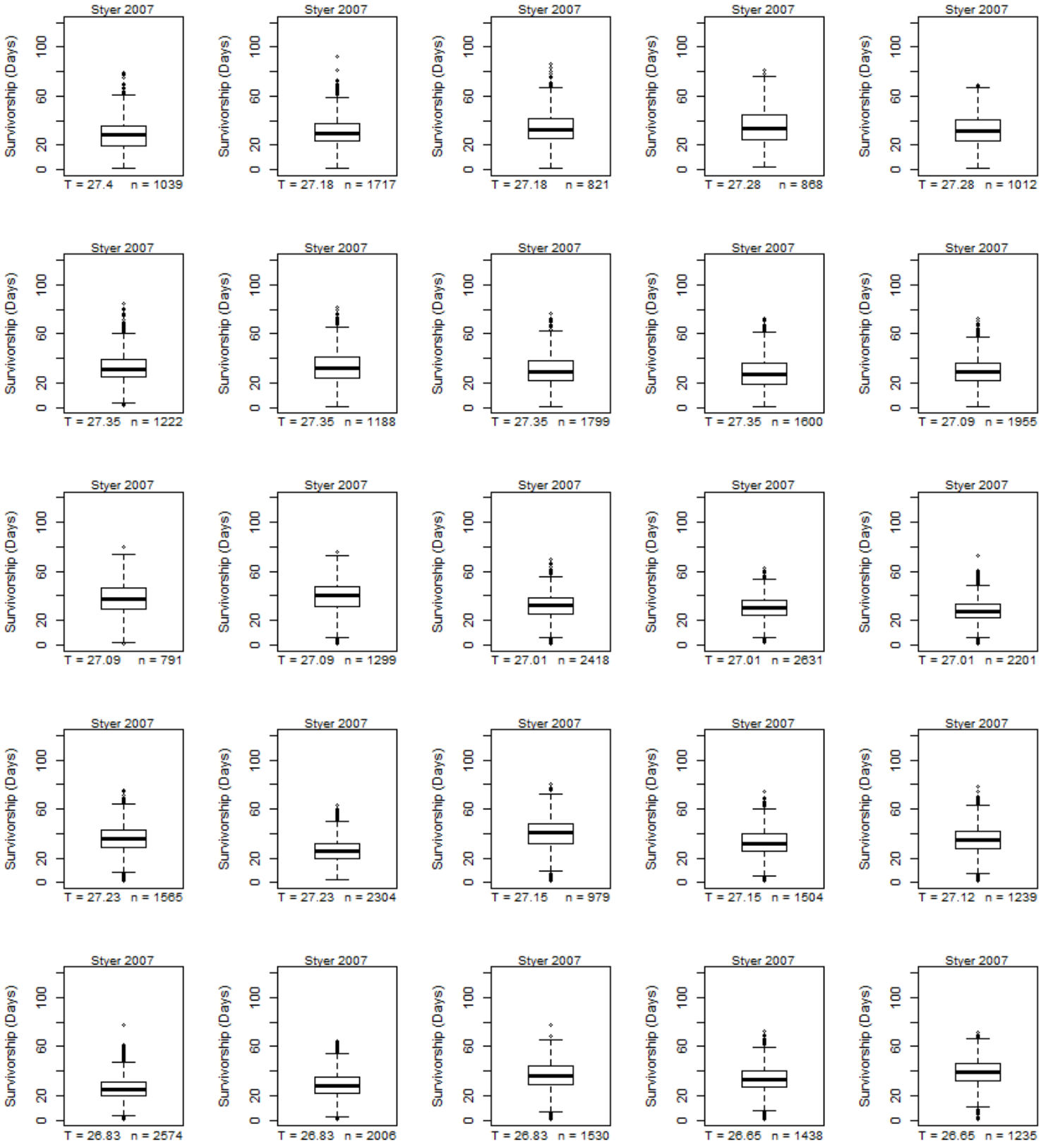
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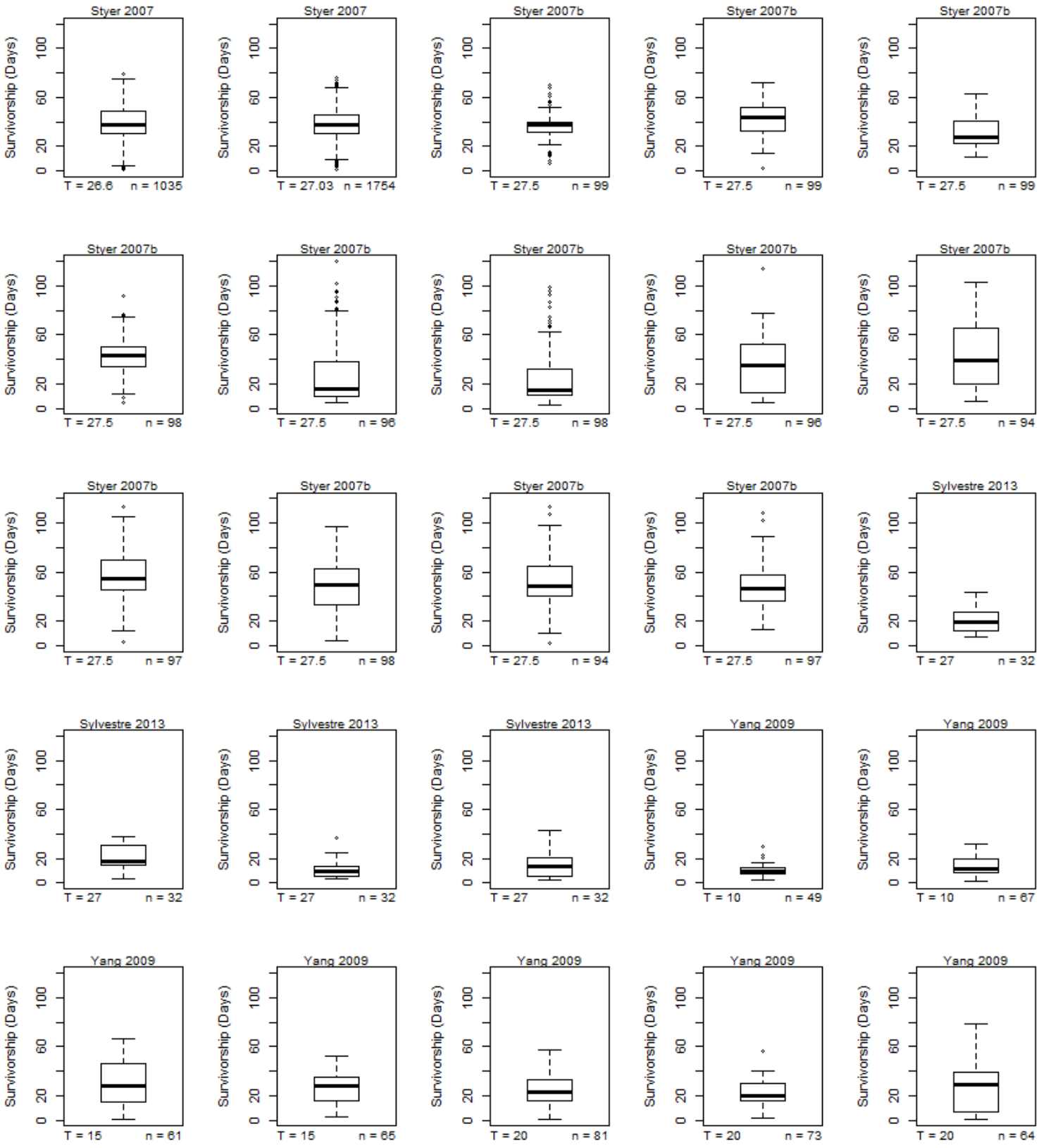
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Styer 2007b
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Styer 2007b
T = 27.5 n = 99
Styer 2007b
T = 27.5 n = 98
Styer 2007b
T = 27.5 n = 96
Styer 2007b
T = 27.5 n = 98
Styer 2007b
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Styer 2007b
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Styer 2007b
T = 27.5 n = 97
Styer 2007b
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Styer 2007b
T = 27.5 n = 94
Styer 2007b
T = 27.5 n = 97
Sylvestre 2013
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Sylvestre 2013
T = 27 n = 32
Sylvestre 2013
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Sylvestre 2013
T = 27 n = 32
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Yang 2009
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Yang 2009
T = 15 n = 65
Yang 2009
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Yang 2009
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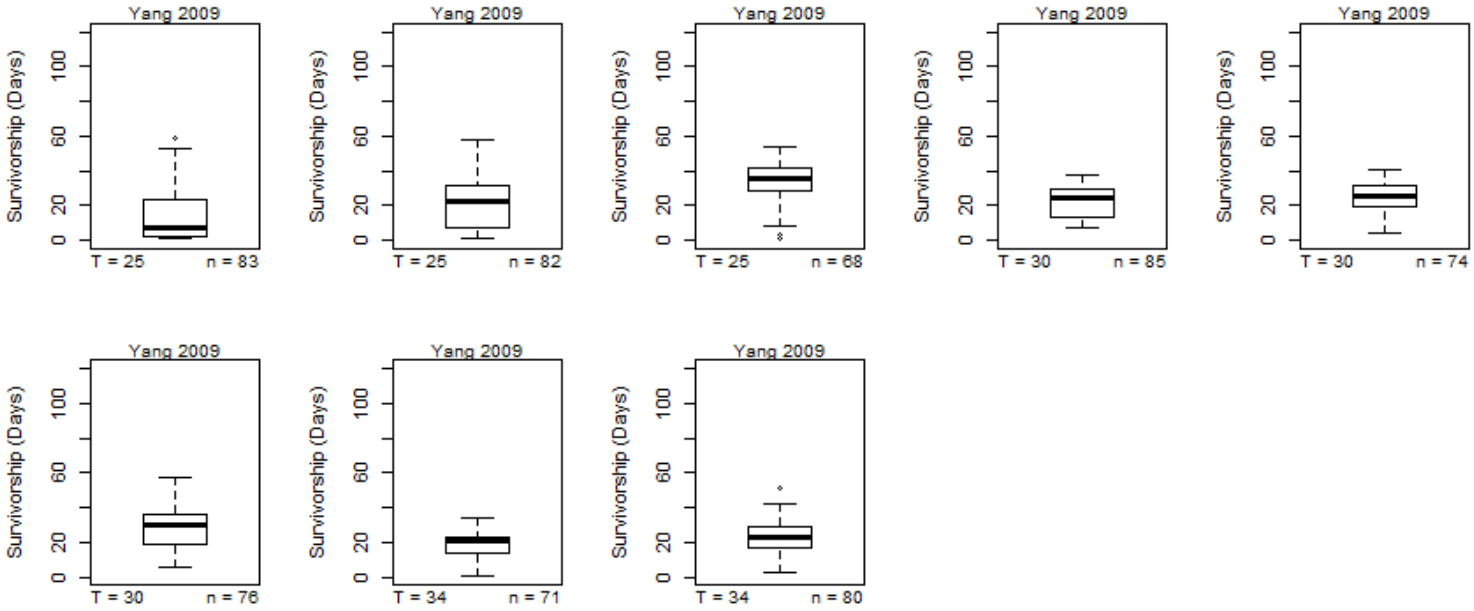

***Ae. aegypti* laboratory experiment bibliography**

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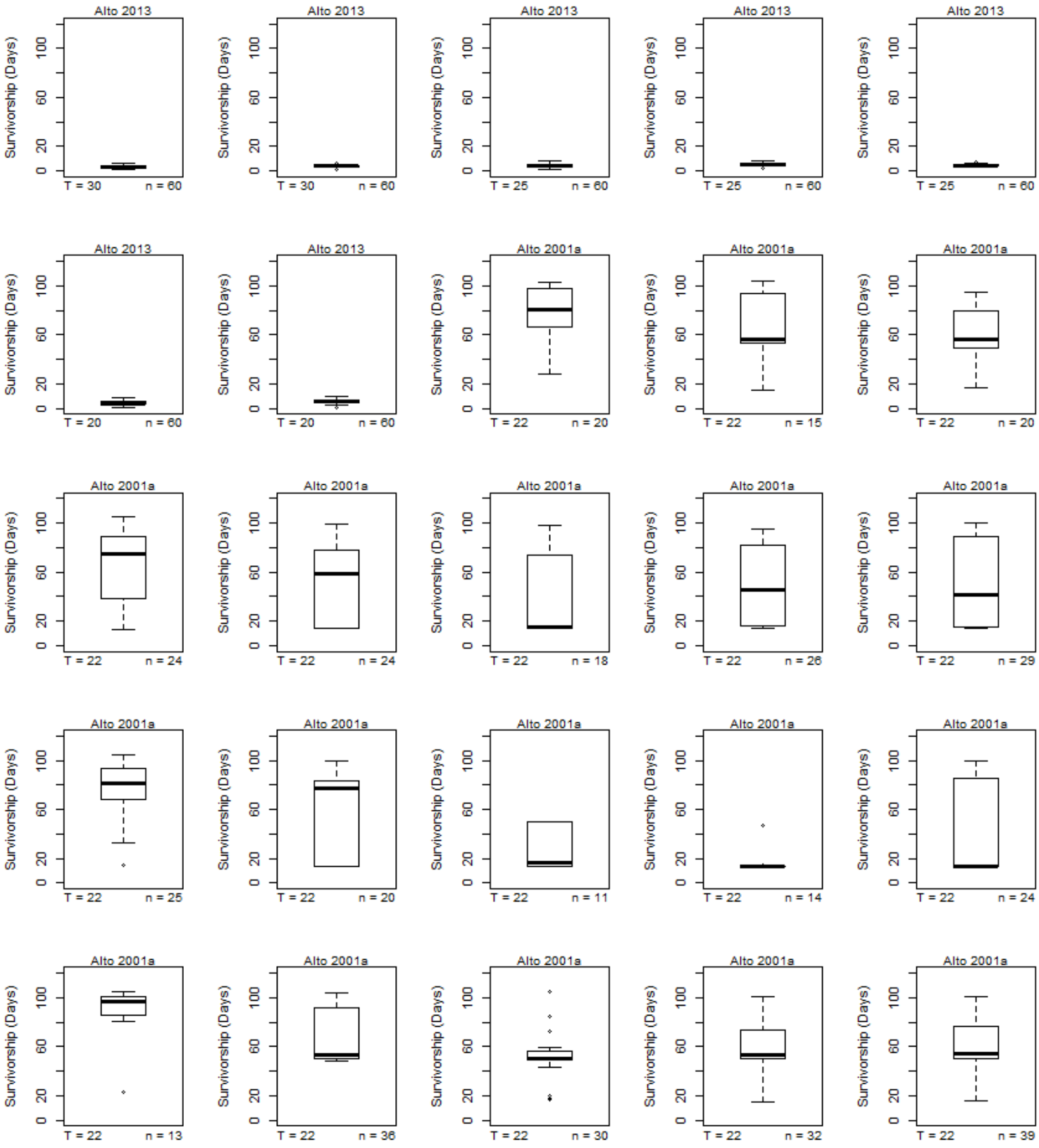
Figure SI2. Boxplots of the *Ae. albopictus* survival under controlled conditions in the laboratory for each experiment used in this study. T = the temperature at which the experiment took place, n = the total number of mosquitoes under observation.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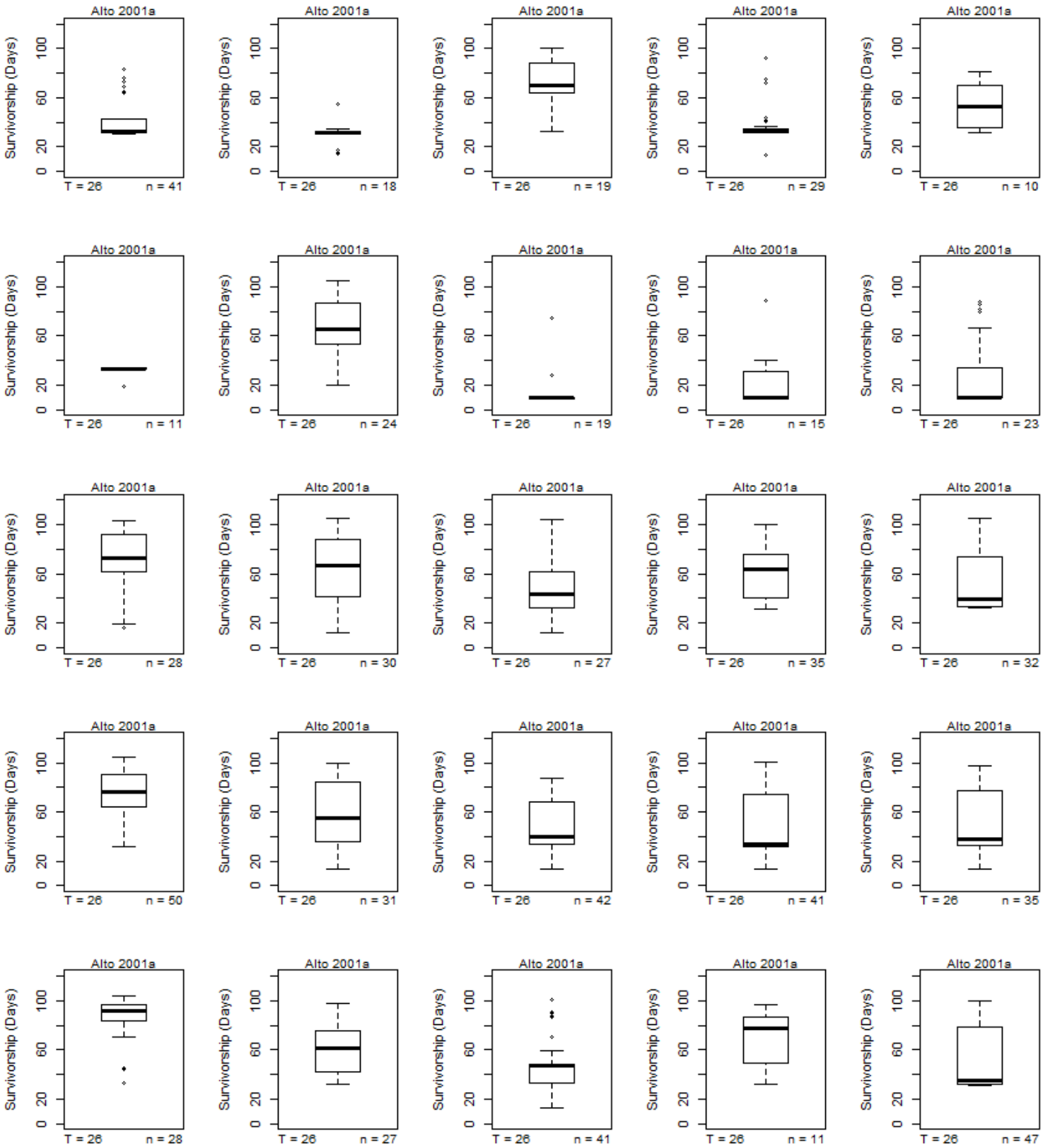
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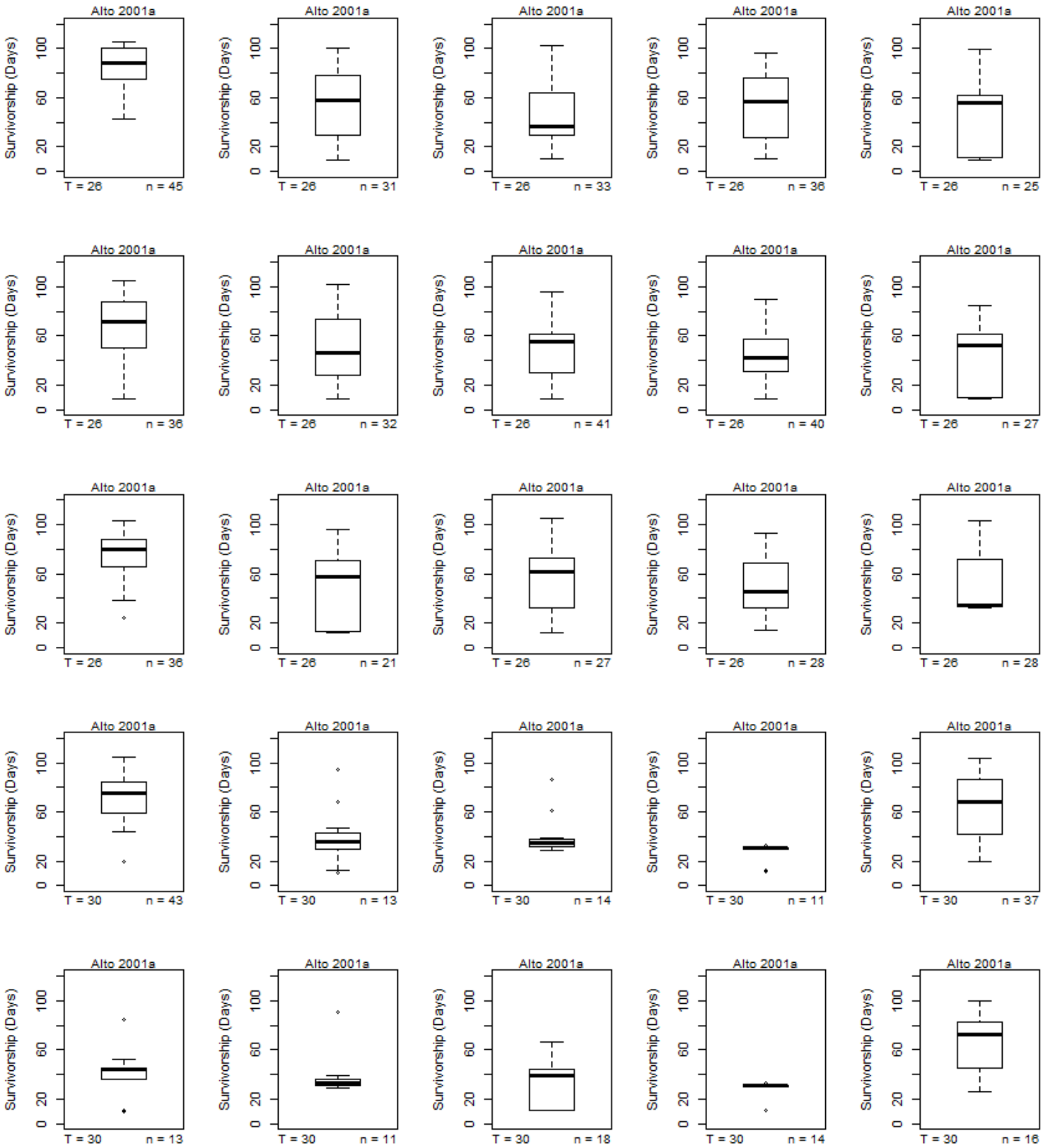
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Survivorship (Days)
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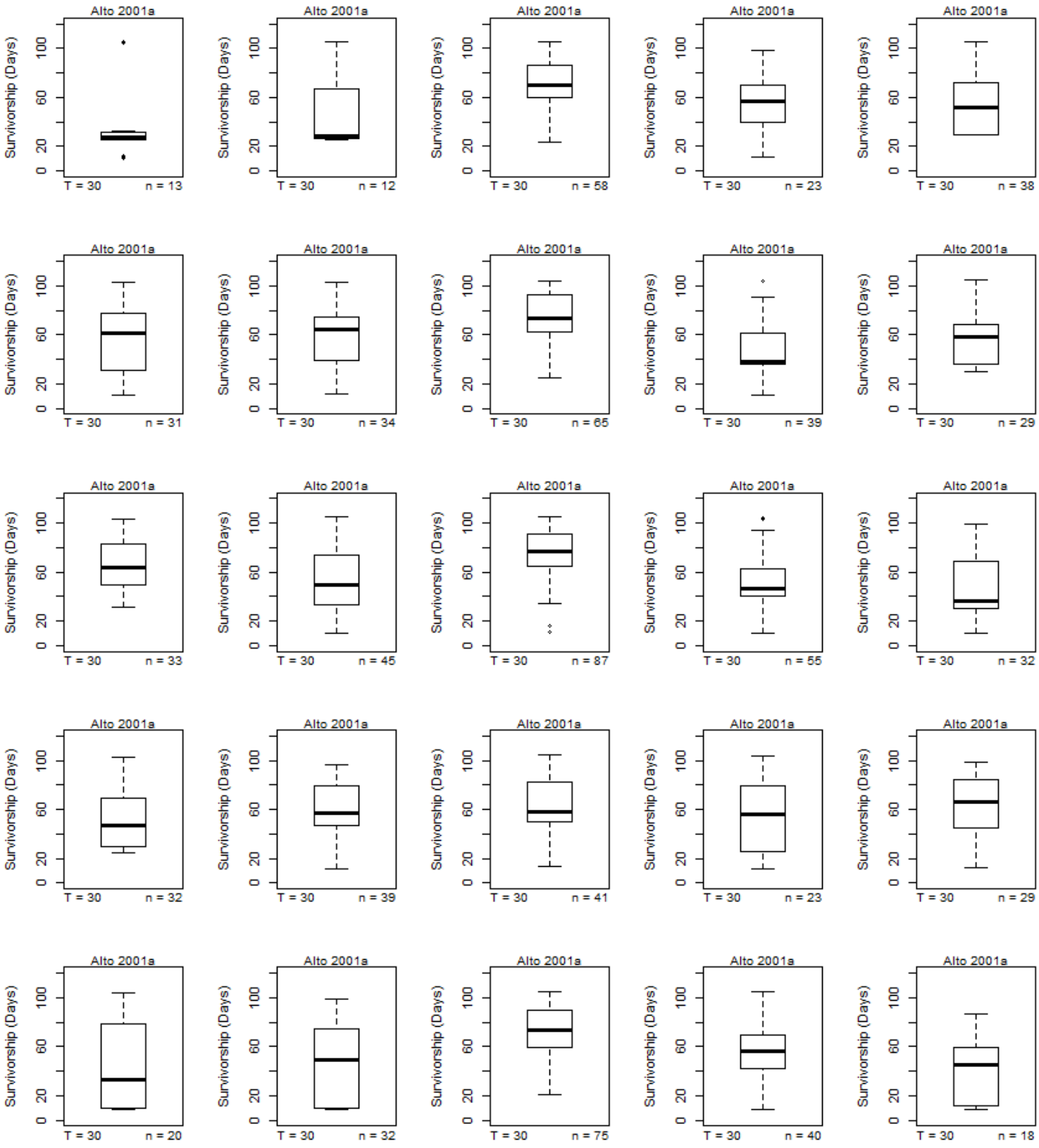
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T = 30 n = 33
Alto 2001a
Survivorship (Days)
0 20 60 100
T = 30 n = 45
Alto 2001a
Survivorship (Days)
0 20 60 100
T = 30 n = 87
Alto 2001a
Survivorship (Days)
0 20 60 100
T = 30 n = 55
Alto 2001a
Survivorship (Days)
0 20 60 100
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Alto 2001a
Survivorship (Days)
0 20 60 100
T = 30 n = 32
Alto 2001a
Survivorship (Days)
0 20 60 100
T = 30 n = 39
Alto 2001a
Survivorship (Days)
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T = 30 n = 41
Alto 2001a
Survivorship (Days)
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T = 30 n = 23
Alto 2001a
Survivorship (Days)
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Alto 2001a
Survivorship (Days)
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Alto 2001a
Survivorship (Days)
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Alto 2001a
Survivorship (Days)
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Alto 2001a
Survivorship (Days)
0 20 60 100
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Alto 2001a
Survivorship (Days)
0 20 60 100
T = 30 n = 18

Alto 2001a
Survivorship (Days)
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T = 30 n = 60
Alto 2001a
Survivorship (Days)
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Survivorship (Days)
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Survivorship (Days)
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Survivorship (Days)
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Survivorship (Days)
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Alto 2001a
Survivorship (Days)
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Alto 2001a
Survivorship (Days)
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Alto 2001a
Survivorship (Days)
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Survivorship (Days)
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Alto 2001a
Survivorship (Days)
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Alto 2001a
Survivorship (Days)
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Alto 2001a
Survivorship (Days)
0 20 60 100
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Alto 2001b
Survivorship (Days)
0 20 60 100
T = 26 n = 105
Alto 2001b
Survivorship (Days)
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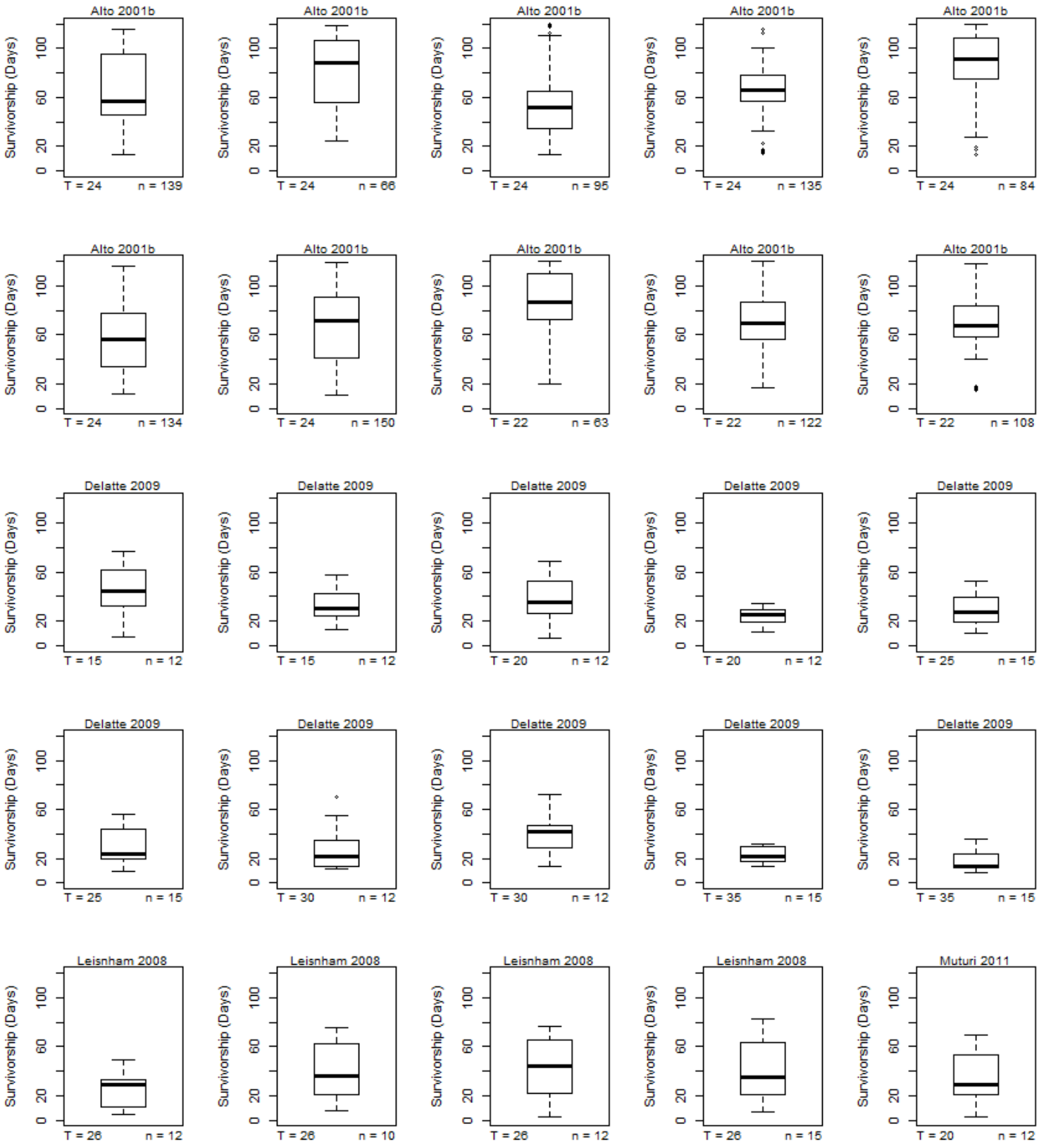
Alto 2001b
Survivorship (Days)
0 20 60 100
T = 26 n = 108
Alto 2001b
Survivorship (Days)
0 20 60 100
T = 26 n = 89
Alto 2001b
Survivorship (Days)
0 20 60 100
T = 26 n = 98
Alto 2001b
Survivorship (Days)
0 20 60 100
T = 26 n = 105
Alto 2001b
Survivorship (Days)
0 20 60 100
T = 24 n = 52
Alto 2001b
Survivorship (Days)
0 20 60 100
T = 24 n = 90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Survivorship (Days)
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Survivorship (Days)
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Alto 2001b
Survivorship (Days)
0 20 60 100
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Alto 2001b
Survivorship (Days)
0 20 60 100
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Alto 2001b
Survivorship (Days)
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Alto 2001b
Survivorship (Days)
0 20 60 100
T = 22 n = 108
Delatte 2009
Survivorship (Days)
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Delatte 2009
Survivorship (Days)
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Delatte 2009
Survivorship (Days)
0 20 60 100
T = 20 n = 12
Delatte 2009
Survivorship (Days)
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Delatte 2009
Survivorship (Days)
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Delatte 2009
Survivorship (Days)
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Survivorship (Days)
0 20 60 100
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Delatte 2009
Survivorship (Days)
0 20 60 100
T = 35 n = 15
Leisnham 2008
Survivorship (Days)
0 20 60 100
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Leisnham 2008
Survivorship (Days)
0 20 60 100
T = 26 n = 10
Leisnham 2008
Survivorship (Days)
0 20 60 100
T = 26 n = 12
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Survivorship (Days)
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Muturi 2011
Survivorship (Days)
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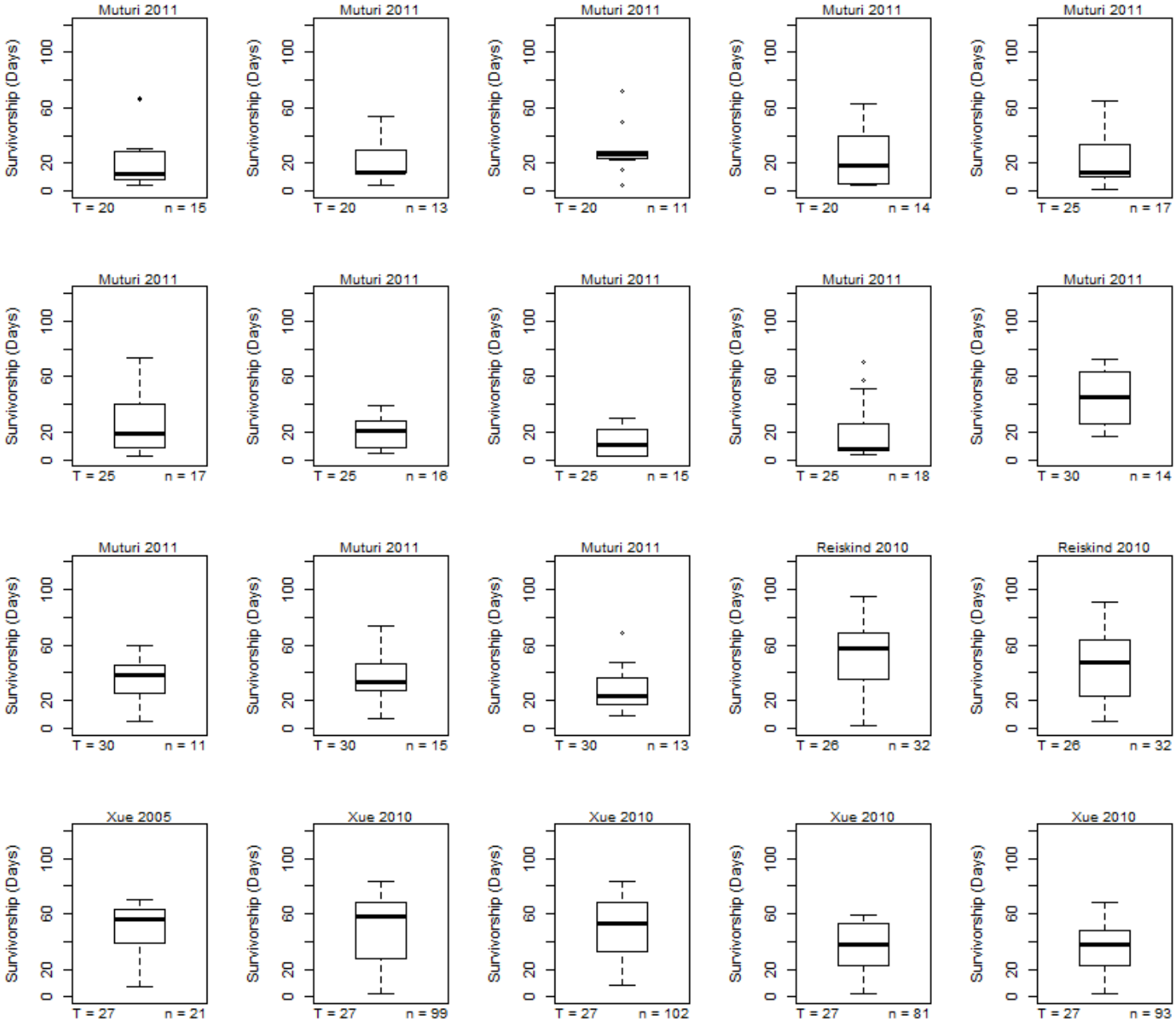

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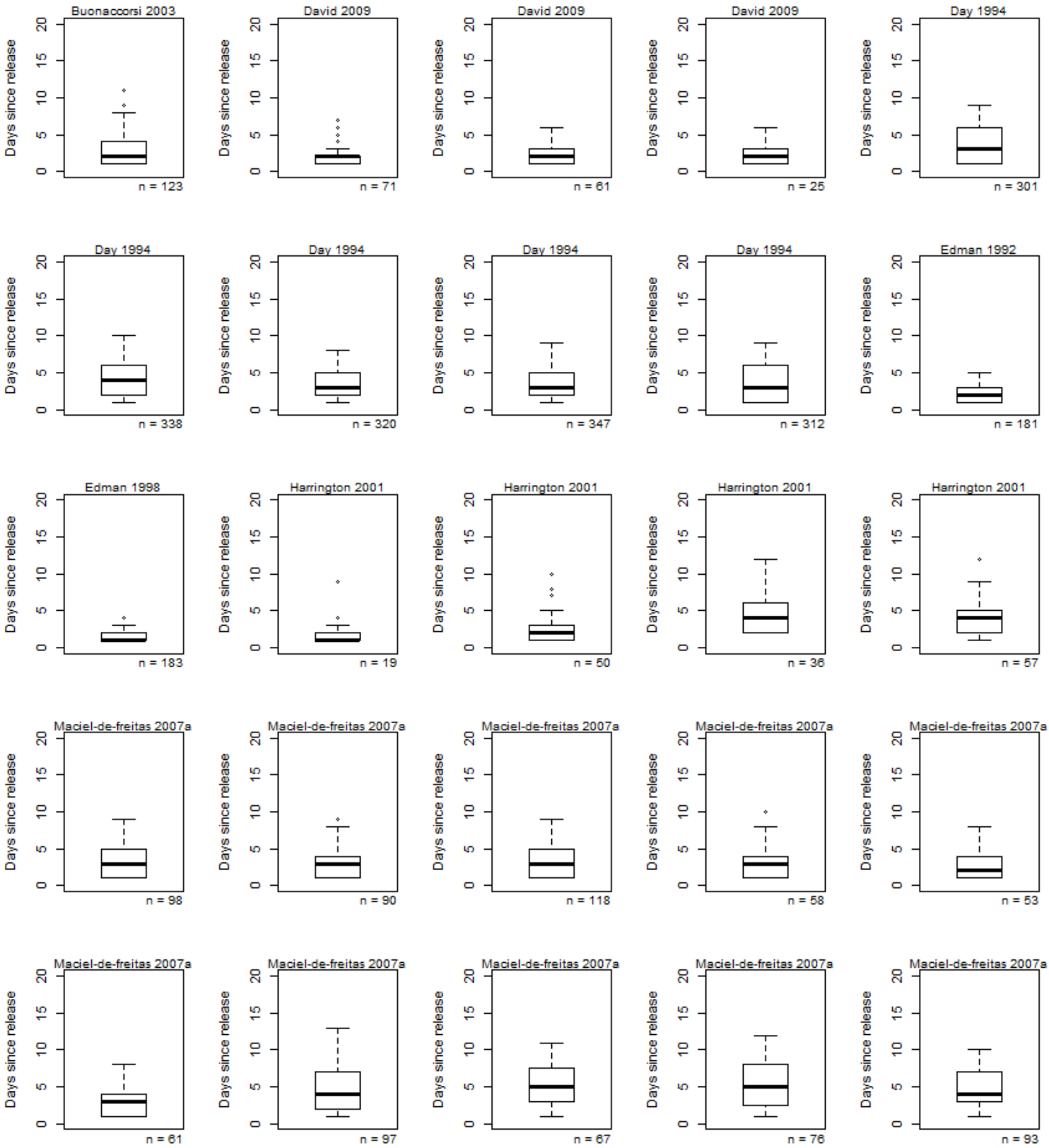
Figure SI3. Boxplots of the proportions of *Ae. aegypti* recaptures on successive days post release for each experiment used in this study, n = the total number of mosquitoes recaptured.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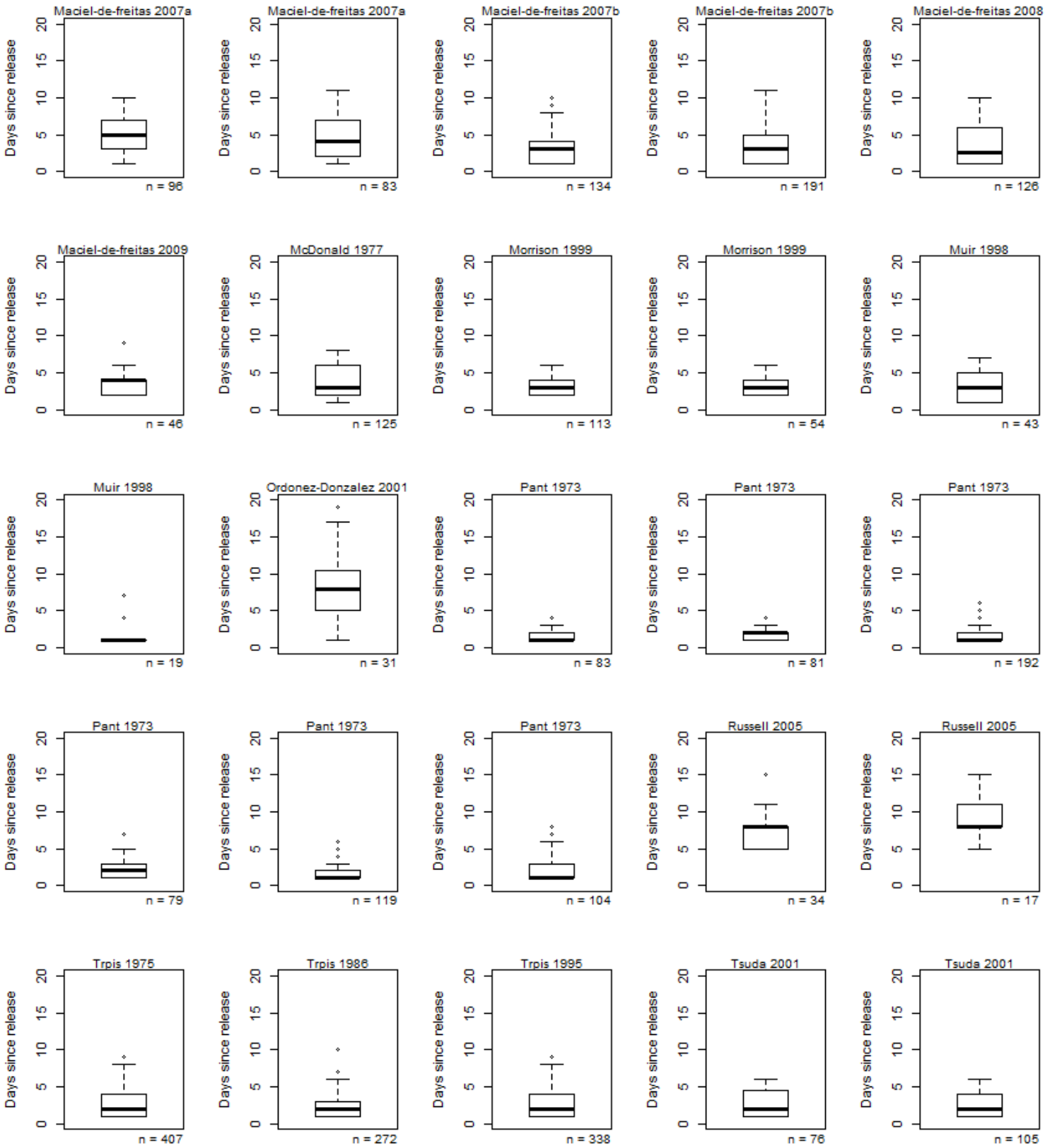
David 2009
n = 71
David 2009
n = 61
David 2009
n = 25
Day 1994
n = 301
Day 1994
n = 338
Day 1994
n = 320
Day 1994
n = 347
Day 1994
n = 312
Edman 1992
n = 181
Edman 1998
n = 183
Harrington 2001
n = 19
Harrington 2001
n = 50
Harrington 2001
n = 36
Harrington 2001
n = 57
Maciel-de-freitas 2007a
n = 98
Maciel-de-freitas 2007a
n = 90
Maciel-de-freitas 2007a
n = 118
Maciel-de-freitas 2007a
n = 58
Maciel-de-freitas 2007a
n = 53
Maciel-de-freitas 2007a
n = 61
Maciel-de-freitas 2007a
n = 97
Maciel-de-freitas 2007a
n = 67
Maciel-de-freitas 2007a
n = 76
Maciel-de-freitas 2007a
n = 93
Days since release
0
5
10
15
20


Maciel-de-freitas 2007a
Days since release
n = 96
Maciel-de-freitas 2007a
Days since release
n = 83
Maciel-de-freitas 2007b
Days since release
n = 134
Maciel-de-freitas 2007b
Days since release
n = 191
Maciel-de-freitas 2008
Days since release
n = 126
Maciel-de-freitas 2009
Days since release
n = 46
McDonald 1977
Days since release
n = 125
Morrison 1999
Days since release
n = 113
Morrison 1999
Days since release
n = 54
Muir 1998
Days since release
n = 43
Muir 1998
Days since release
n = 19
Ordonez-Donzalez 2001
Days since release
n = 31
Pant 1973
Days since release
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Pant 1973
Days since release
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Days since release
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Days since release
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Days since release
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Days since release
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Trpis 1975
Days since release
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Trpis 1986
Days since release
n = 272
Trpis 1995
Days since release
n = 338
Tsuda 2001
Days since release
n = 76
Tsuda 2001
Days since release
n = 105

***Ae. aegypti* mark-release-recapture experiment bibliography**

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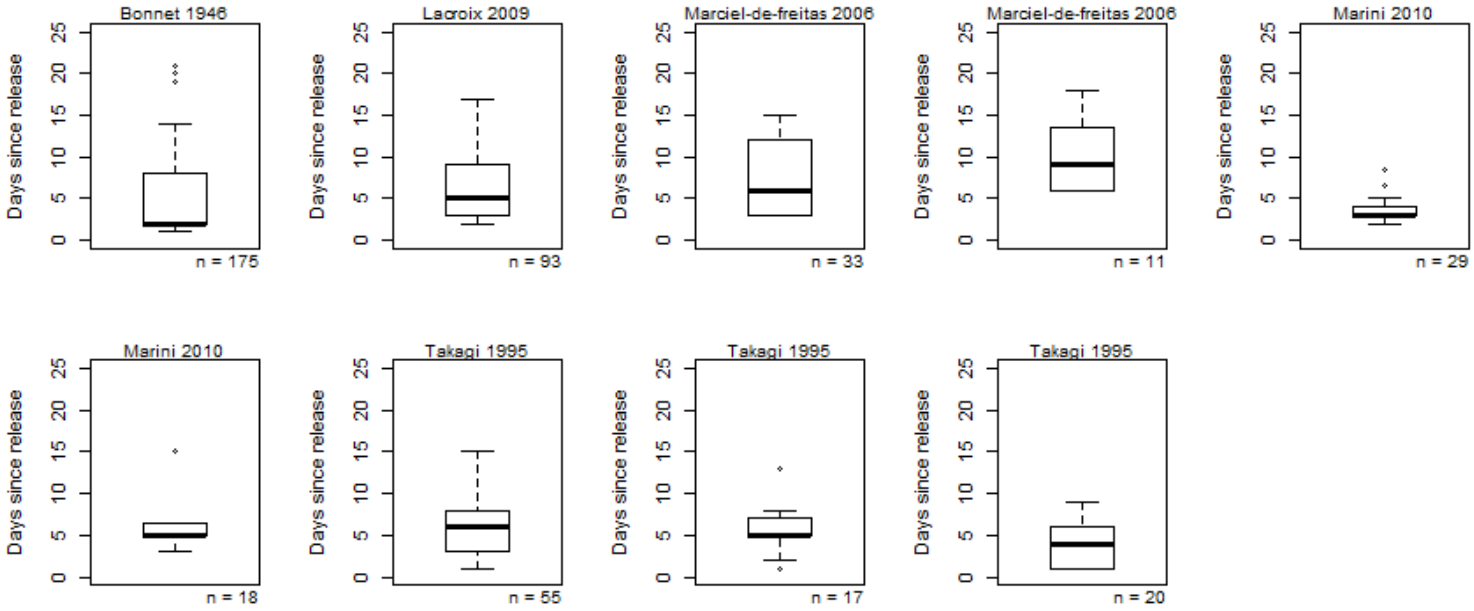
Figure SI4. Boxplots of the proportions of *Ae. albopictus* recaptures on successive days post release for each experiment used in this study, n = the total number of mosquitoes recaptured.



***Ae. albopictus* mark-release-recapture experiment bibliography**

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Appendix to chapter 6 – *Evaluating the potential for dengue transmission in temperate regions*

Additional file 1: Priors used for Bayesian inter-species EIP comparison model.

$$\alpha_{aegypti} \sim N(0, 0.001)$$

$$\alpha_{albopictus} \sim N(0, 0.001)$$

$$\sigma_{EIP_{T,i,j}} \sim lnN\left(\mu_{assay}, {\sigma_{assay}}^2\right)$$

$$\sigma_{EIP_{T,i,aegypti}} \sim lnN(2.14, 0.270)$$

$$\sigma_{EIP_{T,i,albopictus}} \sim lnN(2.14, 0.270)$$

$${\sigma_{study}}^2 \sim Gam(9, 0.9)$$

Additional File 2: Assembly of the supplementary *Aedes aegypti* and *Aedes albopictus* database

AF2.1 Overview

The *Aedes* (Stegomyia) *aegypti* and *Ae.* (Stegomyia) *albopictus* database comprises occurrence data (presence, abundance and absence) linked to point or polygon locations, derived from peer-reviewed literature. Data sources are described in full here. To collate the peer-reviewed database, literature searches were undertaken using the major search engines and the resulting articles were manually reviewed. Geo-spatial information for 5966 occurrences of *Ae. aegypti* (4,397) and *Ae. albopictus* (1,569) were extracted using site descriptions from 1,556 articles. Locations were identified within the articles by searching for specific coordinates or, if not available, small administrative units. All data entries for both point and polygon data were manually checked by the authors and then underwent a series of routine quality control procedures to ensure correct geo-positioning.

AF2.2 Peer-reviewed Literature Search

PubMed (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi) 1960 to 2009 was searched using term “Aedes”, “aegypti” and “albopictus”. The MESH term technology used in the PubMed citation archive ensured all pseudonyms were automatically included (http://www.nlm.nih.gov/mesh/2008/MBrowser.html) in the searches. The same process was repeated for ISI Web of Science (http://wok.mimas.ac.uk) and ProMED (records since 1994; http://www.promedmail.org). The searches were last updated on 3th November 2009. No language restrictions were placed on these searches, however, only those citations with a full title and abstract were downloaded into Endnote version 10 (Thomson ResearchSoft, USA, http://www.endnote.com). 10,314 original citations were scanned and a total of 3,704 references were retrieved with 1,556 unique articles identified as potentially containing useable location data.

In-house language skills allowed processing of all English, French, Portuguese and Spanish articles. Russian articles with data were translated and included in the database. We were unable to extract information from a small number of Turkish, Polish, Hebrew, Italian, German and Chinese articles.

AF2.3 Disease vector data and species type

Confirmed occurrences of *Ae. aegypti* and *Ae. albopictus* found within the peer reviewed literature were recorded as an occurrence datapoint. Species names reported as collected in the site were entered in the database. If both species have been collected at the same site during the same time period, a second data point/polygon has been entered with all respective information. Occurrence points for *Ae. aegypti* were classified in presence, abundance and absence (4,080, 130, 187 respectively) and *Ae. albopictus* in presence and absence (1,469, 100 respectively). No additional information on 'sub-species' or other such as information given in the source pdf e.g. 'temporate' or 'tropical' have been recorded. Beginning and end year of survey was included in the data abstraction. In this analysis only presence and abundance points from any time period were used as they represent a known unique geographic occurrence of the disease.

Both point and polygon was entered into a bespoke PostgreSQL database that links vector data to spatial data. A link to the PDF library was also included in the database.

AF2.4 Geo-positioning occurrence points

All available location information in the peer-reviewed literature was extracted from each article. The site name and contextual information were used together to locate the study site using Microsoft Encarta (Microsoft Corporation, Redmond, WA) and Google Earth 5.1. Place names are often duplicated within a country so the contextual information was used to ensure the right site was selected. Where the site name was not found, the contextual information was used to scan sites in the approximate area to check for names that had been transliterated in Microsoft Encarta/Google Earth in a different way to the published article (e.g. Imichli and Imishly). If the site was not located using Microsoft Encarta or Google Earth, a range of online georeferencing resources were tried including Getty Thesaurus (http://www.getty.edu/research/conducting_research/vocabularies/tgn/index.html), Alexandria (http://middleware.alexandria.ucsb.edu/client/gaz/adl/index.jsp), Falling Rain (http://www.fallingrain.com/world/), National geospatial-intelligence agency (http://geonames.nga.mil/ggmaviewer), and Maplandia (http://maplandia.com/). If the study site could be georeferenced to a specific place, it was recorded as a point location. In total, 5,430 point locations from the peer-reviewed literature were included in the database at this stage. If the study site could only be identified at an

administrative area level (e.g. province or district, etc), it was recorded as a polygon and the centroid (mean centre) was derived to give a latitude and longitude for the site. A total of 536 polygon locations from the peer-reviewed literature were recorded in the database. All point locations and polygon centroids were converted to decimal degrees before entering the quality control process described below. To define the absolute limits of *Aedes* persistence only the point data was used to improve geographic precision.

AF2.5 Automatic Validation and Quality control

All datapoints were crosschecked and validated using a standardised validation procedure. First a multi-polygon shapefile defining land/water boundaries was created as a template to validate geopositioned points. This land/water shape file was rasterised to spatially overlapping raster pixels (the polygon boundaries must cover the centre of the raster pixel) at a 5 x 5 km resolution and was used to ensure all disease occurrence points were positioned on a valid land pixel.

Any points that met the following criteria were excluded from the database:

1. Points found further than 10km from a land boundary. Points located less than 10km from a land boundary were repositioned to the nearest land raster cell.
2. Points representing the centroid of an administrative division having an area greater than 111km^2 (1 degree at the equator).

Once the quality control procedure was complete, the final database contained 5,966 occurrence datapoints (including 5,430 point locations and 536 small polygon centroids) covering a period from 1960 to 2009. Maps displaying the *Ae. aegypti* and *Ae. albopictus* locations are provided in Figures AF2A and AF2B.

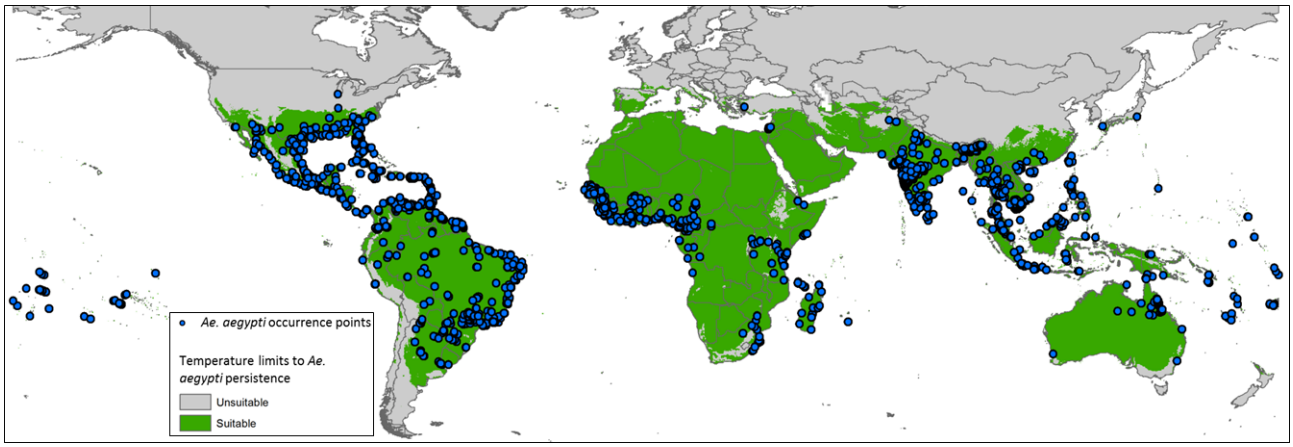


Figure AF2A. The distribution of *Ae. aegypti* occurrence points in the database. The background shows the temperature limits to *Ae. aegypti* persistence where 283 or more days in an average year are suitable for parous females.

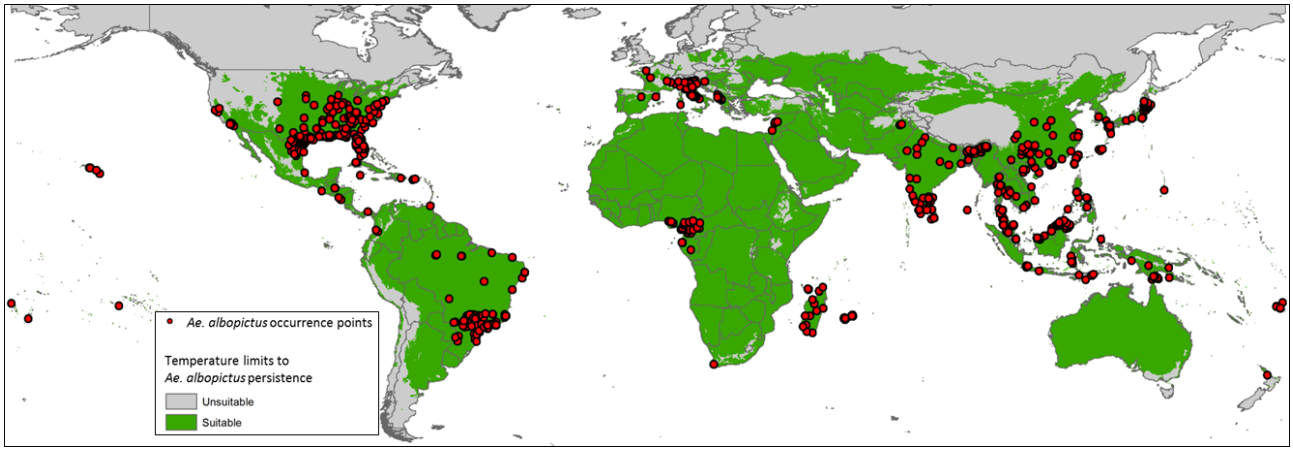


Figure AF2B. The distribution of *Ae. albopictus* occurrence points in the database. The background shows the temperature limits to *Ae. albopictus* persistence where 165 or more days in an average year are suitable for parous females.

S2.6 Bibliography of final endnote library

Due to space constrains this supplementary bibliography is not included in this appendix, but is available here:

http://www.parasitesandvectors.com/content/supplementary/1756-3305-7-338-s2.docx

Additional File 3: Animation of *Ae. aegypti* temperature suitability for oviposition ($X_{ovi}(T)$) throughout an average year.

This animation is available at the following web link:

http://www.parasitesandvectors.com/content/supplementary/1756-3305-7-338-s3.wmv

Additional File 4: Animation of *Ae. albopictus* temperature suitability for oviposition ($X_{ovi}(T)$) throughout an average year.

This animation is available at the following web link:

http://www.parasitesandvectors.com/content/supplementary/1756-3305-7-338-s4.wmv

Additional File 5: Animation of *Ae. aegypti* temperature suitability for introduction of dengue virus transmission ($Z(T)$) throughout an average year.

This animation is available at the following web link:

http://www.parasitesandvectors.com/content/supplementary/1756-3305-7-338-s5.wmv

Additional File 6: Animation of *Ae. albopictus* temperature suitability for introduction of dengue virus transmission ($Z(T)$) throughout an average year.

This animation is available at the following web link:

http://www.parasitesandvectors.com/content/supplementary/1756-3305-7-338-s6.wmv

Additional File 7: Animation of *Ae. aegypti* temperature suitability for persistence of dengue virus transmission ($X(T)$) throughout an average year.

This animation is available at the following web link:

http://www.parasitesandvectors.com/content/supplementary/1756-3305-7-338-s7.wmv

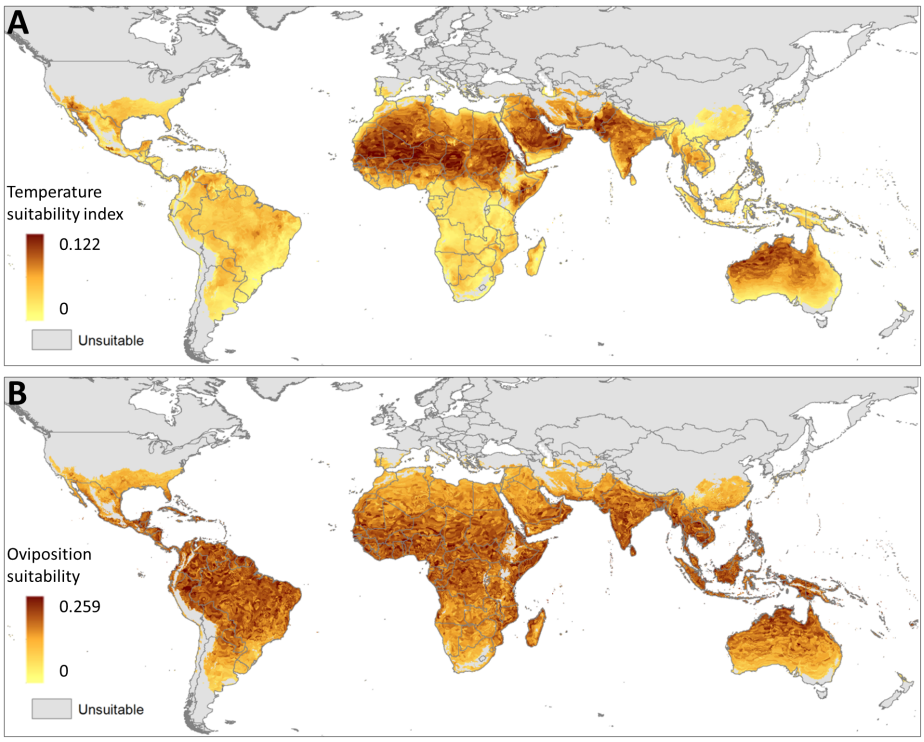
Additional File 8: Animation of *Ae. albopictus* temperature suitability for persistence of dengue virus transmission ($X(T)$) throughout an average year.

This animation is available at the following web link:

http://www.parasitesandvectors.com/content/supplementary/1756-3305-7-338-s8.wmv

Additional File 9: *Ae. aegypti* model prediction uncertainty for the temperature suitability index (A) and oviposition suitability (B).

The output shows the interquartile range in predictions presented in maps 3A and 3D.



Additional File 10: *Ae. albopictus* model prediction uncertainty for the temperature suitability index (A) and oviposition suitability (B).

The output shows the interquartile range in predictions presented in maps 4A and 4D.