The Impact of Temperature on the Bionomics of the Vector Mosquito \textit{Aedes} (\textit{Stegomyia}) aegypti, With Special Reference to the Cool Geographic Range Margins

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Abstract. The mosquito *Aedes* (*Stegomyia*) *aegypti* (L.), which occurs widely in the subtropics and tropics, is the primary urban vector of dengue and yellow fever viruses, and an important vector of chikungunya virus. There is substantial interest in how climate change may impact the bionomics and pathogen transmission potential of this mosquito. This Forum article focuses specifically on the effects of temperature on the bionomics of *Ae. aegypti*, with special emphasis on the cool geographic range margins where future rising temperatures could facilitate population growth. Key aims are to: 1) broadly define intra-annual (seasonal) patterns of occurrence and abundance of *Ae. aegypti*, and their relation to climate conditions; 2) synthesize the existing quantitative knowledge of how temperature impacts the bionomics of different life stages of *Ae. aegypti*; 3) better define the temperature ranges for which existing population dynamics models for *Ae. aegypti* are likely to produce robust predictions; 4) explore potential impacts of climate warming on human risk for exposure to *Ae. aegypti* at its cool range margins; and 5) identify knowledge or data gaps that hinder our ability to predict risk of human exposure to *Ae. aegypti* at the cool margins of its geographic range now and in the future. We first outline basic scenarios for intra-annual occurrence and abundance patterns for *Ae. aegypti*, and then show that these scenarios segregate with regards to climate conditions in selected cities where they occur. We then review how near constant and intentionally fluctuating temperatures impact development times and survival of eggs and immatures. A sub-set of data, generated in controlled experimental studies, from the published literature is used to plot development rates and survival of eggs, larvae, and pupae in relation to water temperature. The general shape of the relationship between water temperature and development rate is similar for eggs, larvae, and pupae. Once the lower developmental zero temperature (10–14 °C) is exceeded, there is a near linear relationship up to 30 °C. Above this temperature the development rate is relatively stable
or even decreases slightly before falling dramatically near the upper developmental zero temperature, which occurs at approximately 38–42 °C. Based on life stage-specific linear relationships between water temperature and development rate in the 15–28 °C range, the lower developmental zero temperature is estimated to be 14.0 °C for eggs, 11.8 °C for larvae, and 10.3 °C for pupae. We further conclude that available population dynamics models for *Ae. aegypti*, such as CIMSiM and Skeeter Buster, likely produce robust predictions based on water temperatures in the 16–35 °C range, which includes the geographic areas where *Ae. aegypti* and its associated pathogens present the greatest threat to human health, but that they may be less reliable in cool range margins where water temperatures regularly fall below 15°C. Finally, we identify knowledge or data gaps that hinder our ability to predict risk of human exposure to *Ae. aegypti* at the cool margins of its range, now and in the future, based on impacts on mosquito population dynamics of temperature and other important factors, such as water nutrient content, larval density, presence of biological competitors, and human behavior.

**Keywords:** *Aedes aegypti*, bionomics, population dynamics, range margins, temperature
The mosquito *Aedes (Stegomyia) aegypti* (L.) is the primary urban vector of dengue and yellow fever viruses, and an important vector of chikungunya virus, in the subtropics and tropics (Gubler 2004, Barrett and Higgs 2007, Pialoux et al. 2007). A recent study estimates that up to 390 million dengue virus infections, including close to 100 million cases of dengue disease manifestations, occur annually (Bhatt et al. 2013). *Ae. aegypti* has a unique life style in that it is very closely associated with human habitation: a wide range of water-holding containers in and around human dwellings are exploited as sites for oviposition of eggs and development of immatures, and the day-time biting females commonly rest and feed indoors (Focks and Alexander 2006, Halstead 2008). The geographic range for *Ae. aegypti* is considered to fall roughly within the low latitude areas equatorward of the average 10 °C winter isotherms in the northern and southern hemispheres (Christophers 1960, WHO 2009). The mosquito persists across a climate suitability gradient ranging from near optimal at the cores of its range in the subtropics and tropics to borderline suitable at the cool range margins, which occur at mid-latitudes or at high elevations at lower latitudes (Eisen and Moore 2013).

patterns is more difficult to predict due to the complicating factor that *Ae. aegypti* immatures are found in a wide range of containers, many of which are kept filled primarily by human action rather than rainfall (Kearney et al. 2009, Tun-Lin et al. 2009).

The core distributional areas for *Ae. aegypti*, where the mosquito and its associated pathogens present a major threat to human health, include parts of the Southern Asia sub-region (particularly India, Sri Lanka, and Bangladesh), parts of the Eastern Asia sub-region (particularly southern China and Taiwan), the entire sub-region of South-Eastern Asia, northeastern Australia, islands of the tropical Pacific Ocean, subtropical and tropical parts of Africa, the Caribbean islands, and large parts of the continental Americas with cool range margins to the north and south as outlined below (WHO 2009). These settings provide potential for accurate prediction of population dynamics of *Ae. aegypti*, now and under climate warming scenarios, based on the combined use of existing biosurveillance data and weather-driven population dynamics models, such as CIMSiM (*Container Inhabiting Mosquito Simulation Model*) and Skeeter Buster (Focks et al. 1993a, b; Magori et al. 2009; Ellis et al. 2011) or other relevant models (Otero et al. 2006, 2008; Chaves et al. 2012; Padmanabha et al. 2012; Tsai et al. 2012; Aznar et al. 2013).

Cool range margins for *Ae. aegypti* in the Americas occur in the U.S. in the northern hemisphere, central and southern Argentina in the southern hemisphere, and in high elevation areas in México’s central highlands and the Andes in western South America (Suarez and Nelson 1981, Ibáñez-Bernal 1987, Darsie and Ward 2005, Rossi et al. 2006, Vezzani and Carbajo 2008, Grech et al. 2012, Lozano-Fuentes et al. 2012, Díaz-Nieto et al. 2013). There also appears to be a cool range margin to the south in Australia (Kearney et al. 2009). Although poorly defined, cool range margins undoubtedly also occur to the north or at high elevations in Southern Asia and Eastern Asia. For these cool range margins, prediction of the population dynamics of *Ae.
aegypti is hindered by limited biosurveillance data and a paucity of experimental data on the
impact of relevant temperature conditions on the bionomics of Ae. aegypti. Moreover, basic
assumptions in weather-driven population dynamics models regarding the impact of temperature
on mosquito development and survival may be less reliable for the lower temperatures typical of
the cool range margins compared to temperatures in the core distributional areas. In this respect,
our lack of understanding of how natural diel temperature fluctuations impact development and
survival of immatures in the lower temperature range is especially concerning. For example, the
development rate of the larval stage may deviate from assumptions in the CIMSiM and Skeeter
Buster models, which are based on enzyme kinetics models (Schoolfield et al. 1971, Sharpe and
DeMichele 1977, Focks et al. 1993a, Magori et al. 2009), when minimum diel temperatures
regularly fall below the temperature threshold at which development is arrested (the lower
developmental zero temperature or ecological zero point).

The aims of this Forum article are to: 1) broadly define intra-annual patterns of
occurrence and abundance of Ae. aegypti, and their relation to climatic conditions; 2) synthesize
the existing quantitative knowledge for how temperature impacts the bionomics of different life
stages of Ae. aegypti, especially with regards to development times and survival of eggs and
immatures; 3) better define the temperature ranges for which existing population dynamics
models for Ae. aegypti are likely to produce robust results; 4) explore potential impacts of
climate warming on human risk for exposure to Ae. aegypti at its cool range margins; and 5)
identify specific knowledge or data gaps that hinder our ability to predict risk of human exposure
to Ae. aegypti at the cool margins of its geographic range now and in the future. We do not
address transmission dynamics of pathogens associated with Ae. aegypti.

Limitations of the presented information
Our Forum article focuses specifically on the effects of temperature on the bionomics of different life stages of *Ae. aegypti*. While temperature is an important driver for *Ae. aegypti* population dynamics, it is not the only one. We therefore caution the reader that the effects described herein for temperature can be confounded by other factors that may impact local mosquito population dynamics. Rainfall, together with human water storage practices, impact the availability of and water dynamics in water-filled containers for egg-laying and development of immatures (Halstead 2008, Kearney et al. 2009, Tun-Lin et al. 2009, Padmanabha et al. 2010). Humidity conditions impact evaporation rates from water-filled containers as well as the activity and longevity of adults (Lewis 1933, Bar-Zeev 1957b, Christophers 1960). Growth and survival of *Ae. aegypti* larvae are impacted by water nutrient conditions, larval abundance, and presence of biological competitors, such as *Aedes (Stegomyia) albopictus* (Skuse), or predators (Christophers 1960; Juliano 1998; Juliano et al. 2004; Lounibos et al. 2010; Padmanabha et al. 2011a,b, 2012). The likelihood of *Ae. aegypti*-human contact is influenced by human behavior as well as the extent of access for the mosquito to indoor environments (Reiter et al. 2003, Hayden et al. 2010). A complete understanding of the coupled natural-human system in which *Ae. aegypti* exists therefore ultimately needs to account for the complex interplay among all of the above-mentioned factors.

**Intra-Annual Occurrence and Abundance Patterns of *Ae. aegypti* In Relation to Climate**

Basic scenarios for intra-annual occurrence and abundance of the active stages (immatures and adults) of *Ae. aegypti* include: 1) year-around activity and potential for high abundance of the active stages; 2) year-around activity but potential for high abundance of the active stages only during the most favorable part of the year, typically when warm temperatures
coincide with substantial rainfall; 3) distinctly seasonal activity where the active stages can reach moderate to high abundance during part of the year but are absent during some part of the year due to unfavorably cold conditions, and where eggs can overwinter and then hatch and produce viable larvae in the spring; and 4) distinctly seasonal activity where the active stages can be found in low numbers during the warm part of the year but are absent during part of the year due to unfavorably cold conditions, and where winter temperatures are so low that overwintered eggs likely either fail to hatch or hatch but fail to produce viable larvae. The mosquito is absent from very high latitudes or elevations due to consistently unfavorable temperature conditions throughout the year. Another possible scenario is distinctly seasonal activity where the active stages are absent during the driest part of the year due to scarcity of water-filled containers.

Such a scenario most likely applies to arid environments in developed parts of the world where water storage by human action is minimal, e.g., in Tucson, AZ or parts of Australia (Hoeck et al. 2003, Kearney et al. 2009, Williams et al. 2010).


Areas in the Americas where the active stages of *Ae. aegypti* are absent during part of the year due to unfavorably cold conditions (scenarios 3 and 4 above) include: most of the geographic area of the U.S. referred to as the “usual range” or “permanent range” (central and northern Florida, Alabama, Mississippi, Louisiana, southeastern Texas, South Carolina, and Georgia) (Focks et al. 1993b, Darsie and Ward 2005); the area of the U.S. referred to as the “extreme range” or “temporary summer region” (Arkansas, Tennessee, Kentucky, North Carolina, Virginia, Maryland, and Delaware; the eastern parts of Texas and Oklahoma; the southern portions of Kansas, Missouri, Illinois, Indiana, Ohio, and Pennsylvania; and isolated areas in coastal New Jersey, New York, Connecticut, and Rhode Island) (Chandler 1945, Bell and Benach 1973, Cookman and Lebrun 1986, Berry et al. 1988, Sweeney et al. 1988, Donnelly 1993, Darsie and Ward 2005, Hutchinson et al. 2008); high elevation areas in México or the South American Andes (Suarez and Nelson 1981, Ibáñez-Bernal 1987, Lozano-Fuentes et al. 2012); and areas of central and southern Argentina (Campos and Maciá 1996, de Garín et al. 2000, Vezzani et al. 2004, Rossi et al. 2006, Grech et al. 2012, Díaz-Nieto et al. 2013, De Majo et al. 2013).

To broadly define the relation between climatic conditions and intra-annual occurrence and abundance patterns for *Ae. aegypti*, Tables 1-2 provide basic climate profiles (long-term monthly and annual average rainfall and 24-hour air temperatures from 1961–1990) for a series of cities that, based on the published literature, fall within the four above-mentioned scenarios for intra-annual occurrence and abundance patterns. It should be noted that the assignation of
cities among these four scenarios is based on the published literature and therefore subject to potential bias due to local surveillance efforts, particularly for assignation to scenarios 2 versus 3. For example, monthly winter (December–February) temperatures are similar for New Delhi (14.2–16.8 °C), where the active stages occur in low numbers during the winter months (Scenario 2; Katyal et al. 1996, Ansari and Razdan 1998, Sharma et al. 2005) and Brownsville, TX (15.2–16.8 °C) where existing surveillance data indicate that the active stages are absent during the coldest months (Scenario 3; Focks et al. 1993b). There are several possible explanations for this observed discrepancy, including more frequent use of the indoor, warmer environment during the winter in New Delhi due to buildings being more penetrable for the female mosquito, or that a more intensive winter surveillance effort in Brownsville could have resulted in detection of the active stages.

Nevertheless, the four city groupings (scenarios) segregate with regards to climate conditions. The ranges for annual average air temperature fall successively, with limited overlap between scenarios 1 (25.4–27.8 °C) and 2 (23.7–26.8 °C), and no overlap for scenarios 3 (16.8–23.2 °C), or 4 (14.5–15.5 °C) (Table 1). A similar pattern is seen for the ranges for the lowest monthly average temperature: 24.6–25.9 °C for scenario 1, all but one city in the 20.5–25.0 °C range for scenario 2, all but one city in the 8.7–15.2 °C range for scenario 3, and three of the four cities in scenario 4 below 6 °C (Table 1). Finally, the differential between the coldest and warmest month is smallest for scenario 1 (<4.5 °C) and increases for scenarios 2 (most cities in the 5–9 °C range) and 3-4 (all but one city >13 °C) (Table 1). The corresponding pattern for rainfall data is less clear, although the cities with year-around potential for high abundance of the active stages of *Ae. aegypti* uniformly have high annual rainfall, >1,450 mm (Table 2).
Moreover, annual rainfall may resolve the overlap in annual average air temperature between scenarios 1 and 2, as all three cities in scenario 1 with annual average air temperature below 27 °C (Singapore City, Djakarta, and Iquitos; 25.4–26.7 °C) have annual rainfall >2,100 mm whereas the three cities in scenario 2 falling within a similar range for annual average air temperature (Chiang Mai, Key West, and San Juan; 25.4–26.8 °C) have annual rainfall <1,400 mm (Tables 1–2). Further quantitative analyses to determine the relationship between temperature or rainfall and peak abundance of *Ae. aegypti* would be of great interest but are hindered by lack of standardization across studies in terms of life stage(s) targeted, temporal sampling scheme, and collection methodology used.

**Specific Impacts of Temperature on the Bionomics of *Ae. aegypti***

We attempt to synthesize the knowledge, accumulated since the late 19th century, of how temperature impacts the bionomics of different life stages of *Ae. aegypti*. The literature initially was queried using a search of the Web of Science database conducted in June 2013 (and repeated in January 2014). The search spanned the years 1898 to present and used the following search strings for topic: 1) “temperature” AND “aegypti”; 2) “temperature” AND “fasciata”; and 3) “temperature” AND “calopus”. The keywords *fasciata* and *calopus* were included to ensure that the search picked up publications using the junior synonyms *Stegomyia fasciata* (Fabricius), *Stegomyia calopus* (Meigen), or *Aëdes calopus* Meigen. Additional searches using the same key words were conducted in PubMed and the Armed Forces Pest Management Board’s Literature Retrieval System. The snowball technique, which identifies additional publications based on referenced materials, was then employed to identify additional publications of interest. The sub-sections below address how near constant or intentionally fluctuating temperature conditions
impact development times and survival of *Ae. aegypti* eggs and immatures, as well as survival, biting activity, and the gonotrophic cycle of females.

**Impact of near constant water temperature on development times of eggs and immatures.** Although early publications on this topic often include observations that are qualitative in nature or present quantitative results for which the underlying experimental design is insufficiently described, they display a basic understanding of how temperature influences development times. For example, Reed and Carroll (1901) not only noted that under “favorable conditions of warmth – i.e., summer and incubator temperature” the accumulated time for development of eggs, larvae, and pupae of *St. fasciata* is 12 days, but also that larvae kept at lower temperature (20 °C) develop slowly and require about 20 days to reach the pupal stage. A large number of books or papers have presented quantitative data on development times of eggs or immatures of *Ae. aegypti* kept under near constant water temperatures (Reed and Carroll 1901; Marchoux et al. 1903; Francis 1907; Newstead and Thomas 1910; Howard et al. 1912; Bacot 1916; Fielding 1919; Buxton and Hopkins 1927; Bonne-Wepster and Brug 1932; Bliss and Gill 1933; Shannon and Putnam 1934; Johnson 1937; Trager 1937; Headlee 1940, 1941, 1942; De Meillon et al. 1945; Farid 1949; Horsfall 1955; Bar-Zeev 1958; Christophers 1960; Surtees 1961; Ofuji 1963; Fay 1964; Keirans and Fay 1968; Hoffman 1971; McCray and Schoof 1972; Hien 1975a, b; Gilpin and McClelland 1979; Smith et al. 1988; Rueda et al. 1990; Wu and Chang 1993; Sames 1999; Tun-Lin et al. 2000; Kamimura et al. 2002; Lounibos et al. 2002; Chang et al. 2007; Dickerson 2007; Farnesi et al. 2009; Mohammed and Chadee 2011; Padmanabha et al. 2011a, b; Richardson et al. 2011, Farjana et al. 2012; Carrington et al. 2013a).

In Figures 1A–3A, we present data points for mean development rate (1 / development time in hours) of eggs, larvae, or pupae in relation to temperature from a subset of the above-

Here we present data based on mean development time, rather than median development time (such as presented by Gilpin and McClelland 1979), because data for mean development time are far more plentiful than for median time. Moreover, a study by Rueda et al. (1990) that presents both mean and median development times for larvae and pupae across a series of temperatures ranging from 15–34 °C show the data for mean and median times to be similar (typically within 0.15 days for larvae and 0.05 days for pupae) and strongly correlated ($R^2 > 0.99$ for both larvae and pupae). When there was no survival for a given life stage at a given temperature, the development rate is considered to be zero.

The general shape of the relationship between temperature and development rate is similar for eggs, larvae, and pupae: once the lower developmental zero temperature (10–14 °C) is exceeded there is a near linear relationship up to 30 °C above which the development rate is relatively stable or even decreases slightly before falling dramatically near the upper developmental zero temperature, which occurs at approximately 38–42 °C (Figures 1A–3A).

Despite incorporating data from numerous different studies that cover nearly 100 years and
include a wide range of *Ae. aegypti* strains, the plotted relationships for larvae and pupae are remarkably clear. The primary drawback to merging data from multiple studies is that experimental conditions will differ, especially in terms of food provided for the larvae and the density of the larvae in their rearing containers, both of which can impact development time as well as survival (Bacot 1916; Atkin and Bacot 1917; Young 1922-23; Buxton and Hopkins 1927; Bonne-Wepster and Brug 1932; Shannon and Putnam 1934; Trager 1937; De Meillon et al. 1945; Bar-Zeev 1957a; Christophers 1960; Fay 1964; Keirans and Fay 1968; Southwood et al. 1972; Gilpin and McClelland 1979; Subra and Mouchet 1984; Arrivillaga and Barrera 2004; Barrera et al. 2006; Maciá 2006; Legros et al. 2009; Padmanabha et al. 2010, 2011b; Walsh et al. 2011; Farjana et al. 2012). On the other hand, individual studies often use a single strain of *Ae. aegypti* that could be adapted to specific temperature conditions and therefore may produce results that are outliers in the “bigger picture” for the species. Larval development rates, in relation to temperature, are shown in Figure 4 for four separate experimental series that use different *Ae. aegypti* strains (Bar-Zeev 1958, Wu and Chang 1993, Rueda et al. 1990, Tun-Lin et al. 2000). The plotted data suggest that the strains have different larval development rates in the 20–36 °C range; for example, the development rate per hour varies from 0.0017–0.0045 at 20 °C and 0.0062–0.0084 at 30 °C. This variability among strains underscores the inherent danger in basing a temperature-development rate relationship used in models for *Ae. aegypti* population dynamics on data for a single strain of the mosquito.

Based on the data shown in Figures 1A–3A, and additional observations from the literature not included as data points in these figures (from Fielding 1919, Davis 1932, Bar-Zeev 1958, and Christophers 1960), it appears that the lower water temperature at which development is arrested for a given life stage and specimens fail to progress to the next life stage (the
developmental zero temperature) is in the 10–14 °C range for eggs, 13–14 °C range for larvae, and 10–12 °C range for pupae. Under the assumption of a near linear relationship between temperature and development rate from the lower developmental zero temperature to 28 °C, we can use the data plotted in Figures 1A–3A to first determine the linear relationship for a given life stage in the 15–28 °C range and then calculate the lower temperature at which the development rate reaches zero. Using this method, the estimate for the lower developmental zero temperature is 14.0 °C for eggs ($y = -0.01652 + 0.00118x$; ANOVA, $F_{1,2} = 268.75, r^2 = 0.992, P = 0.004$), 11.8 °C for larvae ($y = -0.004682 + 0.0003978x$; $F_{1,25} = 85.69, r^2 = 0.774, P < 0.001$), and 10.3 °C for pupae ($y = -0.012324 + 0.0012x$; $F_{1,38} = 284.21, r^2 = 0.882, P < 0.001$). Our estimate for the developmental zero water temperature for larvae of 11.8 °C, based on data for multiple strains of *Ae. aegypti*, is lower than the corresponding estimates for single strains of 13.4 and 12.1 °C reported by Gilpin and McClelland (1979) and Ofuji (1963), respectively, but higher than the estimates reported by Kamimura et al. (2002) for three different strains (averages of 8.4, 8.7, and 9.6 °C for larvae resulting in males and females combined) or the air temperature estimate of 8.5–9.1 °C given by Tsuda and Takagi (2001). Our estimate for the developmental zero temperature for pupae of 10.3 °C is similar to the estimates reported by Kamimura et al. (2002) (averages of 10.5, 10.8, and 11.3 °C for pupae resulting in males and females combined).

The relationship between temperature and mean development rate (Figures 1A–3A) is near linear in the 15 to 30–31 °C range for eggs ($F_{1,3} = 610.03, r^2 = 0.995, P < 0.001$) as well as larvae ($F_{1,31} = 147.57, r^2 = 0.826, P < 0.001$) and pupae ($F_{1,50} = 518.87, r^2 = 0.912, P < 0.001$). This agrees with the finding by Gilpin and McClelland (1979) of a linear relationship between temperature and median development rate for larvae in the 14–31 °C range. The development rates appear to reach their maximums around 30 °C and further increases to 35–36 °C result in
similar or even slightly decreasing development rates before they drop dramatically at higher temperatures (Figures 1A–3A). The higher water temperatures at which development is arrested for a given life stage appear to be in the 36–38 °C range for eggs, 36–42 °C range for larvae, and 38–42°C range for pupae. A few studies (Macfie 1920, Farid 1949, Christophers 1960, and Smith et al. 1988) give additional data for survival in the 38–44 °C range, but these were based on short exposures (minutes to 24 hours) after which the exposed life stage was moved to an optimal temperature rather than exposed to high temperature over the full development period. Most recently, Richardson et al. (2011) conducted stress tolerance tests over a 26–45 °C range which showed that, for Queensland strains, larvae were knocked down (ceased moving) at 43–44 °C.

Impact of near constant water temperature on egg hatching success and survival of immatures. There is a substantial literature on the impact of near constant water temperatures on the egg hatching success and survival of immatures of Ae. aegypti (Reed and Carroll 1901; Howard et al. 1912; Bacot 1916; Fielding 1919; Macfie 1920; Buxton and Hopkins 1927; Davis 1932; Ramsay and Carpenter 1932; Bliss and Gill 1933, Shannon and Putnam 1934; Headlee 1940, 1941, 1942; Hatchett 1946; Farid 1949; Woodhill 1949; Gander 1951; Horsfall 1955; Bar-Zeev 1957b, 1958; Christophers 1960; Surtees 1961; Ofuji 1963; Fay 1964; Keirans and Fay 1968; Mulla and Chaudhury 1968; Hoffman 1971; Crovello and Hacker 1972; McCray and Schoof 1972; Hien 1975c; Kasule 1986; Smith et al. 1988; Rueda et al. 1990; Wu and Chang 1993; Sames 1999; Tun-Lin et al. 2000; Kamimura et al. 2002; Lounibos et al. 2002; Chang et al. 2007; Dickerson 2007; Farnesi et al. 2009; Mohammed and Chadee 2011; Richardson et al. 2011; Carrington et al. 2013a). However, many of these studies are difficult to interpret in terms of field relevance as they present data for short exposures at a given temperature followed by
transfer to a near optimal temperature and subsequent documentation of survival. The data for eggs are particularly problematic as they include results based on use of freshly laid as well as mature (conditioned) eggs. In Figures 1B–3B, we present data points for survival of eggs, larvae, or pupae to the following life stage in relation to water temperature from a subset of the above-mentioned studies (eggs: Christophers 1960, Hoffman 1971, Farnesi et al. 2009; larvae Fielding 1919, Shannon and Putnam 1934, Bar-Zeev 1958, Keirans and Fay 1968, Smith et al. 1988, Wu and Chang 1993, Sames 1999, Tun-Lin et al. 2000, Kamimura et al. 2002, Mohammed and Chadee 2011, Richardson et al. 2011; pupae: Fielding 1919, Shannon and Putnam 1934, Farid 1949, Bar-Zeev 1958, Christophers 1960, Surtees 1961, Smith et al. 1988, Richardson et al. 2011). The included data points fulfill the following criteria: eggs, data based on examination of ≥100 eggs in a trial where the eggs were kept in water; larvae, data based on ≥25 larvae in a trial that starts with the first instar; pupae, data that are stated or can be reasonably assumed to be based on ≥20 pupae. In addition, Figure 5 shows data points for survival from the larval to adult stage in relation to temperature for a set of studies where both the larval and pupal stages were kept in water of a near constant temperature (Fielding 1919; Bar-Zeev 1958; Rueda et al. 1990; Sames 1999, AegH series; Tun-Lin et al. 2000; Richardson et al. 2011).

The lower and higher developmental zero temperatures, where eggs, larvae, or pupae fail to proceed to the next life stage, were already discussed in: *Impact of near constant water temperature on development times of eggs and immatures*. Between these developmental zero (no survival) temperatures, the proportion of eggs that hatched was ≥0.75 from 15–32 °C and ≥0.90 from 22–28 °C (Figure 1B). At higher temperatures, hatching success decreased rapidly from 0.90 at 32 °C to 0.48 at 35 °C and 0 at 36–38 °C (Figure 1B). For the larval stage, there appears to be a more gradual increase in survival with rising temperature. The proportion of
larvae surviving to the pupal stage reaches 0.10–0.77 at 15–16 °C (average of 0.52 within this temperature range), 0.54–0.92 at 20 °C (0.69), 0.73–0.97 at 24–26 °C (0.88), and typically exceeds 0.90 at 26.5–34 °C (0.92) (Figure 2B). The proportion of surviving larvae then falls at 35–36 °C, with two of four data points ≤0.06 (Figure 2B). For the pupal stage, the proportion emergence to adults near uniformly exceeds 0.90 in the 13–34 °C range (average of 0.96 within this temperature range) (Figure 3B). There are precipitous decreases in the proportion emergence to adults when temperatures fall to 11–12 °C or rise to 36–38 °C (Figure 3B).

Finally, the plot for proportion survival from the larval to adult stage is similar to the one for the larval stage, with the proportion of adult emergence reaching 0.03–0.24 at 14–15 °C (average of 0.17 within this temperature range), 0.21–0.72 at 16 °C (0.55), 0.63–0.95 at 20–26 °C (0.86), and 0.66–0.97 at 27–32 °C (0.88) before falling to 0.59–0.90 (0.74) at 34–35 °C and 0–0.81 (0.39) at 36–37 °C (Figure 5).

**Impact of fluctuating water temperature on the bionomics of Ae. aegypti immatures.** It has long been recognized that fluctuating temperatures impact the bionomics of *Ae. aegypti*. Reed and Carroll (1901) noted that, compared to a constantly high temperature, moving eggs into a cooler chamber at 20 °C for two hours daily resulted in delayed adult emergence (27 days rather than 15–18 days). Headlee (1940, 1941, 1942) and Keirans and Fay (1968) compared survival and development times for *Ae. aegypti* immatures kept under series of near constant water temperatures versus temperatures that broadly (± ~8 °C), moderately (± ~5.5 °C), or more narrowly (± ~3 °C) fluctuated cyclically around the near constant ones. The results of these pioneering studies are summarized in Table 3. The data for near constant temperatures, which fall within the 15–32 °C range, agree with the data plotted in Figures 2A–3A for this temperature range: increasing temperatures result in shorter development times and higher development rates
(Table 3). Not surprisingly, the strongest impact of fluctuating temperatures, compared to the corresponding near constant ones, occurred for the temperature ranges that approached or crossed the lower or higher developmental zero temperatures. For example, with broadly fluctuating cyclical temperatures survival from first instar larvae to adults was highest (60.0%) and development time to maximum emergence of adults shortest (20 days) for the 15.6–32.2 °C range, whereas exposure to the lower 10.0–26.7 °C range resulted in reduced survival (32%) and longer development time (23 days), and exposure to the higher 21.1–37.8 °C range resulted in minimal survival (2%) and an even longer development time (28 days) (Headlee 1940; Table 3).

Recent experimental studies confirm the older findings for development times and survival of *Ae. aegypti* immatures held under near constant versus intentionally fluctuating temperatures (Mohammed and Chadee 2011, Carrington et al. 2013a). Data from Mohammed and Chadee (2011) within the 24.5–34.5 °C range show minor reductions in larval survival and small increases in development time for fluctuating compared to near constant water temperatures (Table 3). Carrington et al. (2013a) examined the effects of an increasing daily temperature range (DTR) around a mean temperature of 26 °C on the immature stages and found that a large DTR of 18.6 °C resulted in slightly, but significantly, extended average development times from larva to female (12.4 days) compared to a smaller DTR of 7.6 °C (10.6 days) or the baseline DTR of 0 °C (10.6 days) (Table 3). Although survival to the pupal stage was high (> 0.85) for all examined conditions, there was a slight reduction for the large DTR relative to the smaller DTR or the constant temperature of 26 °C (Carrington 2013a; Table 3). Notably, these renewed studies on the impact of fluctuating temperatures on the bionomics of *Ae. aegypti* immatures were based on fluctuations around mean temperatures typical of the mosquito’s core distributional areas rather than mean temperatures typical for the cool range margins. They also
do not account for intra-container temperature gradients, which may occur commonly in larger
containers in the field and allow larvae to minimize exposure to unfavorable temperatures.

In a combined laboratory and field experiment, Richardson et al. (2011) compared
development times for immatures kept under near constant temperatures to those for immatures
placed under natural temperature conditions in Cairns, Queensland, Australia during October;
there were only minor differences (<0.5 days) in mean time to pupation or adult emergence for
immatures exposed to naturally fluctuating temperatures versus the prediction resulting from
data for constant temperatures being fitted to the Sharpe-Schoolfield model from Schoolfield et
al. (1981). Finally, Yang et al. (2009) examined development times and survival for immatures
kept under different temperatures for light (day; 10–14 hours) and dark (night; 10–14 hours)
conditions, with temperature pairs for light and dark ranging between a low extreme of 13.5 and
7.5 °C and a high extreme of 42 and 37 °C (i.e., 2–3.5 °C above and below 10, 15, 20, 25, 30, 35,
or 40 °C). A fitted curve for development rate reached its peak value around 25 °C and
approached zero values at 10–12 °C and 38–40 °C, and a fitted curve for survival indicated low
mortality (<10%) from 15–36 °C but rapidly increasing mortality for temperatures <15 °C or >36
°C.

**Impact of air temperature on egg hatching success.** The impact of air temperature on
egg hatching success is of interest because eggs may need to persist in a dry state for long
periods of time, during dry or cold parts of the year, before being exposed to water of a
temperature that allows for egg hatch to occur. The literature includes experimental laboratory
studies where eggs were exposed, for some period of time, to air of a given temperature before
being submerged in water of a temperature that allows for egg hatch to occur (Bacot 1916,
Fielding 1919, Buxton and Hopkins 1927, Bonne-Wepster and Brug 1932, Davis 1932, Shannon

Based on highly variable experimental conditions in the laboratory studies, including the time the eggs were allowed to mature before exposure to air of a given temperature (ranging from 0–14 days), different exposure times (ranging from 2 minutes to 69 days), or different relative humidity conditions (ranging from ~10 to 90–100%), it was not possible to meaningfully merge data across studies. However, individual studies still provide important insights, especially in terms of combinations of exposure times, temperatures, and humidity conditions.

Mulla and Chaudhury (1968) presented data for three different exposure temperatures (26.7, 32.2, or 37.8 °C), three exposure times (3, 4, or 5 days), and four relative humidity conditions (90–100, 67, 32, or 11% RH) for freshly laid eggs. For the highest air temperature of 37.8 °C, only the combination of the shortest (3–day) exposure time and the most humid conditions (90–100%) resulted in any of the eggs subsequently hatching (0.14 hatch rate). For 32.2 °C, eggs were found to subsequently hatch if the exposure RH was 90–100% (hatch rate of 0.51–0.79 across exposure times) or 67% (0–0.28) but none hatched if the RH was ≤32%. For the lowest examined air temperature of 26.7 °C, the subsequent egg hatch rate was high if the exposure RH was 90–100% (0.78-0.95 across exposure times) but decreased gradually when the RH decreased to 67% (0.28–0.82), 32% (0.05–0.13), or 11% (0–0.04). Dickerson (2007) presented data for five different exposure temperatures (15, 21, 27, 32, or 35 °C), six exposure
times (1, 2, 3, 4, 8, or 12 weeks) and three relative humidity conditions (95, 75, or 35% RH) for mature eggs. There were trends toward decreased egg hatch rates at 35% RH, compared to 95 or 75% RH, but this was not consistent across exposure temperatures. The decrease in egg hatch rate at lower RH appears to be less pronounced for mature eggs (Dickerson 2007) compared to fresh eggs (Mulla and Chaudhury 1968), likely resulting from mature eggs being less sensitive to low humidity. Moreover, for mature eggs Woodhill (1949) reported a hatch rate of 0.90 after 69 days exposure at 25–27 °C and 70–80 %, and Hien (1975c) recorded hatch rates of 0.60 after 2 months exposure at 25–26 °C and 60–70% RH, 0.46 after 3 months, and 0.14 after 4 months.

Other laboratory studies have examined the effect of extreme air temperatures on egg survival. For high temperatures, uniform mortality was reported following very short exposures (2–10 minutes) to 46–52 °C and for longer exposures (1–7 days) to 40–45 °C (Davis 1932, Smith et al. 1988). For low temperatures, mortality rates ≥0.99 were reported following air exposures of mature eggs to -5.5 °C for 48 hours, 1 °C for 9 days, 2.8 °C for 28–56 days, and 7 °C for 21 days (Davis 1932, Christophers 1960, McCray and Schoof 1972). However, eggs hatched following exposure to 1.1 °C for 3 days (hatch rate of 0.92), 2.8 °C for 1–4 or 7–14 days (0.41–0.73 and 0.07–0.34, respectively), 4.4 °C for 8 or 14 days (0.70 and 0.27, respectively), or to 7 °C for 11 days (0.25) (Woodhill 1949, Christophers 1960, McCray and Schoof 1972).

Some field studies on overwintering of eggs also deserve mention. Rozeboom (1939) established a colony of locally collected Ae. aegypti in Stillwater, OK in the summer of 1937 and in November-December of the same year placed mature eggs from this colony in containers kept within an old shed (exposed to outside temperatures but protected from rain and snow) or outdoors (exposed to the elements). The eggs were recovered in April of the following year, after experiencing several periods during which the minimum temperatures dropped considerably
below freezing for four to nine days in succession, and then immersed in water in the laboratory. Large numbers of eggs hatched from the container that had been kept in the shed, and the resulting larvae developed into vigorous adults, whereas only a single egg hatched from the container kept outdoors and the resulting specimen died in the pupal stage. Rozeboom (1939) noted that the great mortality among the eggs kept outdoors perhaps was due not so much to winter temperatures as it was to exposure to rain, snow and repeated freezing and thawing.

Similarly, Hatchett (1946) found that eggs in containers fully exposed to the elements over the winter of 1944–45 in Houston, TX had a lower hatch rate when brought into the laboratory and immersed in water (0.25) compared to those from containers placed in partially protected places such as in open sheds or under grass (0.31). Although substantial overwinter survival of eggs was reported from Houston, TX during the winters of 1943–44 (noted only as high) and 1944–45 (25–31%), and from Nagasaki, Japan during the winter of 1960–61 (46%), the resulting larvae were found to only rarely reach the adult stage (Chandler 1945, Hatchett 1946, Ofuji 1963). In Buenos Aires City, Argentina, overwinter survival of eggs for a 3-month period in 2008, with a mean temperature of ~13 °C, was 70% but the viability of the resulting larvae was not examined (Fischer et al. 2011).

**Impact of near constant or fluctuating temperatures on females.** The mobility and extensive use of indoor environments by *Ae. aegypti* females allow them to seek out a wide range of microhabitats to mitigate the effect of adverse temperature or humidity conditions. In addition, Xu et al. (2010) concluded that uncertainty in the estimate of nominal survival rate for females is the most important source of uncertainty for the prediction of population densities of all life stages by the Skeeter Buster simulation model, and called for more accurate and precise empirical estimates of this parameter. Although the field relevance of results from experimental
laboratory studies on the impact of temperature on the bionomics of females is highly uncertain, there are still observations from the literature worth mentioning. The broad impact of temperature on the females was clear early on: Finlay (1886) noted that the limits for functional activity were in the 15–38 °C range. Other early writers (Reed and Carroll 1901; Marchoux et al. 1903; Macfie 1915–1916, 1920; Connor 1924; Lewis 1933; Lumsden 1947) observed that the female is unlikely to bite when the air temperature is below 16.7–17 °C and that a temperature above 37 °C shortens life, diminishes the blood-sucking capability, and destroys fertility. Later studies confirmed that even brief exposure of females, kept at high humidity, to very high temperatures is detrimental: females uniformly survived 60 minutes of exposure to 39–40 °C but exposure for 15–30 minutes to 42–43 °C caused >65% mortality and exposure for 30 minutes to 45–51 °C was uniformly lethal (Christophers 1960, Smith et al. 1988). At the lower temperature extreme, adults can survive exposure to 4.4 °C for 24–72 hours (Woodhill 1949) and can survive for weeks at constant temperatures of 7–9 °C (Otto and Neumann 1905). The duration of forced experimental flight also is impacted by temperature, as it increases >10-fold from 10 to 15 °C, peaks from 15–27 °C, and then decreases to 35 °C where it is similar to the duration for 10 °C (Rowley and Graham 1968).

With regards to survival time for females, Lewis (1933) presented a series of data that demonstrated: 1) a gradual decrease in mean survival time with decreasing RH for starved females kept at 23 °C; and 2) increased mean survival time for starved females kept at 100% RH as the temperature decreased from 23–30 to 10 °C, perhaps due to decreasing levels of activity at lower temperatures. Bar-Zeev (1957b) presented a comprehensive data series for mortality of starved females kept under different combinations of temperature (0.5, 4, 8, 12, 28, 35, or 40 °C) and humidity (0, 43–47, 83–86, or 100% RH). At the lowest and highest temperatures (0.5 and
the time to reach 50% mortality was similar across the examined RH conditions, whereas for all other temperatures (4–35 °C) there was a consistent trend of increased time to reach 50% mortality with increasing RH for a given temperature condition. Finally, Lansdowne and Hacker (1975) observed strain-related variability in mean survival time for sugar-fed females, held at 27 °C and 70% RH, for strains from Houston, TX (20.8 days); Ocala, FL (25.0 days); and Carrizal, Venezuela (13.0 days).

For the length of the gonotrophic cycle, there is a dearth of controlled experimental studies comparing the time elapsed from blood meal to oviposition across different temperatures. Christophers (1960) noted that most references up to that point were of a rather general nature, and that estimation of the length of the gonotrophic cycle is complicated by that *Ae. aegypti* does not usually lay all its eggs in a single act of oviposition. Although the shape of the relationship between temperature and development rate for the gonotrophic cycle remains poorly defined, there seems to be agreement across studies that the length of the gonotrophic cycle decreases (the development rate increases) with temperature within the 18–30 °C range (Marchoux et al. 1903, Haddow and Gillett 1957, Christophers 1960, Focks et al. 1993a, de Almeida Costa et al. 2010).

Recent experimental studies have examined the effects of near constant versus fluctuating temperatures on female survival and reproductive success. Lambrechts et al. (2011) reported that an increasing DTR around a mean temperature of 26 °C resulted in decreased survival over the experimental period, from ~70% for a DTR of 0 °C to 50% for a DTR of 10 °C and 30% for a DTR of 20 °C. Follow-up studies comparing DTRs around a mean temperature of 26 °C of 0 °C, 7.6 °C and 18.6 °C demonstrated that the largest DTR of 18.6 °C, compared to a DTR of 0°C, resulted in reduced female survival and reproductive output (Carrington et al. 2013a, c). In contrast, there were no differences in the proportion of surviving females when comparing a near
constant temperature of 20 °C with a DTR of 18.6 °C around a mean temperature of 20 °C (range, 11.7–30.3 °C) or a near constant temperature of 30 °C with a DTR of 7.6 °C around a mean temperature of 30 °C (range, 27.1–34.7 °C) (Carrington et al. 2013b).

**Limitations for Existing Models to Predict Population Dynamics of *Ae. aegypti* at Its Cool Range Margins**

One of our aims was to compile published data on how temperature impacts development time and survival of eggs and immatures at lower water temperatures, in order to assess how well these data match up with the temperature-dependent development and survival curves used in two simulation models for population dynamics of *Ae. aegypti* – CIMSiM and Skeeter Buster (Focks et al. 1993a, b; Magori et al. 2009; Ellis et al. 2011). For development rates, the temperature-dependent curves used in CIMSiM and Skeeter Buster are based on enzyme kinetics models (Schoolfield et al. 1971, Sharpe and deMichele 1977). In the 16–35 °C range for water temperature, the shape of the plotted data for development rates and survival of eggs, larvae, or pupae in relation to temperature presented herein (Figures 1-3) agrees reasonably well with the shapes of the corresponding temperature-dependent curves for development rates and survival presented for CIMSiM by Focks et al. (1993a). Moreover, CIMSiM and Skeeter Buster have been field validated for cities with year-around potential for high abundance of *Ae. aegypti* (Scenario 1 in Tables 1-2; Bangkok, Thailand; and Iquitos, Peru) as well as for cities with year-around activity but only seasonal potential for high abundance (Scenario 2; Cairns and Townsville, Australia; and Key West, FL) and cities with only seasonal occurrence of the active stages but where overwintered eggs can hatch in the spring and produce viable larvae (Scenario 3; Jacksonville, FL; New Orleans, LA; Brownsville, TX; Charleston, SC; Memphis, TN; and Buenos Aires City, Argentina) (Focks et al. 1993b, Maguire et al. 1999, Williams et al. 2008,
Based on the data from Table 1, these cities have annual average air temperatures in the 16.8–27.8 °C range and monthly average temperatures ≥16 °C for 7–12 months of the year.

The utility of existing versions of CIMSiM and Skeeter Buster is less certain in the lower 10–16 °C water temperature range, which represents temperatures that *Ae. aegypti* is likely to encounter in cool range margin cities where annual average air temperatures often are below 15 °C and monthly average temperatures in most cases reach 16 °C for no more than 5 months of the year (Scenario 4 in Table 1). There are several potential problems associated with use of the current versions of CIMSiM and Skeeter Buster at low temperatures. First, the plotted data for development rates of eggs and immatures in relation to water temperature in the 10–19 °C range (Figures 1A–3A) are suggestive of linear relationships that abruptly are interrupted at lower temperature thresholds where development is arrested, ~12–14 °C, rather than the more gradually decreasing enzyme kinetics model-based development rate curves that are used in CIMSiM and Skeeter Buster for the 10–14 °C range under the assumption of 100% daily survival at these temperatures.

Second, the plotted data for survival of eggs and immatures over the full developmental period in relation to temperature (Figures 1B–3B) differs from the temperature-dependent daily survival curves used in CIMSiM. Specifically, Focks et al. (1993a) present daily survival curves based on 100% survival for eggs above 0 °C and for larvae and pupae at temperatures ≥10 °C, which also implies 100% survival over the full developmental period above these thresholds. The data presented herein instead indicate that survival over the full developmental period at 15–16 °C already has fallen to ~80% for eggs, 10–77% for larvae, ~90% for pupae, and 3–72% when both the larval and pupal stages are considered together (Figures 1B–3B, 5). Although
data for the 10–14 °C range are very limited, they clearly indicate very low survival for the larval and pupal stages combined (0% for 10–13 °C and ~25% for 14 °C; Figure 5) in this temperature range. For eggs, the data are even more scarce but Christophers (1960) noted that exposure to 7 °C inhibits development.

Third, we still have very poor knowledge of the impact of naturally fluctuating temperatures on development times and survival of immatures when the lower end of the temperature fluctuations approach or fall below the lower developmental zero temperature. Repeated fluctuations across the lower developmental zero temperature may induce stress at the organismal level that is not adequately captured by the enzyme kinetics models used in CIMSiM and Skeeter Buster. We speculate that this could be manifested by a delayed re-start of the developmental process, compared to what is possible at the enzymatic level, when the temperature first approaches and then exceeds the lower developmental zero temperature. Should this occur, it will result in a longer development time compared to the prediction from the enzyme kinetics models. Moreover, stress induced by repeated fluctuations across the lower developmental zero temperature also may lead to reduced survival compared to the expectations based on experimental data where immatures were held at near constant temperature.

A final consideration is our minimal knowledge of the extent to which *Ae. aegypti* populations persisting at cool range margins can adapt to develop in low water temperatures. Such adaptation could allow larvae to develop more rapidly, compared to the prediction from the enzyme kinetics models used in CIMSiM and Skeeter Buster, and lead to immatures being more likely to reach adult emergence at low water temperature. Experimental laboratory data suggest that adaptation, with increased survival of the larval stage, can occur after short-term exposure of larvae from multiple generations to low temperatures of 5 °C (Chang et al. 2007). In conclusion,
available population dynamics models for *Ae. aegypti* likely produce robust predictions for water
temperatures in the 16–35 °C range, which includes the geographic areas where *Ae. aegypti* and
its associated pathogens present the greatest threat to human health, but may be less reliable in
cool range margins where water temperatures regularly fall below 15°C.

Buenos Aires City is of particular interest because it is an example of a city: 1) located
near the margin of the core distributional area for *Ae. aegypti* (Vezzani and Carbajo 2008, Díaz-
Nieto et al. 2013); 2) characterized by distinctly seasonal activity with moderate abundance for
the active stages of the mosquito, which are absent during part of the year due to unfavorably
cold conditions, but where eggs can overwinter (de Garín et al. 2000, Vezzani et al. 2004,
Fischer et al. 2011, De Majo et al. 2013); and 3) for which Skeeter Buster as well as another
population dynamics model for *Ae. aegypti* have been validated (Otero et al. 2006, 2008; Legros
et al. 2011). Downstream, we plan to compare more detailed temperature profiles for the two
coolest cities under Scenario 3 (Seasonal potential for moderate to high mosquito abundance and
successful overwintering of eggs; Buenos Aires City and Memphis) with those for the cities
under Scenario 4 (Seasonal potential for low mosquito abundance and no or minimal
overwintering of eggs; Stillwater; Baltimore; Puebla City, México; and Neuquén City,
Argentina). Based on the data in Table 1, Buenos Aires City and Memphis have a combination
of annual average temperatures >16.5 °C, lowest monthly average temperatures >4 °C, and
highest monthly average temperatures >24 °C. None of the four cities listed under Scenario 4
fulfill more than one of these three criteria (Table 1). The temperature profiles for this suite of
six cities may prove useful in a first step to extrapolate where hypothetical cool range margins
for *Ae. aegypti* occur in different parts of the world.
Potential Impacts of Climate Warming or Changes to Rainfall on Human Risk for Exposure to *Ae. aegypti* Females at the Cool Range Margins

Assuming that availability of water-filled containers to serve as development sites for immatures, water nutrient content, larval density, or presence of biological competitors do not become limiting factors, the risk for human exposure to *Ae. aegypti* females can be impacted in two basic ways by climate warming: 1) increased number of annual days with females being active and potentially biting humans (extended active season); and 2) increased peak abundance of females (higher upper limit for population growth). For areas where *Ae. aegypti* is forced to overwinter in the egg stage because cold temperatures during part of the year prevent activity by the immature or adult stages, rising temperatures could lead to increased overwinter survival of eggs, earlier hatching of eggs in the spring, and thus earlier emergence of host-seeking females, and later cessation of blood-feeding activity by females in the fall. Increased temperatures also may reduce development times for eggs, larvae, and pupae, as well as reduce the duration of the gonotrophic cycle of the female, with the overall effect of a shortened generation time and, consequently, increased potential for population growth (see references in: *Specific Impacts of Temperature on the Bionomics of Ae. aegypti*). One potentially very important, but poorly understood, confounder is the extensive use of indoor environments by females and development of immatures in water-filled containers stored indoors.

2006, García-Rejón et al. 2008, Codeço et al. 2009, Azil et al. 2010, Oo et al. 2011, Campos et al. 2012, de Melo et al. 2012). However, as the availability of water-filled containers depends not only on rainfall but also is influenced by water storage through human action, the extent of the impact of increased or decreased rainfall on population dynamics of *Ae. aegypti* likely will be varied and location-specific (Moore et al. 1978, Kuno 1995, Rodhain and Rosen 1997, Halstead 2008).

To quantify risk for human exposure to *Ae. aegypti* females, we should consider both the number of days when females are active and can bite, and their temporal abundance pattern. The relative impact of climate warming on the cumulative abundance of *Ae. aegypti* females over the active season reasonably will be greatest at the cool range margins and smallest in core distributional areas with already near optimal temperature conditions for the mosquito to proliferate. However, it is important to remember that the absolute impact on the cumulative abundance of *Ae. aegypti* females (and thus the basic risk for human bites) may be greatest in settings with intermediately suitable temperature conditions, or perhaps even in settings with near optimal temperature conditions, rather than at the cool margins. Overall risk for human exposure to *Ae. aegypti* females can be viewed as the product of entomological risk for human exposure to *Ae. aegypti* females (their abundance) and the degree of mosquito-human contact (the proportion of females that contact and bite humans). The impact of climate change on the likelihood that mosquito-human contact occurs undoubtedly will be varied and location-specific. Mosquito-human contact is influenced by socioeconomic conditions (e.g., use of air conditioning or intact screens to preclude mosquitoes from the indoor environment or water storage resulting from unreliable access to piped water) as well as human behavior (e.g., use of repellents or specific behaviors that result in increased or decreased contact with mosquitoes) and diel...
microclimatic conditions (e.g., the portions of the diel period during which indoor or outdoor microclimatic conditions favor mosquito host-seeking activity).

Needs for Biosurveillance and Research at the Cool Range Margins for *Ae. aegypti*

There are several notable knowledge or data gaps that hinder our ability to predict risk of human exposure to *Ae. aegypti* at the cool margins of its geographic range now and in the future. The locations of the cool range margins for *Ae. aegypti* remain poorly defined. This unfortunate situation arises partly from, understandably, limited biosurveillance in areas where *Ae. aegypti* is scarce and presents only a minor threat to human health, and partly from a lack of field research studies specifically designed to determine occurrence and abundance of the mosquito along geographic transects perceived to bisect the cool range margin, such as the study along an elevation gradient in México presented by Lozano-Fuentes et al. (2012). As the locations of the cool range margins are not well defined, it is very difficult to generate data for water and air temperature conditions – and for other important factors such as key container types, water nutrient conditions, larval abundance, presence of biological competitors, and access for adults to indoor environments – characteristic of the cool range margins. There also is a dearth of data from controlled experimental studies for how water temperatures in the 10–16 °C range, especially for fluctuating temperatures that repeatedly fall below the life stage-specific lower developmental zero temperatures, impact development time and survival of *Ae. aegypti* eggs and immatures. Such studies also need to account for a range of different container types with variable intra-container temperature gradients as well as variable nutrient conditions and larval densities. One unfortunate result of these data gaps is that they prevent us from assessing the reliability of existing models for population dynamics of *Ae. aegypti* at low water temperatures.
We also have a very poor understanding of the specific climatic conditions that allow for eggs to overwinter and produce viable larvae in the following spring. This is of concern because overwinter survival of eggs has tremendous impact on the potential for population growth during the warm part of the year, including to determine whether local populations of *Ae. aegypti* can persist from year-to-year or if they die off every winter and then need to be re-established annually through accidental importation of eggs or immatures through human action (i.e., movement of infested containers). Moreover, an improved understanding of the extent to which local mosquito populations in the cool margins result from locally derived specimens versus ones originating from eggs or immatures brought in from warmer areas would enhance our understanding of the potential for adaptation to develop and survive in cooler settings.

Overcoming the above-mentioned knowledge and data gaps will require a combination of: 1) field biosurveillance designed to better define the locations of the cool range margins for *Ae. aegypti*; 2) field studies to clarify the air and water temperatures, key container types, water nutrient conditions, larval densities, and biological competitors and predators that the mosquito encounters at the cool range margins; 3) laboratory studies where strains of *Ae. aegypti* originating from different geographic areas, including cool range margins, are assessed for how field-relevant near constant and fluctuating water temperatures, in concert with water nutrient conditions and larval densities, impact development rates and survival of eggs and immatures in different container types with variable intra-container temperature gradients; 4) experimental studies to assess overwinter survival of eggs under field conditions where the microclimate of the container environment is closely monitored and in the laboratory for simulated field-relevant winter temperature and humidity conditions; and 5) potentially adapting simulation models for population dynamics of *Ae. aegypti* to more accurately account for development rates and
survival of eggs and immatures for low water temperatures, and then to field validate model predictions.

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(*Stegomyia*) *aegypti* (L.) and *Aedes* (*Stegomyia*) *albopictus* Skuse in selected areas in


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Table 1. Long-term monthly and annual average 24-hour air temperatures for selected cities from 1961–1990.

<table>
<thead>
<tr>
<th>City</th>
<th>Monthly average air temp. (°C)</th>
<th>Average 24-hour air temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Jan</td>
<td>Feb</td>
</tr>
<tr>
<td><strong>Scenario 1: Year-around potential for high abundance of Ae. aegypti</strong></td>
<td></td>
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<tr>
<td>Bangkok, Thailand®</td>
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<tr>
<td>Manhattan, Philippines®</td>
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<td></td>
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<tr>
<td>Singapore City, Singapore®</td>
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<tr>
<td>Djakarta, Indonesia®</td>
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<tr>
<td>Iquitos, Peru®</td>
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<tr>
<td><strong>Scenario 2: Year-around activity by Ae. aegypti but only seasonal potential for high abundance</strong></td>
<td></td>
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<tr>
<td>Chiang Mai, Thailand®</td>
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<td>New Delhi, India®</td>
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<td>Cairns, Australia®</td>
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<td>Key West, FL®</td>
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<td>Veracruz, Mexico®</td>
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<tr>
<td>Rio de Janeiro, Brazil®</td>
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<tr>
<td><strong>Scenario 3: Only seasonal occurrence of Ae. aegypti active stages (moderate to high abundance) but overwintered eggs can hatch in the spring and produce viable larvae</strong></td>
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<td>New Orleans, LA®</td>
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<td>Charleston, SC®</td>
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<td>Memphis, TN®</td>
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<tr>
<td>Buenos Aires City, Argentina®</td>
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</tr>
<tr>
<td><strong>Scenario 4: Only seasonal occurrence of Ae. aegypti active stages (in low numbers) and with overwintered eggs unlikely to both hatch and produce viable larvae</strong></td>
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<tr>
<td>Stillwater, OK®</td>
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<tr>
<td>Baltimore, MD®</td>
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<td>Puebla City, Mexico®</td>
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<tr>
<td>Neuquén City, Argentina®</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

aData from the Global Historical Climatology Network version 3 (Lawrimore et al. 2011); bData from the National Climatic Data Center’s TD 9641 Clim 81 1961–1990 Normals dataset.
Table 2. Long-term monthly and annual average rainfall for selected cities from 1961–1990.

<table>
<thead>
<tr>
<th>City</th>
<th>Average rainfall (mm)</th>
<th>Monthly average rainfall (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Jan</td>
<td>Feb</td>
</tr>
<tr>
<td>Scenario 1: Year-around potential for high abundance of Ae. aegypti</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bangkok, Thailand^a</td>
<td>9</td>
<td>30</td>
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<tr>
<td>Manila, Philippines^a</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Singapore City, Singapore^a</td>
<td>198</td>
<td>154</td>
</tr>
<tr>
<td>Djakarta, Indonesia^a</td>
<td>390</td>
<td>288</td>
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<tr>
<td>Iquitos, Peru^a</td>
<td>279</td>
<td>227</td>
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<tr>
<td>Scenario 2: Year-around activity by Ae. aegypti but only seasonal potential for high abundance</td>
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<td></td>
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<tr>
<td>Chiang Mai, Thailand^a</td>
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<td>5</td>
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<td>New Delhi, India^a</td>
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<td>Cairns, Australia^a</td>
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<td>422</td>
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<td>Key West, FL^b</td>
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<td>46</td>
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<td>San Juan, Puerto Rico^a</td>
<td>71</td>
<td>55</td>
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<tr>
<td>Veracruz, México^a</td>
<td>22</td>
<td>17</td>
</tr>
<tr>
<td>Rio de Janeiro, Brazil^a</td>
<td>114</td>
<td>105</td>
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<tr>
<td>Scenario 3: Only seasonal occurrence of Ae. aegypti active stages (moderate to high abundance) but overwintered eggs can hatch in the spring and produce viable larvae</td>
<td></td>
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<tr>
<td>Jacksonville, FL^b</td>
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<td>100</td>
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<td>New Orleans, LA^b</td>
<td>128</td>
<td>153</td>
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<td>Brownsville, TX^b</td>
<td>40</td>
<td>27</td>
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<tr>
<td>Charleston, SC^b</td>
<td>88</td>
<td>84</td>
</tr>
<tr>
<td>Memphis, TN^b</td>
<td>95</td>
<td>110</td>
</tr>
<tr>
<td>Buenos Aires City, Argentina^a</td>
<td>119</td>
<td>118</td>
</tr>
<tr>
<td>Scenario 4: Only seasonal occurrence of Ae. aegypti active stages (in low numbers) and with overwintered eggs unlikely to both hatch and produce viable larvae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stillwater, OK^b</td>
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<td>39</td>
</tr>
<tr>
<td>Baltimore, MD^a</td>
<td>80</td>
<td>81</td>
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<td>Puebla City, México^a</td>
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<td>7</td>
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<tr>
<td>Neuquén City, Argentina^a</td>
<td>16</td>
<td>15</td>
</tr>
</tbody>
</table>

^aData from the Global Historical Climatology Network version 2 (Peterson and Vose 1997); ^bData from the National Climatic Data Center’s TD 9641 Clim 81 1961–1990 Normals dataset.
Table 3. Impact of near constant versus fluctuating temperatures on development time and survival of *Ae. aegypti* immatures.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Starting to final life stage</th>
<th>Near constant temperature</th>
<th>Fluctuating temperature</th>
<th>Differential (data for fluctuating temp. minus data for constant temp.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headlee 1940</td>
<td>Larva (1^st^ instar) to Adult</td>
<td>18.3 (°C) 0.17 / 0.32</td>
<td>10.0 / 26.6 (°C) 0.32</td>
<td>23.0 / 0.15 (°C) -1.5</td>
</tr>
<tr>
<td>Headlee 1940</td>
<td>Larva (1^st^ instar) to Adult</td>
<td>23.9 (°C) 0.74 / 0.86</td>
<td>15.6 / 32.2 (°C) 0.60</td>
<td>20.0 / 0.35 (°C) -0.37</td>
</tr>
<tr>
<td>Headlee 1940</td>
<td>Larva (1^st^ instar) to Adult</td>
<td>29.4 (°C) 0.73 / 0.91</td>
<td>21.1 / 37.7 (°C) 0.91</td>
<td>17.7 / 0.05 (°C) -6.5</td>
</tr>
<tr>
<td>Headlee 1941</td>
<td>Larva (1^st^ instar) to Adult</td>
<td>18.3 (°C) 0.79 / 20.0</td>
<td>14.4 / 24.0 (°C) 0.77</td>
<td>32.0 / -0.02 (°C) 1.5</td>
</tr>
<tr>
<td>Headlee 1942</td>
<td>Larva (1^st^ instar) to Adult</td>
<td>18.9 (°C) 0.90 / 0.91</td>
<td>22.5 / 24.0 (°C) 0.91</td>
<td>22.0 / 0.01 (°C) -0.5</td>
</tr>
<tr>
<td>Headlee 1942</td>
<td>Larva (1^st^ instar) to Adult</td>
<td>23.6 (°C) 0.83 / 0.91</td>
<td>20.0 / 27.2 (°C) 0.89</td>
<td>13.0 / 0.06 (°C) -1.5</td>
</tr>
<tr>
<td>Headlee 1942</td>
<td>Larva (1^st^ instar) to Adult</td>
<td>30.0 (°C) 0.69 / 0.77</td>
<td>27.2 / 32.8 (°C) 0.77</td>
<td>10.5 / 0.08 (°C) 0</td>
</tr>
<tr>
<td>Keirans &amp; Fay 1968(^a)</td>
<td>Larva (1^st^ instar) to Pupa</td>
<td>15.6 (°C) 0.46 / 0.61</td>
<td>10.0 / 21.1 (°C) 0.61</td>
<td>21.0 / -0.15 (°C) -12/ -5</td>
</tr>
<tr>
<td>Keirans &amp; Fay 1968(^a)</td>
<td>Larva (1^st^ instar) to Pupa</td>
<td>21.1 (°C) &gt;0.90 / 0.90</td>
<td>15.6 / 26.7 (°C) &gt;0.90</td>
<td>9 / 0.15 (°C) --</td>
</tr>
<tr>
<td>Keirans &amp; Fay 1968(^a)</td>
<td>Larva (1^st^ instar) to Pupa</td>
<td>26.7 (°C) &gt;0.90 / 0.90</td>
<td>5 / 8 (°C) &gt;0.90</td>
<td>6 / 0 (°C) --</td>
</tr>
<tr>
<td>Keirans &amp; Fay 1968(^a)</td>
<td>Larva (1^st^ instar) to Pupa</td>
<td>32.2 (°C) &gt;0.90 / 0.90</td>
<td>26.7 / 37.8 (°C) &gt;0.90</td>
<td>5 / 0 (°C) --</td>
</tr>
<tr>
<td>Mohammed &amp; Chadee 2011(^b)</td>
<td>Larva (1^st^ instar) to Pupa</td>
<td>27 (°C) 0.98 / 0.98</td>
<td>25.4 / 29.5 (°C) 0.86</td>
<td>6 / -0.12 (°C) 1</td>
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<tr>
<td>Mohammed &amp; Chadee 2011(^b)</td>
<td>Larva (1^st^ instar) to Pupa</td>
<td>30 (°C) 0.97 / 0.97</td>
<td>24.5 / 32.5 (°C) 0.89</td>
<td>7 / -0.08 (°C) 3</td>
</tr>
<tr>
<td>Mohammed &amp; Chadee 2011(^b)</td>
<td>Larva (1^st^ instar) to Pupa</td>
<td>33 (°C) 0.92 / 0.92</td>
<td>24.5-34.5 (°C) 0.86</td>
<td>6 / -0.06 (°C) 1</td>
</tr>
<tr>
<td>Carrington et al. 2013(^a)</td>
<td>Larva (1^st^ instar) to Adult</td>
<td>26 (°C) 0.92 / 0.92</td>
<td>10.6 / 9.8 (°C) 0.92 / 0.91</td>
<td>10.6 / 0.01 (°C) 0 / -0.2</td>
</tr>
<tr>
<td>Carrington et al. 2013(^a)</td>
<td>Larva (1^st^ instar) to Adult</td>
<td>26 (°C) 0.92 / 0.92</td>
<td>10.6 / 9.8 (°C) 0.92 / 0.92</td>
<td>12.4 / 11.3 (°C) -0.01 / -0.02 (°C) 1.8 / 1.5</td>
</tr>
</tbody>
</table>

\(^a\)Based on data for the full food condition presented in Table 1 of the paper; \(^b\)Near constant and fluctuating temperatures (mid-points for the 1 °C ranges for minimum and maximum values) were matched based on the data presented in Table 1 of the paper; \(^c\)To the pupal stage for generation 2 / 23; \(^d\)To maximum emergence for adults; \(^e\)To first pupation / 90% pupation; \(^f\)To ≥ 50% pupation; \(^g\)To Female / Male; \(^h\)Based on data presented in Figure 1 of the paper.
**Figure legends**

**Figure 1.** Development rates for *Ae. aegypti* eggs (A) and proportion of eggs hatching (B) when held at near constant temperature, ranging from 1–38 °C, based on a compilation of previously published data (Christophers 1960, Hoffman 1971, Farnesi et al. 2009).

**Figure 2.** Development rates for *Ae. aegypti* larvae (A) and proportion of survival from the larval to pupal stage (B) when held at near constant temperature, ranging from 9–52 °C, based on a compilation of previously published data (Fielding 1919, Shannon and Putnam 1934, Bar-Zeev 1958, Keirans and Fay 1968, Smith et al. 1988, Rueda et al. 1990, Wu and Chang 1993, Sames 1999, Tun-Lin et al. 2000, Kamimura et al. 2002, Mohammed and Chadee 2011, Richardson et al. 2011).

**Figure 3.** Development rates for *Ae. aegypti* pupae (A) and proportion of survival from the pupal to adult stage (B) when held at near constant temperature, ranging from 7–52 °C, based on a compilation of previously published data (Fielding 1919, Shannon and Putnam 1934, Farid 1949, Bar-Zeev 1958, Christophers 1960, Surtees 1961, Ofuji 1963, Smith et al. 1988, Rueda et al. 1990, Tun-Lin et al. 2000, Kamimura et al. 2002, Richardson et al. 2011).

**Figure 4.** Comparison of development rates for *Ae. aegypti* larvae of different strains – Raleigh, NC (Rueda et al. 1990); Thursday Island, north Queensland, Australia (Tun-Lin et al. 2000); and two strains of unknown origin (Bar-Zeev 1958, Wu and Chang 1993) – held at near constant temperature, ranging from 15–36 °C.
Figure 5. Proportion of survival from the larval to adult stage for *Ae. aegypti* held at near constant temperature, ranging from 9–40 °C, based on a compilation of previously published data (Fielding 1919, Bar-Zeev 1958, Rueda et al. 1990, Sames 1999, Tun-Lin et al. 2000, Richardson et al. 2011).
Figure 1
Figure 2
Figure 4
Figure 5