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Effects of different temperature regimens on the development of *Aedes aegypti* (L.) (Diptera: Culicidae) mosquitoes

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ABSTRACT

This study was conducted to determine the effects of increased water temperatures on the development of *Aedes aegypti* immatures under laboratory conditions in Trinidad, West Indies using temperature regulated water baths to cover a range of temperatures from 24–25 °C to 34–35 °C at a relative humidity of 80%. Two experiments were designed: (1) at constant temperature regimens and (2) under diurnal temperature regimens ranging from 24–25 °C to 34–35 °C. At 24–25 °C egg hatching success was 98% at 48 h, however at 34–35 °C egg hatching rates declined to 1.6% after 48 h. *Ae. aegypti* larvae reared under constant temperature regimens showed pupation on day 4 with highest pupation occurring at 30 °C (78.4%) However, under diurnal temperature regimens, pupation began on day 4 but only at the higher temperatures of 30–35 °C. Under diurnal temperature regimens ranging from 24 °C to 35 °C significantly more females emerged at higher temperatures, than males. In contrast, at constant temperatures of 24–35 °C no significant difference in M/F ratios were observed. The body size of *Ae. aegypti* reared at constant temperature regimens was significantly larger than males and females larvae reared under diurnal temperature regimens of 25–30 °C. The results of this study are discussed in the context of changing or increasing water temperatures, seasonal changes in vector populations and vector competence. Using these key factors control strategies are recommended to manage vector populations as expected increases in temperatures impact the Caribbean region.

1. Introduction

Advances in the science of climate change and in the epidemiology of dengue fever (DF) have demonstrated marked changes in disease transmission patterns (Gubler and Kuno, 1997; Guha-Sapir et al., 2005; Chadee et al., 2007) and changes in the behaviour and ecology of the dengue vector, *Aedes aegypti* (L.) mosquitoes (Chadee and Martinez, 2000; Chen et al., 2006; Hemme et al., 2010). Within the Caribbean and Latin American region outbreaks of dengue fever and its hemorrhagic manifestations were known to occur in cycles, occurring every 5–10 years (Gubler and Kuno, 1997) but recent changes in the epidemiology of the disease have resulted in an inc rease in the frequency of outbreaks.

The mosquito vector *Ae. aegypti* is known to be able to adapt to varying environmental conditions, as it is poikilothermic and has shown seasonal increases in the vector population and seasonal variability in vector competence (Paupy et al., 2003). Multiple anthropogenic and biological factors have been attributed to the changing epidemiology of DF and ecology and behaviour of the vector: including demographic changes in the human populations (Gubler and Kuno, 1997; Chadee, 2004), urbanization (Chadee, 2010), speed and volume of international traffic (Gubler and Kuno, 1997; Chadee and Martinez, 2000), introduction of new dengue genotypes (Rico-Hesse, 1990), failure of vector control programs due to insecticide resistance (Rawlins, 1998; Polson et al., 2011), poor management practice (Rosenbaum et al., 1995; Chadee et al., 2004) and seasonal peaks in the vector population and in dengue occurrence (Chen et al., 2006; Chadee et al., 2007).

Recent studies on climate variability and climate change forecast a global increase in temperatures of 1.4–5.8 °C (IPCC, 2007) and these changes can impact development times and the vectorial capacity of the *Ae. aegypti* mosquito. The speed and duration of larval development is governed by a series of internal and external factors (Christophers, 1960; Clements, 1999) but one of the most important external drivers is temperature. Some studies have shown that the age at pupation and adult size of various mosquito species may reflect the environmental conditions during growth of the larval stages (Reisen et al., 1984; Fish, 1985; Haramis, 1985; Lyimo et al., 1992). Laboratory studies have also shown that larvae reared at high temperatures and under food stress conditions develop into small adults and experience high mortality (Reisen et al., 1984; Siddiqui et al., 1976). Conversely, larvae reared at high temperatures and fed optimally developed into large adults (Tun-Lin et al., 2000). Kamimura et al. (2002) reported that larval

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rearing temperatures can have a major impact on disease transmission by affecting body size, development time and production. It is therefore important to conduct studies on the impact of different temperatures on the duration of the immature stages of *Ae. aegypti* in order to explain the finding from previous studies some of which included confounding factors like differential food supply and crowding of rearing containers (Southwood et al., 1972) and to be able to forecast impacts of future climate change events on the mosquito populations. This study examines the effects of different temperatures on the development of *Ae. aegypti* and their impact on adult emergence under laboratory conditions.

2. Materials and methods

2.1. Mosquitoes

The *Ae. aegypti* mosquitoes used in this study were obtained as eggs using modified ovitraps (Fay and Eliason, 1966) in the field in Curepe (10°38′N; 60°24′W), Trinidad, West Indies. Eggs collected were examined under a microscope (at 40×) for the chorionic pattern which is reported to be characteristic of *Ae. aegypti* (Pratt and Kidwell, 1969). These eggs were identified in the Parasitology Laboratory, Department of Life Sciences, University of the West Indies, St. Augustine, Trinidad. All mosquito eggs collected from the field in Curepe were identified as *Ae. aegypti.*

The duration of immature development times of *Ae. aegypti* was assessed under: (1) constant temperature regimens and (2) under a diurnal temperature regimen, for a range of temperatures from 25 to 35 °C at 80% relative humidity.

2.2. Egg hatching experiment

Temperature regulated water baths were set up to cover a range of temperature; 24–25 °C, 26–27 °C, 29–30 °C, 32–33 °C and 34–35 °C. Six replicate 250 mL beakers containing 150 mL of water was placed in each bath and allowed to acclimatize for 24 h. A paper strip containing 30–40 eggs collected from the field was placed in each beaker and allowed to incubate and hatch for 24 h. At the end of this period, the number of eggs hatched was counted and the remaining eggs on the strips allowed to hatch for a further 24 h, after which the number of eggs hatched was again determined. The percentage of eggs hatched after 24 and 48 h was determined for each temperature treatment.

2.3. Effects of constant temperatures on larval and pupal development

Temperature regulated water baths were set up to capture the range of temperature 24–25 °C, 26–27 °C, 29–30 °C, 32–33 °C and 34–35 °C. At each temperature 6–8 1 L beakers containing 800 mL of water were allowed to acclimatize for 24 h. Circa 100 newly hatched *Ae. aegypti* larvae were placed in each beaker and fed daily with 0.1 g of ground fish food. Pupae were removed on a daily basis, and placed in individual chambers until the adults emerged. The rate of pupation was calculated for each treatment temperature, based on the total number of pupae obtained at the end of the development period. The number of adult males and females emerging was enumerated and used to determine the male/female (M/F) ratio for emergent adults for each treatment temperature. The wings of both males and females were dissected out and mounted on glass slides in a drop of saline solution. Using a dissecting microscope with an ocular micrometer wing lengths (Fig. 1) were measured from the apical notch to the axillary margin, excluding the wing fringe for each mounted wing as measured by Nasci (1986) and Schneider et al. (2011).

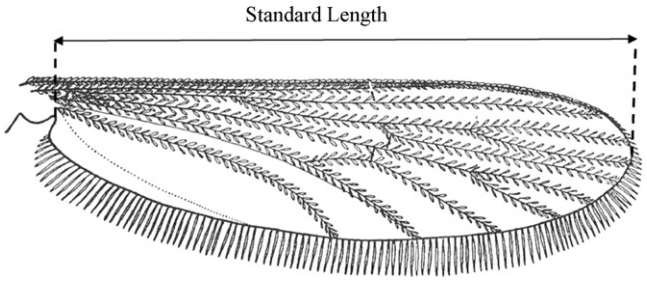

Fig. 1. Diagram showing the standard length measurement for a wing of *Ae. aegypti*.

2.4. Effects of diurnal temperatures on larval and pupal development

This experiment was repeated with the water baths turned on at 8 am and off at 5 pm, thus gradually heating up to the designated maximum temperature level set and turned off at 5 pm to allow the water to cool to room temperature (24–25 °C). This approach mimicked the diurnal heating and cooling cycle often observed in the field and was conducted during this study for a range of temperatures; ambient or 24–25 °C, 26–27 °C, 29–30 °C, 32–33 °C and 34–35 °C. These heating and cooling cycles closely coincided with temperatures observed in the field (see Fig. 2) similar to those reported by Hemme et al. (2009).

2.5. Data analysis

Wing-length measurements collected for each temperature treatment were analysed using an ANOVA and Tukey HSD analysis (SYSTAT Ver.5.0) to determine whether any significant differences occurred between and among different temperatures and at different temperature regimens. In addition, hatching rates, rates of pupation and sex ratio data were transformed and converted into contingency tables and subjected to a *G*-test (Petrie and Sabin, 2000) to determine whether any significant differences were observed among the various treatments.

3. Results

3.1. Egg hatching

The percentages of eggs which hatched following submergence in water heated at different temperatures are shown in Fig. 2. At 24–25 °C hatching success was 95% after 24 h and 98% at 48 h. This was significantly ($P < 0.05$) higher than the hatching success at 26–27 °C (37% – 24 h; 57% – 48 h); 29–30 °C (10%, 24 h; 20% – 48 h): 32–33 °C (3.7% – 24 h; 3.7% – 48 h) and 34–35 °C (1.6%, 24 h; 1.6%, 48 h) as shown in Fig. 3.

3.2. Rearing at constant temperature regimens

Ae. aegypti larvae reared under the constant temperature regimens showed greater than 90% survival across all test temperatures. Pupation generally started on day 4, with the highest pupation rate occurring at 30 °C (78.4%) and the lowest at 25 °C (0.5%) (Table 1). This pattern continued until days 7 and 8, resulting in 80–99% pupation across the different temperature regimens (Table 1 and Fig. 4).

The length of the *Ae. aegypti* pupal stage showed no significant difference between temperatures 25–33 °C, however at 35 °C, pupation was only 80%, significantly ($P < 0.05$) less than those of the other temperatures (Fig. 4). Adults emerged within 2–3 days and the ratio of males/females (M/F) generally ranged from 0.9 at 30 °C to 1.16 at 27 °C and 35 °C (Fig. 5A). There was no significant dif-