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Aedes aegypti disregard humidity-related conditions with adequate nutrition

Canyon, D.V.[1*], Muller, R.[2] and Hii, J.L.K.[3]
[1]Department of Public Health Sciences, John A. Burns School of Medicine, University of Hawaii at Manoa, 1960 East-West Rd, Biomed Building #D104B. Honolulu HI 96822 USA
[2]Tropical Health Solutions P/L and James Cook University, PO Box 1072, Malanda 4885 Qld Australia
[3]Taman Damai, Jalan Fung Yei Ting 88300 Kota Kinabalu, Sabah, Malaysia
[*]Corresponding author email: dcanyon@hawaii.edu
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Abstract. Weather variations have clear associations with the epidemiology of dengue fever and populations of *Aedes aegypti* mosquitoes. Data on humidity associations, however, lags with respect to its effect on host-biting, nectar-seeking and survival. This experimental study on *Ae. aegypti*, sourced from the arid tropics, investigated the effect of low and high relative humidity and diet in relation to host-biting, temporal variations in feeding frequency, and mosquito mortality. In each environmental setting, 10 replicates, containing one male and five female mosquitoes, were challenged with different nutritional sources every six hours over 12 days. Results showed that host-biting did not diminish in low humidity and was six times higher than expected. Sucrose feeding was observed to significantly moderate host-biting and water alone was inadequate for survival. The high host-biting rates help to explain the intensity of dengue epidemics, while the ability of the mosquito to disregard adverse humidity-related conditions helps to explain how dengue epidemics in arid tropical regions can be just as devastating as those in the wet tropics.

INTRODUCTION

Increasing concerns over global climate change has prompted considerable research and speculation as to the impact on biological systems, infectious diseases and subsequent health outcomes. It has been asserted, primarily by modelers, that vectored diseases will increase in frequency and distribution due to laboratory data showing that vector biology and viral replication are responsive to temperature modulation (Thai & Anders, 2011). There is some support for this since in Kaohsiung City, Taiwan, temperature and relative humidity were identified as major determinants in the fluctuation of dengue fever incidence (Wu *et al.*, 2007) and in Cambodia, larval indices and dengue incidence correlated with periods of higher temperature and greater rainfall (Pontes *et al.*, 2000).

The global spread of dengue, most likely due to increased people movement, is poorly understood and the influence of climatic variables on vector capacity requires clarification. The distribution of *Aedes aegypti* and dengue has been historically limited by the 10°C January and July isotherms (to 15°C in South America) (Otero *et al.*, 2006), so small climate changes are unlikely to have a large effect at the global level. However, temperature variations may have consequences at regional or local levels (Johansson *et al.*, 2009).

It has long been thought that host seeking is reduced and mosquitoes become inactive during periods of low humidity for the purpose of conserving energy and maintaining body fluids (Rudolfs, 1925; Mayne, 1930; Lewis, 1933). Diptera are thought to achieve this either by developing a degree of dehydration tolerance (Mogi *et al.*, 1996) or by reducing metabolic processes (Hoffmann & Parsons, 1989). *Aedes aegypti* possesses significant tolerance to dehydration which is related to geographic origin and environmental conditions (Machado-Allison & Craig, 1972; Mogi *et al.*, 1996). Although they are maintained in what should be a constant environment, domestic mosquito strains can exhibit a greater degree of desiccation tolerance than feral strains (Mogi *et al.*, 1996). Not all insectaries have strict humidity, temperature or light control and some are even exposed to outdoor variations.

This study thus attempted to determine if a laboratory strain of *Ae. aegypti* from Charters Towers, an arid tropical region in northern Australia with a long history of multiple dengue outbreaks (Canyon, 2008), would become inactive and decrease biting frequency in environments with strictly controlled low humidity or if it would replenish lost fluid by maintaining its normal rate of host feeding. In addition to host biting frequency being used as an indicator for blood feeding, it was also used as an indicator for metabolic speed, because mosquitoes experiencing a reduction in metabolic processes would experience a reduction in sugar and blood requirements. The importance of water and sugar in low and high humidity was also assessed.

MATERIALS AND METHODS

Aedes aegypti larvae were collected from a house gutter in Charters Towers, Northern Australia, and were reared according to methods outlined by Foster (1980). Charters Towers is situated in the arid tropics. In January (summer) and July (winter) the mean min-max annual temperatures are 21.9-33.6 and 11.0-24.5°C, respectively. Mean annual relative humidity is 65-68% RH at 0900 h and 39-44% RH at 1500 h, and the mean annual rainfall is 659.5 mm with the wettest and driest parts of the year being January (mean rainfall: 135 mm) and July (mean rainfall: 17 mm). Larvae were collected at this location because mosquitoes from a dry and hot climate are more likely to have well-developed desiccation tolerance (Machado-Allison & Craig, 1972) and so may evince more distinct responses to low humidity conditions than mosquitoes from a wet climate.

Observations were made in seven experimental settings: 1) periodic host availability in 34% RH, 2) periodic host availability in 84% RH, 3) periodic host availability in 84% RH + permanent 3% sucrose supplement, 4) periodic water availability in 34% RH, 5) periodic water availability in 84% RH, 6) periodic 10% sucrose availability in 34% RH, and 7) periodic 10% sucrose availability in 84% RH (Table 1). The provision of sucrose in 34% RH was not possible because the experimental environment could not be held constant in a small 50 ml settings. In each experiment, 10 replicates of five female and one male mosquito were placed in 50 ml specimen vials. Mosquitoes were two to four days old and had emerged into the insectary environment with water and 3% sucrose as food sources. Females were presumed to be mated. Each vial had a 1 cm diameter hole cut into the side for placement of sucrose-soaked cotton pads for supplementary feeding. Netting was placed over one end of the vial through which biting and probing took place. The low humidity environment consisted of a glass tank (45 cm x 45 cm) with two circular holes (12 cm diameter) fitted with gauze sleeves and sealed with plastic. A temperature and humidity probe (Protimeter, PCWI) was inserted into one hole and the other was used for access. Silicone gel beads were used to maintain a stable low humidity 33.9 ± 0.3 (SE) % RH at a temperature of 27.2 ± 0.1 (SE) °C and a 14:10 (L:D) photoperiod. Saturation deficiency (SD), in millimeters of mercury, corresponds to certain values of temperature and humidity. In settings 1, 4 and 6, the SD was 18 mm, and in settings 2, 3, 5 and 7, the SD was 4 mm. The high humidity environment was maintained in an environmental cabinet (Lindner and May Pty. Ltd.) at 27.2 ± 0.1 (SE) °C, 84.1 ± 0.5 (SE) % RH and a 14:10 (L:D) photoperiod.

Host-biting is used as an indicator of blood feeding since the actual ingestion of blood, water and sucrose was not quantified. Even though fluid consumption was not always observed following successful probing, host biting is a valid epidemiological measurement. Feeding observations (biting and probing) were of 10 m duration (Canyon *et al.*, 1999). The author acted as the host for 10 min on each vial every 6 h at 0600, 1200, 1800 and 2400 h for 12 days. Host-biting was observed by strapping a vial onto each finger of one hand and counting the number of bites obtained. A bite was only counted if a female had successfully probed and had remained stationary for at least 10 s while deeply penetrating the skin or if blood was imbibed. Females were allowed to feed to repletion and multiple bites by the same mosquito were not included.

A stationary position after successful probing on sucrose or water soaked Teri Wipers (Kimberly-Clark) was interpreted as an indicator of sucrose or water feeding. No sucrose probing observations were made in Setting 3 which contained a permanent source of sucrose with periodic access to a host. Sucrose probing almost always resulted in observable fluid intake and was easy to detect. Mosquitoes did not feed for long on water so a probe followed by a stationary period of at least 3 seconds was recorded as a successful probe.

The distributions of the number of bites/probes with respect to the seven experimental settings as well as with respect to the feeding times proved to be skewed. Therefore, in the description, medians were used as measures of central tendencies and quartiles as measures of dispersion. Consequently, comparisons of median bites/probes between settings were tested by means of the non-parametric unpaired Mann-Whitney test. Comparisons between the four feeding times were tested using the non-parametric Friedman test for related samples. For all statistical tests, the alpha level was set to 0.05.

RESULTS

Median bites/probes per mosquito in the seven settings over the whole 12 day period are presented in Table 1. Biting rates in mosquitoes maintained on a host alone were not significantly different between low and high humidity, however, significantly lower biting was observed in high humidity with a sucrose supplement. Water probing was significantly higher in low humidity than in high humidity, however, significantly more sucrose probing was observed in high humidity as compared to low humidity.

Median bites/probes per mosquito and percentage mortality in the four time periods daily in the seven settings are presented in Figures 1 and 2. In Settings 1 and 2, host biting remained fairly constant with a slight decline from 2.0 to 1.5 daily bites per mosquito over the 12 days. This did not match mortalities which increased on day 8 in both settings, and ended on day 12 with 50% and 27% survival in Settings 1 and 2, respectively. In

Table 1. Median host bites and water and sucrose probes per female *Aedes aegypti* mosquito (exposed to diet source for 10 minutes every six hours for 12 days in seven settings) in response to low and high relative humidity

Experimental Setting	Food Source	Relative Humidity	Bites & probes every 6 h*	(quartiles)
1	Host	34% RH	0.47 b	(0.42 – 0.57)
2	Host	84% RH	0.51 b	(0.41 – 0.58)
3	Host	Sucrose + 84% RH	0.26 a	(0.25 – 0.32)
4	Water	34% RH	0.36 b	(0.35 – 0.42)
5	Water	84% RH	0.16 a	(0.11 – 0.18)
6	Sucrose	34% RH	0.29 a	(0.23 – 0.35)
7	Sucrose	84% RH	0.40 b	(0.36 – 0.46)

*Rows within the same diet with different letters were significantly different ($P<0.05$)

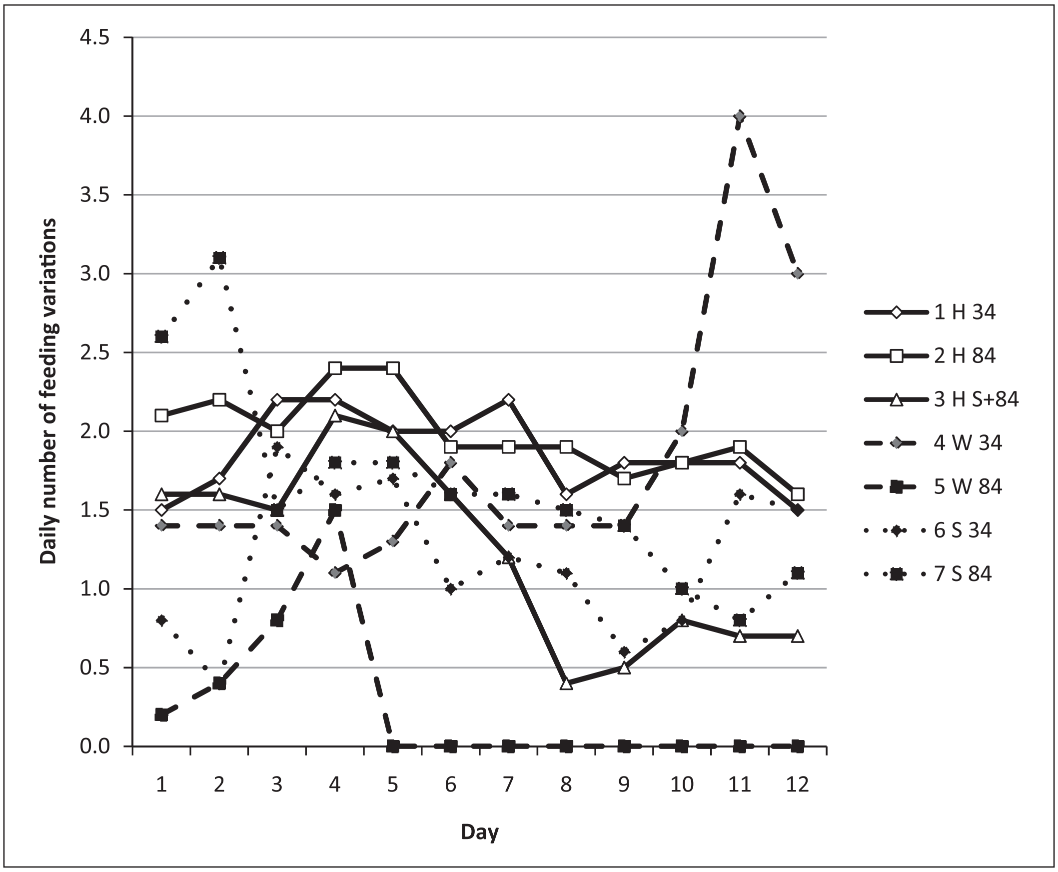

Figure 1. Daily variations in (H)ost biting, (W)ater probing and (S)ucrose probing in 34 and 84% RH in seven experimental settings presented as: median bites or probes by a female *Aedes aegypti* per day

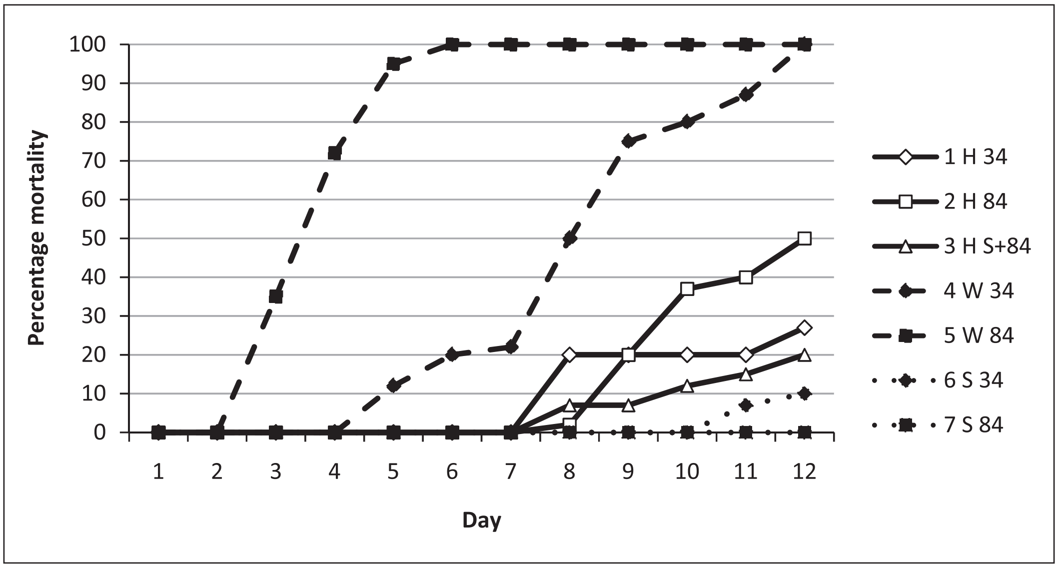

Figure 2. Percentage longevity of female *Aedes aegypti* in 34 and 84% RH on (H)ost, (W)ater and (S)ugar diets in seven experimental settings

Setting 3, a decline in host biting from 1.6 to 1.2 daily bites per mosquito was observed from days 1–7 with a sucrose supplement in high humidity. Host biting declined further to 0.4–0.7 daily bites per mosquito from days 8–12. Mortalities were similarly to Setting 1 and reached 20% by day 12. In Settings 4 and 5, females lived for 5 days and 11 days on water at high and low humidity respectively. Water probing rates remained constant while mosquitoes were alive. In setting 5, water probing in high humidity was delayed and only really started on day 3 and ended on day 4 with the death of most mosquitoes. In Setting 6, sucrose probing in low humidity was initially low but peaked on days 3–5 and days 11–12. In Setting 7, a different pattern was observed in high humidity where probing was initially high at 2.60 daily probes per mosquito, but declined steadily to 1.12. Survival on sucrose remained at close to 100% over the 12 days regardless of humidity.

Median bites/probes per female mosquito in each of the four times and seven settings are displayed in Figure 3. Friedman analysis showed time (dawn, noon, dusk, midnight) to be significantly different within both water and sucrose probing at 34% RH. A dusk (1800–2000 h) peak was observed in all settings except Setting 1, where midnight biting occurred more frequently than dusk biting.

DISCUSSION

This study aimed to determine if a domestic, arid region strain of *Ae. aegypti* would become inactive and decrease biting frequency in low humidity or if it would remain active by replenishing lost fluid by continuing to feed on available sources of moisture and nutrition. While the observation that host seeking is reduced and mosquitoes become inactive during periods of low humidity for the purpose of conserving energy and maintaining body fluids (Rudolfs, 1925; Mayne, 1930; Lewis, 1933) may be true where hosts are not easily available, it is not true when a host is easily available. Thus mosquitoes cohabiting with human hosts would continue to blood feed at the same rate regardless of variations in humidity. The ability of a mosquito to remain active in the presence of hosts in low humidity is thus not related to desiccation tolerance. Host biting frequency may be used as an indicator for metabolic speed since mosquitoes showing a reduction in blood requirements would experience diminished metabolic activity. In the comparison of Settings 1 and 2, metabolic processes were not affected in an observable manner. There was also no indication to suggest that feeding frequency increased to maintain water balance. Therefore it may be concluded that inactivity is employed when hosts are unavailable and that mosquitoes become opportunistically active and behave without consideration of humidity when hosts are readily available.

If the presence of sustenance negates the effect of humidity then Setting 3 (periodic host + continuous sugar) represents the impact of other food sources in a mosquito's environment on its blood-feeding pattern. In this case, host-biting was comparable with Settings 1 and 2 until day 7 when host-biting decreased from an average of 2.0 blood meals per day to 0.5-1.0 meals per day (Figure 1). While it is common knowledge that sugar feeding depresses blood feeding (Foster, 1995), it has also not been previously determined that sugar feeding may have the effect of reducing disease transmission by halving blood-feeding.

In high humidity, this study found a stronger signal to feed on sucrose which corresponded to its greater availability in the wet tropics (Shuel, 1955) and findings that sucrose feeding frequently occurs in the wet tropics (Van Handel *et al.*, 1994; Martinez-Ibarra *et al.*, 1997). Rainfall has a considerable indirect effect on *Ae. aegypti* since it regulates the amount of available vegetative sugar sources and the availability of breeding sites (Hayden *et al.*, 2009). Nevertheless, it is important to note that nectar sources remain available, although reduced in the arid tropics which experience low rainfall and low humidity (Shuel, 1955). While the test mosquito was assumed to be somewhat 'adapted' to desiccation, this result suggests that it is opportunistic when it comes to sugar which negates humidity effects.

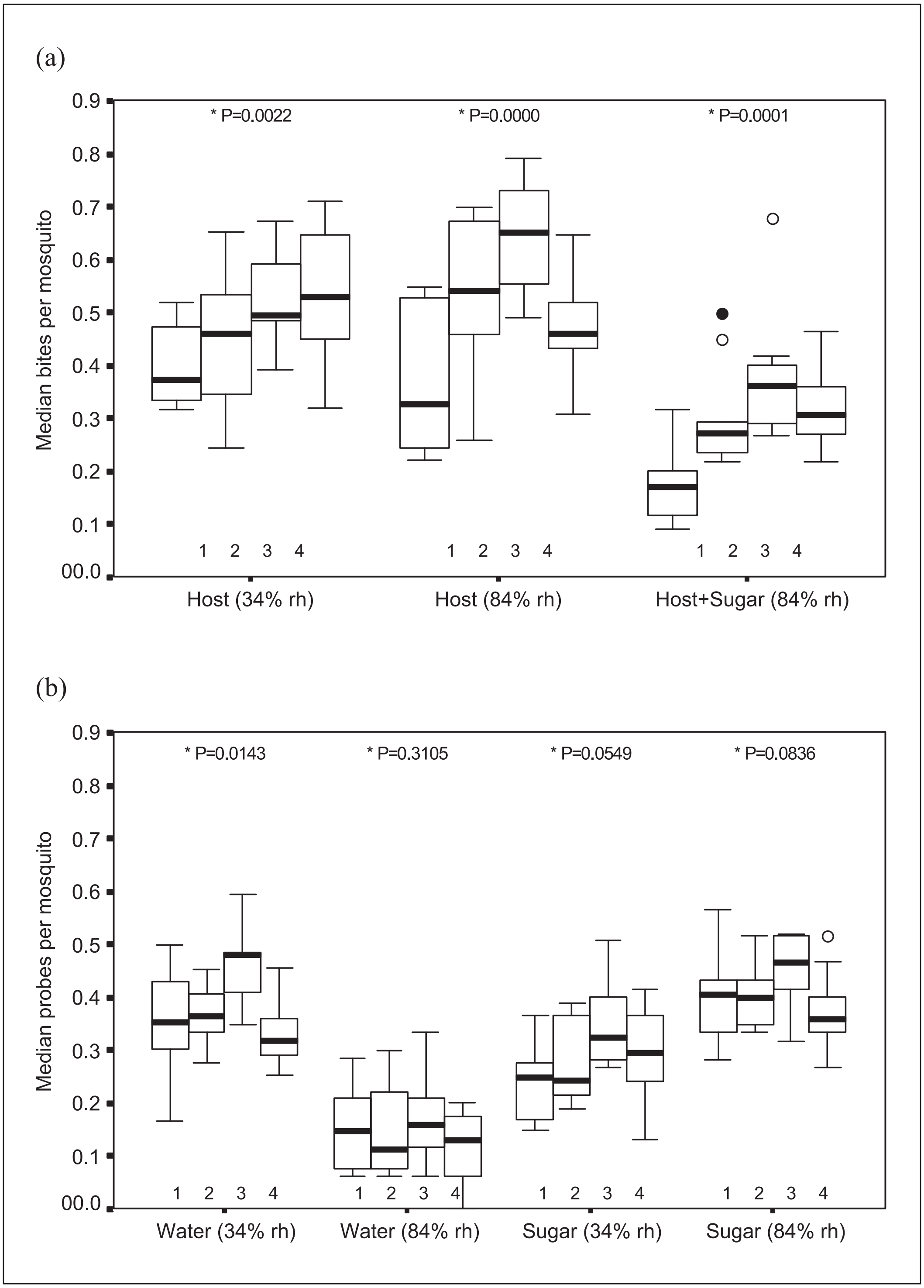

Figure 3ab. Median diel activity (bold horizontal lines) in (3a) host biting at 34% (Setting 1) and 84% RH (Setting 2), host-biting with sucrose at 84% RH (Setting 3), (3b) water or sucrose probing at 34% and 84% RH (Settings 4 to 7), by female *Ae. aegypti*. Boxes indicate interquartiles and the whiskers indicate ranges. Also indicated are outliers (o) and extremes (·). 1: 0600 h. 2: 1200 h. 3: 1800 h. 4: 2400 h. * Significant differences between feeding times within diet were tested by Friedman test

Sugar also acts to confer greater longevity on mosquitoes which is essential for them to become more effective disease vectors. On blood alone, only 50% of mosquitoes survived in 84% RH and the addition of sugar increased survival to 80%. These results contradicted the conclusions of Scott *et al.* (1997), who suggested that *Ae. aegypti* incurred a fitness advantage when they fed on blood alone.

Water alone was insufficient for body fluid maintenance and was ineffective in prolonging life. This experiment revealed low humidity to be a necessary signal required to initiate water feeding because mosquitoes at 84% RH (Setting 5) did not appear to receive this signal. Consequently, a surprisingly low number of water probes, which usually did not cause a change in abdomen size, were observed and all mosquitoes died by day 5. Similar results were observed by Costello & Brust (1972), who found that mortality of female *Ae. vexans* (Meigen) on a water diet was positively correlated with increasing humidity. These results indicate that water is not an important component in a mosquito's natural diet.

Diel biting behavior varied significantly within experimental setting when host-biting was available and was mostly not significant in the other settings. Typically, interest in feeding was lowest at dawn rising to a peak at dusk. This variation from the normal dusk and dawn periods of biting intensity is most likely due to the mosquitoes never being exposed to natural exogenous environmental conditions.

And finally, the extent of host-biting discovered in this study was extreme when one considers that the vector capacity formula usually ascribes a single blood feed per three-day gonotrophic cycle. Even when the moderating effect of sucrose is taken into account, the host-biting rate discovered in this paper decreases from 6 (2.0/day) to 3 (1.0/day) per gonotrophic cycle and is still three times higher than the usually touted figure of one blood-feed per cycle.

In conclusion, regardless of possible desiccation mechanisms, during periods of moisture availability in high humidity conditions, mosquitoes survive and remain active by replenishing transient depletions of body fluid as they seek out sustenance in the form of plant sugars and host blood. This feeding behavior persists in low humidity environments albeit to a lesser extent. Indeed, blood feeding occurs with a higher frequency than is required to account for reproductive productivity (Holstein, 1954; Omer & Cloudsley-Thompson, 1970). Therefore blood feeding is driven by a combination of physiological requirements including reproduction, hunger, nutrient replacement and maintenance of body fluid balance. In a host-only environment, this results in two blood meals per day, which is important in epidemiological terms. However, in an environment where nectar or sugar is available, the blood-feeding rate decreases by more than half. Field research is now required to determine the extent of sugar-feeding in *Ae. aegypti* that cohabit with their hosts to clarify the exact blood-feeding rate.

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