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Relationship between mosquito (Diptera: Culicidae) landing rates on a human subject and numbers captured using CO_2-baited light traps

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Abstract

Capture rates of insectary-reared female *Aedes albopictus* (Skuse), *Anopheles quadrimaculatus* Say, *Culex nigripalpus* Theobald, *Culex quinquefasciatus* Say and *Aedes triseriatus* (Say) in CDC-type light traps (LT) supplemented with CO_2 and using the human landing (HL) collection method were observed in matched-pair experiments in outdoor screened enclosures. Mosquito responses were compared on a catch-per-unit-effort basis using regression analysis with LT and HL as the dependent and independent variables, respectively. The average number of mosquitoes captured in 1 min by LT over a 24-h period was significantly related to the average number captured in 1 min by HL only for *Cx. nigripalpus* and *Cx. quinquefasciatus*. Patterns of diel activity indicated by a comparison of the mean response to LT and HL at eight different times in a 24-h period were not superposable for any species. The capture rate efficiency of LT when compared with HL was ≤15% for all mosquitoes except *Cx. quinquefasciatus* (43%). Statistical models of the relationship between mosquito responses to each collection method indicate that, except for *Ae. albopictus*, LT and HL capture rates are significantly related only during certain times of the diel period. Estimates of mosquito activity based on observations made between sunset and sunrise were most precise in this regard for *An. quadrimaculatus* and *Cx. nigripalpus*, as were those between sunrise and sunset for *Cx. quinquefasciatus* and *Ae. triseriatus*.

Keywords: sampling, trapping, bias, diel activity

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Introduction

Battery-operated CDC-type light traps (Sudia & Chamberlain, 1962) supplemented with CO_2 are commonly used in mosquito surveillance programs (Moore *et al.*, 1993). These devices capture a greater number and variety of adult mosquitoes than other trap types (e.g. resting boxes, malaise traps, ovitraps) (Williams & Gingrich, 2007) and provide faunal composition and abundance data that are important for the implementation and evaluation of mosquito control activities (Amoo *et al.*, 2008). Similarly, risk assessment models for disease transmission and depictions of mosquito distribution produced by spatial analysis methods and mapping systems software rely on data provided by light traps (Diuk-Wasser *et al.*, 2006), including inputs that are used to estimate mosquito density; biting activity on humans; and age-structure, survivorship and pathogen infection rate(s) in

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the mosquito population (Garrett-Jones, 1964; Reisen & Pfuntner, 1987; Gu *et al.*, 2003; Kilpatrick *et al.*, 2005; Eisen & Eisen, 2008).

Light traps have been studied extensively as a human host surrogate for the estimation of mosquito landing/biting rates. These evaluations have targeted primarily malaria vectors and used unbaited CDC-type traps. Odetoyinbo (1969) in The Gambia, and later Gunasekaran *et al.* (1994) in India and Hii *et al.* (2000) in Papua were unable to demonstrate a relationship between the numbers of landing/biting mosquitoes on humans and those captured by light trap. However, Garret-Jones & Magayuka (1975) combined mosquito responses to three indoor trapping techniques (the CDC portable trap and the Monks Wood light trap fitted with white light and mercury vapour tubes) and the use of bed nets by humans to estimate the 'man-biting density per person per night' for *Anopheles* spp. in Tanzania. The same workers showed that light traps did not increase the number of hungry mosquitoes entering human sleeping areas but rather intercepted those otherwise present and prevented from feeding by bed nets. Yet other studies in Thailand (Ismail *et al.*, 1982), Tanzania (Lines *at al.*, 1991; Davis *et al.*, 1995), Kenya (Mbogo *et al.*, 1993), Venezuela (Rubio-Palis & Curtis, 1992), Burkina-Faso (Costantini *et al.*, 1998) and Sierra Leone (Magbity *et al.*, 2002) have demonstrated a correspondence between the numbers of *Anopheles* and *Culex* mosquitoes collected in light traps placed near bed net-protected human hosts and the numbers of mosquitoes biting human collectors.

The capture rates of mosquitoes in CDC-type light traps have been compared directly with those landing on human hosts in two additional studies. Vaidyanathan & Edman (1997) explained 18% of landing *Cx. salinarius* Coquillett responses on a human host from the numbers of females trapped in CDC light traps in Massachusetts, USA. Strickman *et al.* (2000), in The Republic of Korea, compared mosquito landing responses to human hosts with those to CO_2-baited CDC light traps and developed a series of density thresholds based on the latter that were used to estimate attainment of a minimum significant potential biting rate by *An. sinensis* Wiedemann.

From a sampling perspective, light traps are useful for catching large numbers of certain mosquito species and for measuring relative changes in abundance of these species in time and space. They have limited value as an ecological tool, however, because they sample species and sub-populations within species unequally (Service, 1993; Southwood & Henderson, 2000). In the latter case, group testing-based estimates of virus infection rate in pools of light-trapped mosquitoes (Chiang & Reeves, 1962; Lanciotti *et al.*, 2000; Gu *et al.*, 2003), while effective for documenting virus transmission, can lead to an upwardly biased estimate of infection rate in the vector population (Katholi & Unnasch, 2006). This appears to be a critical issue, given an implicit assumption of equivalency between light trap-captured and natural mosquito population parameters (Reisen & Pfuntner, 1987).

The study reported here was made under controlled conditions to compare, as precisely as possible, the capture rate of adult mosquitoes by light trap (LT) with a baseline capture rate determined using the human landing (HL) collection method. The objective of the study was to develop the procedures and techniques needed to observe, analyze and interpret mosquito responses to LT and HL and to propose hypotheses that can be tested using these methodologies with field populations of mosquitoes.

Materials and methods

Test arena

Observations were made in an aluminum-framed building (12.9 m L × 4.3 m W × 2.8 m [average] H) with a fiberglass window screen (approximately seven openings per linear cm) on four sides and a white-enameled aluminum sheet metal roof. Flooring comprised 20-cm-deep 'pea gravel' (0.50–0.75-cm dia) throughout. The building was partitioned (by a translucent vinyl-fiberglass screen panel) into two equal-sized rooms (6.4 m L × 4.3 m W × 2.8 m H) each with an external door. Window screen was fitted into channels in the exterior frame members of the building and held in place with rubber stripping. All joints and other openings in the building were sealed to prevent entry/escape of mosquitoes and invertebrate or vertebrate predators. The volume of each room was 77.7 m^3.

Mosquitoes

The mosquito species selected for this study, while of laboratory origin, were intended to represent diverse taxa. The test populations were of finite density and were confined to screened enclosures to eliminate emigration/recruitment effects. Mosquitoes were reared outdoors so that we could observe their responses within the context of exposure to natural cycles of light and temperature.

Capture rate responses were studied for *Aedes albopictus* (Skuse) (Gainesville strain, 1992), *Ae. triseriatus* (Say) (Gainesville strain, 1996), *Anopheles quadrimaculatus* Say (Orlando strain, 1952), *Culex nigripalpus* Theobald (Vero Beach strain, 1999) and *Cx. quinquefasciatus* Say (Gainesville strain, 1995). Cohorts of eggs of each species were reared to the adult stage outdoors under ambient light and temperature conditions using the techniques described by Gerberg *et al.* (1994). An approximately equal number of 4–9-day-old nulliparous female mosquitoes were available in each room at the beginning of a given test, although this number ranged among the tests from approximately 800 to 2200 females (density: 12.8–28.3 $♀\,m^{-3}$), depending on the time of year in which tests were made (March through October) and the water temperature during mosquito development. Adult mosquitoes were released into each room from the holding cages 24 h in advance of the beginning of a test. During this time, they were provided 10% sucrose solution (in H_2O) via cotton wick.

Collection methods

Each test lasted 24 h and comprised a matched pairs comparison of the numbers of mosquitoes captured by a continuously-operating miniature (CDC-type) light trap (LT) with the numbers captured using the human landing (HL) collection method in eight separate 15-min-long intervals (spaced throughout the 24-h period). The LT was operated in the center of one (randomly selected) room of the screened building. At the same time, in the center of the second room, mosquitoes that landed on the exposed forearm of a human subject were collected with a mechanical aspirator (Hausherr's Machine Works, Toms River, NJ). For LT collections, a programmable (model 4012, John W. Hock Company, Gainesville, FL) collection bottle rotator, fitted with a single CDC-type light trap (with light) (model 512, John W. Hock Company, Gainesville, FL) and eight collection bottles, was used to capture adult mosquitoes in each of eight consecutive collection intervals of variable duration (see below) in a 24-h period. Compressed CO_2 gas was released continuously at the rate of 250 ml min^{-1} from the end of 0.5 mm O.D. Tygon® tubing attached 1.5 cm below the LT intake. HL collections were made using a mechanical aspirator to vacuum mosquitoes that alighted on the skin and commenced immediately to probe (i.e. touched the skin with their proboscis) or that remained on the skin for five seconds. None was allowed to bite. Approximately 415 cm^2 of forearm skin was exposed for this purpose, comprising the area from a line of circumference 3 cm below the elbow to a line of circumference 3 cm above the wrist. To capture landing mosquitoes, the exposed forearm was extended forward in front of the body of the test subject and held approximately 45 cm above the ground throughout the collection period. The exposed skin surface was observed for landed mosquitoes during this time as the arm was rotated in a counterclockwise then clockwise fashion. A 6000 candle power *Visor*LIGHT™ (Model LT06, Donegan Optical Company, Lenexa, KS, USA), attached to the top of the aspirator and fitted with a red acetate lens cover, provided on-demand night-time illumination. All HL collections were made from the same human subject (DRB).

In each 24-h test, the schedule for LT operation and for HL collections was arranged according to the times of sunset and sunrise within the diel period and the length (min) of the corresponding photophase (sunrise to sunset) and scotophase (sunset to sunrise) (local times for each event were obtained from the *Nautical Almanac*, US Naval Observatory, for longitude W 82°20′ and latitude N 29°40′). To do this, the 24-h day was divided into eight 'periods' (periods 1 through 8). Periods 1 and 5 incorporated the two light-transition events in the diel period (day-sunset-night and night-sunrise-day, respectively). The day portion of these two periods comprised one-eighth of all minutes in the photophase, the night portion one-eighth of all minutes in the scotophase. In the same manner, periods 2, 3 and 4 (night) each comprised one-fourth of all minutes in the scotophase and periods 6, 7 and 8 (day) each comprised one-fourth of all minutes in the photophase. Thus, for example, in the case of tests made in June when the photoperiod was 14L:10D, the duration of periods 2, 3 and 4 was 150 min each, the duration of periods 6, 7 and 8 was 210 min each, and the duration of periods 1 and 5 was 180 min each. For period 1, the time intervals preceding and following sunset were 105 min and 75 min, respectively; and, for period 5, the time intervals preceding and following sunrise were 75 min and 105 min, respectively.

The LT was operated (and CO_2 released) continuously for 24 h. The collection bottle rotator was programmed to move a new collection bottle in position beneath the light trap at the beginning of each period. Collections using the HL method were made for 15 min in each period. In periods 1 and 5, these were made at sunset and sunrise, respectively. In periods 2, 3 and 4 (night) and 6, 7 and 8 (day), HL collections were made midway through each period. Studies commenced July 2004 and ended October 2006. During this time, five matched-pair comparisons (replicates) of responses to the LT and HL collection methods were made for each mosquito species.

Catch per unit effort

The total number of female mosquitoes captured (nf) by each collection method in each period (i) in each replicate was transformed to $\log_{10}(nf_i+1)$. Operating time (t) for the HL method in each period was 15 min. Operating time (t) for LT in a period ranged between 150 and 210 min. For analysis purposes, we calculated a catch-per-unit-effort response (R) (Southwood & Henderson, 2000) for the nf_i observed for each species according to collection method and replicate as the number of mosquitoes captured after 1 min of collection time:

$$R = \frac{\log_{10}(nf_i + 1)}{t_i}$$

Capture rate by period

Differences in mean R between periods were compared for each species according to collection method using the model: $R = \beta_0 + \beta_1(\text{period})$. For each species, the pattern of diel activity indicated by LT and HL was compared by rank ordering all eight periods according to mean R (rank = 1 for the period with highest R; rank = 8 for the period with lowest R) and then testing the difference in ranks assigned LT and HL in the same period for departure from 0 using Student's t-test.

Capture efficiency index

A capture efficiency index ($\text{CEI} = R_{\text{LT}}/R_{\text{HL}}$) was used to compare the mosquito capture rate by LT with the capture rate using HL. A mean CEI ≥ 1 indicated equivalent or greater efficiency for LT compared with HL for that mosquito species in that period. An index <1 indicates the LT collection method was less efficient than HL.

Daily capture rate

The daily capture rate is a commonly used operational index of mosquito density, but the comparability of LT and HL data for depicting seasonal trends in mosquito population density is unknown. Given the total LT operating time ($T_{\text{LT}} = 1440$ min) and the total HL collection time ($T_{\text{HL}} = 120$ min) used in each of our 24-h tests, we calculated the mean daily capture rate (R_D) for each species according to collection method ($R_{D(\text{LT})}$ or $R_{D(\text{HL})}$) as:

$$R_D = \frac{\sum_{i=1}^{8} \log_{10}(nf_i + 1)}{T}$$

The model: $R_{D(\text{LT})} = \beta_0 + \beta_1(R_{D(\text{HL})})$ (Neter *et al.*, 1983) was used to determine if change in $R_{D(\text{LT})}$ is related to change in $R_{D(\text{HL})}$ and to evaluate the comparability of seasonal population data indicated by each collection method.

Relationship of capture rates by LT to HL

Initially, a linear regression model ($R_{\text{LT}} = \beta_0 + \beta_1(R_{\text{HL}})$ (Neter *et al.*, 1983) was used to evaluate the relationship of R_{LT} to R_{HL} for the following sampling regimens: (a) all eight periods, (b) period 1, (c) period 5, (d) periods 1 and 5, (e) periods 1 through 5, (f) periods 5 through 1, (g) periods 6 through 8, (h) periods 2 through 4, (i) periods 2 through 5, (j) periods 6 through 1, (k) periods 8 through 2 and (l) periods 4 through 6. We evaluated the linear and curvilinear response in each case by successive additions of quadratic ($\beta_2(R^2_{\text{HL}})$) and cubic effect coefficients ($\beta_3(R^3_{\text{HL}})$) to the linear model.

Data analysis

Statistical analysis (SAS Institute, 2003) utilized tabulation (PROC MEANS), analysis of variance (PROC ANOVA, PROC GLM) and regression (PROC REG, PROC GLM) procedures.

Pre-planned comparisons of means were made using the Least Significant Difference (LSD) test at the 5% level of significance.

Results

Catch per unit effort

When R_{LT} responses were compared with R_{HL} responses, fitted models for *Ae. albopictus*, *Ae. triseriatus* and *An. quadrimaculatus* were not significant (fig. 1). High LT responses generally corresponded with high HL responses, although R_{LT} at or near 0 were observed at $R_{HL} \geq 0.8$ for *Ae. albopictus*, at $R_{HL} \geq 0.2$ for *An. quadrimaculatus* and at $R_{HL} \geq 0.5$ for *Ae. triseriatus*.

Coefficients (±SE) for the fitted linear model for *Cx. nigripalpus* ($s^2 = 0.0014$) were: $\beta_0 = 0.0085$ (0.0097) and $\beta_1 = 0.0508$ (±0.0182) (fig. 1). For *Cx. quinquefasciatus*, the fitted regression line indicated a curved response when $R_{HL} < 0.75$ and a straight line response at higher values. Fitted model coefficients ($s^2 = 0.0365$) were: $\beta_0 = 0.1678$ (±0.1030), $\beta_1 = -0.4851$ (±0.6144), $\beta_2 = 0.7948$ (±0.9388) and $\beta_3 = -0.2076$ (±0.4032) (fig. 1).

Capture rate by period

There was no significant difference in the response to LT by *Ae. albopictus* from one period to the next. In contrast, average capture rates by HL were highest ($F_{7,32} = 5.74$, $P < 0.001$) in periods 6–7–8–1 and lowest in period 4 (table 1). HL responses for *Ae. albopictus* increased between early-morning and sunset (periods 6–7–8), but 25% of all landing females were collected at night (periods 2–3–4) compared with 31% at night by LT.

The effects of period were significant ($F_{7,32} = 5.11$, $P < 0.001$) for *Ae. triseriatus* responses to LT but not to HL (table 1). Most females were collected at sunrise and after sunset (periods 5, 2), whereas fewest were captured in daytime (periods 6–7–8, 1). In contrast, HL capture rates for *Ae. triseriatus* were highest in periods 7–8, 1 and 5 and lowest following sunrise (period 6).

The number of *An. quadrimaculatus* captured in LT increased before and at sunset (periods 8–1) and at sunrise (period 5). Mean responses differed only for periods 7–8 (LSD, $P = 0.05$) (table 1). Period effects were significant for HL responses ($F_{7,32} = 6.93$, $P < 0.001$) with collections highest before sunset (periods 7–8) and higher between sunrise and sunset (periods 5–6–7–8–1) than at night (period 4). Sixty-five percent of *An. quadrimaculatus* were collected by HL during daylight (periods 5–6–7–8) compared with 50% of all females by LT during this time.

Culex nigripalpus responses to LT were not significantly influenced by period. Daily activity patterns indicated by this collection method were bimodal with highest capture rates at, and following, sunset (periods 1–2) and at sunrise (period 5) (table 1). The HL response pattern was unimodal with significant period effects ($F_{7,32} = 5.51$, $P < 0.001$). Landing rates were highest in periods 8–1 but decreased thereafter through sunrise (periods 2–3–4–5–6).

Response patterns of *Cx. quinquefasciatus* to LT and HL indicated a single peak of activity in each case but significant period effects for LT only ($F_{7,32} = 5.01$, $P < 0.001$) (table 1). In this case, responses were higher in periods 8–1–2 than in periods 4–5–6–7, whereas HL responses were higher at sunset (period 1) than before, during or after sunrise (periods 4–5–6) (LSD, $P = 0.05$).

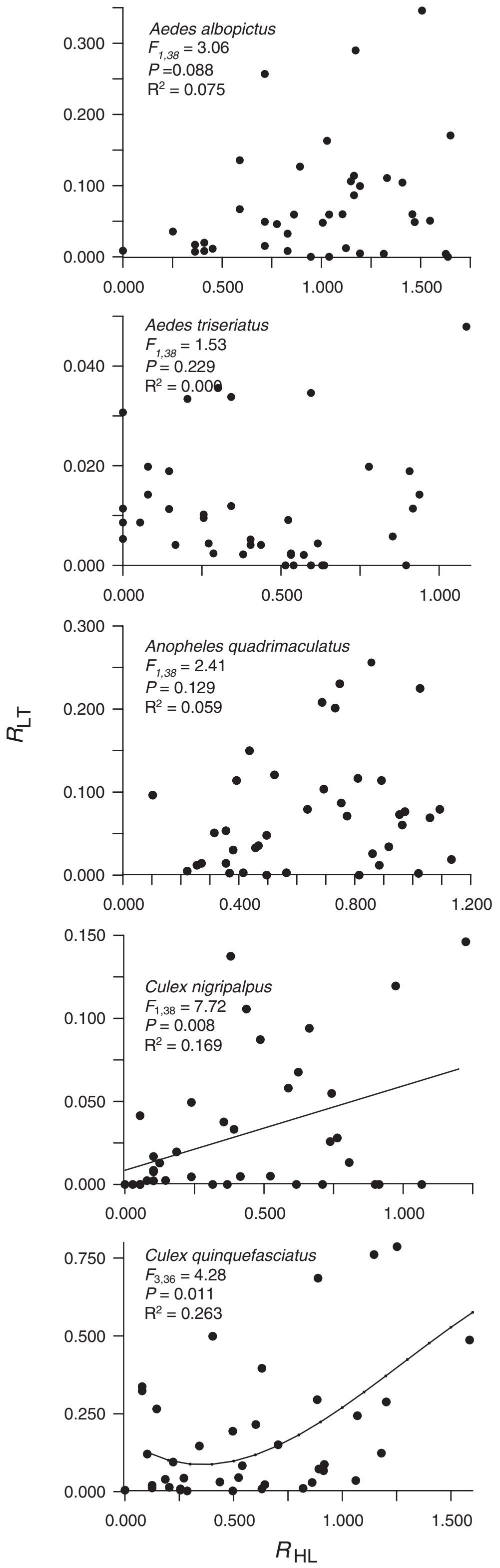

Fig. 1. Relationship of mosquito capture rate by LT (R_{LT}) to mosquito capture rate by HL (R_{HL}) for five mosquito species based on catch per one minute of collection effort.

Table 1. Mean capture rate (±SE) of five mosquito species by LT and HL collection methods.

	Period							
	1	2	3	4	5	6	7	8
Aedes albopictus								
LT	0.26 (0.17)a	0.32 (0.30)a	0.06 (0.06)a	0.08 (0.05)a	0.24 (0.39)a	0.09 (0.07)a	0.36 (0.50)a	0.18 (0.20)a
HL	11.6 (3.3)ab	6.2 (4.4)bc	4.9 (3.4)bc	3.2 (4.0)c	6.9 (4.9)bc	18.7 (9.9)a	22.0 (15.6)a	31.2 (12.9)a
Aedes triseriatus								
LT	0.02 (0.02)ab	0.06 (0.03)c	0.04 (0.03)ac	0.02 (0.01)ab	0.06 (0.04)c	0.01 (0.01)b	0.01 (0.01)b	0.01 (0.01)b
HL	4.1 (3.1)ab	2.3 (3.0)abc	1.5 (2.6)c	1.3 (2.2)c	3.0 (4.6)abc	1.0 (0.6)bc	2.4 (0.7)abc	3.8 (1.8)a
Anopheles quadrimaculatus								
LT	0.30 (0.25)ab	0.13 (0.16)ab	0.17 (0.25)ab	0.13 (0.14)ab	0.22 (0.22)ab	0.16 (0.13)ab	0.10 (0.08)b	0.38 (0.33)a
HL	4.8 (4.0)ab	1.7 (0.6)bc	1.9 (1.2)bc	1.5 (0.9)c	4.5 (4.0)ab	5.9 (1.6)ad	8.0 (1.0)d	7.6 (3.4)ad
Culex nigripalpus								
LT	0.21 (0.09)a	0.15 (0.04)ab	0.08 (0.06)ab	0.08 (0.04)ab	0.18 (0.08)a	0.01 (0.01)b	0.01 (0.01)b	0.08 (0.03)ab
HL	8.1 (2.6)a	1.7 (0.7)bcd	0.6 (0.4)cd	1.1 (0.7)cd	1.0 (0.5)cd	0.5 (0.2)cd	3.0 (1.5)bc	3.8 (0.7)ab
Culex quinquefasciatus								
LT	2.12 (1.85)a	0.93 (0.68)ab	0.42 (0.40)bc	0.13 (0.11)c	0.12 (0.10)c	0.17 (0.33)c	0.02 (0.02)c	1.24 (1.93)ab
HL	11.5 (13.3)a	3.8 (5.5)ab	3.9 (5.2)ab	3.3 (4.3)b	3.1 (3.4)b	2.3 (2.4)b	2.8 (2.1)ab	7.7 (5.6)ab

Tabulated means for LT and HL based on non-transformed data. Row means followed by the same letter are not significantly different ($P=0.05$, LSD test using $\log_{10}(nf_i+1)$).

The daily modes of activity indicated by LT and HL compared poorly within species when ranked by period (table 2). There were eight instances of subjective correspondence (i.e. $P \geq 0.59$) among the 80 rankings and nine significant ($P=0.05$) departures from correspondence. Significant departures from correspondence indicate a disparate response (in terms of mean R) to each collection method in the same period. Aedine species accounted for a majority of these differences (64%). In the case of *Ae. albopictus* (table 2), for example, HL collections indicated period 8 as the time of peak activity (rank = 1.2/8), whereas LT collections for period 8 were ranked 3.8/8. Similarly, for *Ae. triseriatus*, the period 8 rank for LT was 7.3/8 and 2.2/8 for HL. The overall comparability of ranks was greatest (based on P) for *Cx. quinquefasciatus*, particularly in periods 4–5–6 and 8.

Capture efficiency index

The CEI varied widely for each mosquito species (table 3). The range was greatest (3.5–108%) for *Cx. quinquefasciatus* and least (0.2–12.4%) for *Ae. triseriatus*. Period effects were significant only for the latter species ($F_{7,32}=2.67$, $P=0.027$). The CEI was highest on average in period 2, lowest in period 7, and higher between sunset and sunrise (periods 1–2–3–4–5) than during the day (6–7–8).

Daily capture rate

The fitted daily capture rate model for *An. quadrimaculatus* was significant, but factors other than collection method influenced variability of R_D for all other species (table 4). The results indicate that changes in the daily capture rate according to LT are not well correlated with changes indicated by HL.

Relationship of capture rates by LT to HL

None of the single or multi-period models evaluated for *Ae. albopictus* was significant at the 5% level (table 5). For *An. quadrimaculatus*, there was a significant curvilinear relationship between R_{LT} and R_{HL} in periods 1–5, but neither the model for periods 5–1 (>85% of R_{HL} responses) nor for other sampling intervals for this species was significant. Four sampling interval models were fitted ($P \leq 0.05$) for *Cx. nigripalpus* (table 4), including a single period model for sunset (period 1). All multiple period models for this species included period 1 and period 2 responses, with the most robust model encompassing the time between sunset and sunrise. For *Cx. quinquefasciatus*, neither the period 1 nor the period 5 model was significant; conversely, fitted multiple period models included the daytime through sunset periods (table 5). For *Ae. triseriatus*, fitted multiple period models (table 5) included daytime through sunset (periods 6–7–8–1) and sunrise through sunset (periods 5–6–7–8–1).

Discussion

Daily capture rate

The daily capture rate (R_D) is a commonly used operational index of mosquito density. Our results suggest this index may lack meaning with respect to seasonal trends in mosquito landing rates when determined on the basis of responses to LT. The disparity can also potentially impact daily and seasonal estimates of the minimum infection rate, use of the maximum

Table 2. Mean rank of period based on R_{LT} compared with mean rank of period based on R_{HL} (i.e. $R_{\mathrm{LT}} \mid R_{\mathrm{HL}}$).

Mosquito species	Period							
	1	2	3	4	5	6	7	8
Aedes albopictus								
Mean rank	2.4 | 3.6	2.8 | 6.1	5.8 | 5.9	5.2 | 7.5	6.0 | 6.1	5.7 | 2.8	4.3 | 2.8	3.8 | 1.2
Probability > |*t*|	0.071	0.025*	0.704	0.043*	0.927	0.061	0.274	0.003*
Aedes triseriatus								
Mean rank	4.6 | 2.8	1.8 | 4.6	3.2 | 6.2	3.8 | 6.6	2.6 | 4.2	5.1 | 6.0	7.6 | 3.4	7.3 | 2.2
Probability > |*t*|	0.053	0.101	0.050*	0.060	0.120	0.498	0.011*	0.007*
Anopheles quadrimaculatus								
Mean rank	1.6 | 4.4	5.6 | 6.4	5.6 | 6.8	5.8 | 6.4	4.0 | 4.6	5.3 | 2.8	6.5 | 2.0	1.6 | 2.6
Probability > |*t*|	0.073	0.282	0.170	0.734	0.552	0.080	0.001*	0.230
Culex nigripalpus								
Mean rank	1.8 | 1.3	3.3 | 3.4	5.5 | 6.5	5.0 | 6.0	2.8 | 6.0	7.1 | 6.5	6.6 | 3.5	4.0 | 2.5
Probability > |*t*|	0.182	0.495	0.252	0.308	0.099	0.537	0.224	0.103
Culex quinquefasciatus								
Mean rank	2.2 | 1.4	2.5 | 5.5	3.4 | 5.1	5.3 | 5.6	5.6 | 5.2	6.3 | 5.5	7.8 | 5.2	3.0 | 2.5
Probability > |*t*|	0.448	0.021*	0.156	0.638	0.692	0.597	0.017*	0.611

* Difference in mean ranks significantly different from 0 ($P = 0.05$, Student's *t*-test).

Table 3. Mean capture efficiency indices (CEI) for LT collection method for five mosquito species.

	Period							
	1	2	3	4	5	6	7	8
Aedes albopictus								
CEI	0.096	0.159	0.040	0.065	0.075	0.028	0.083	0.044
Aedes triseriatus								
CEI	0.013	0.124	0.105	0.043	0.068	0.027	0.002	0.002
Anopheles quadrimaculatus								
CEI	0.163	0.124	0.118	0.259	0.115	0.077	0.042	0.148
Culex nigripalpus								
CEI	0.065	0.091	0.089	0.095	0.264	0.020	0.014	0.039
Culex quinquefasciatus								
CEI	0.448	1.084	0.861	0.286	0.124	0.337	0.035	0.271

Tabulated means based on $\log_{10}(nf_i + 1)$.

likelihood estimation procedure and the determination of mosquito population size in calculations of vectorial capacity (Garrett-Jones, 1964; Dye, 1986; Gu *et al.*, 2003) because each of these computational methods depends on estimates of mosquito density acquired using relative sampling methods, most often light traps. Sample representativeness is a concern in such cases, particularly when group-based pathogen assay methods are used to quantify infection rates in captured mosquito populations (Katholi & Unnasch, 2006).

Patterns of diel activity and the relationship of capture rates by R_{LT} *to* R_{HL}

Patterns of diel activity in mosquito populations are used to target the application of insecticides in time and space, measure repellency in field tests and to determine the risk of infection with mosquito-borne pathogens. The baseline HL responses observed here indicate a single peak of diel activity for all mosquito species except *Ae. triseriatus*. In contrast, responses to LT at sunrise by *An. quadrimaculatus* and *Cx. nigripalpus*, which compare poorly with HL responses at the same time, are likely the result of stimuli other than human host presence. Similarly, patterns of diel activity indicated by each collection method, when compared by the rank-order of periods, lacked congruency for all species except *Cx. quinquefasciatus*. For example, the highest ranked R_{HL}-based periods indicate maximum activity before sunset for *Ae. albopictus*, whereas LT responses indicate peak activity after sunset. For *An. quadrimaculatus* and *Cx. nigripalpus*, discordance in the patterns of activity indicated by LT and HL was observed for the midday and sunrise periods. Taken in sum, these observations suggest that the patterns of diel activity indicated by LT collection do not accurately reflect temporal modes of mosquito landing on human hosts and that, in the field, such activity should be verified by observation of HL responses.

Trap efficiency

In a strict sense, trap efficiency indicates the number of mosquitoes available for capture that are actually captured. Under the proper conditions (i.e. mosquito availability to capture is constant; the rate of mosquito capture per unit of

Table 4. Mean daily capture rates for five mosquito species by LT ($R_{D(\mathrm{LT})}$) and HL ($R_{D(\mathrm{HL})}$) collection methods.

Mosquito species		Mean daily capture rate					$F_{1,4}$	P
Aedes albopictus	Date*	05/05	06/05	06/05	07/05	07/05		
	$R_{D(\mathrm{LT})}$	0.086	0.032	0.104	0.081	0.052	0.61	0.492
	$R_{D(\mathrm{HL})}$	1.053	0.628	1.075	0.859	1.264		
Aedes triseriatus	Date	07/04	08/04	08/04	04/06	07/06		
	$R_{D(\mathrm{LT})}$	0.006	0.011	0.013	0.014	0.014	2.14	0.239
	$R_{D(\mathrm{HL})}$	0.273	0.293	0.474	0.356	0.756		
Anopheles quadrimaculatus	Date	05/05	06/05	06/05	06/06	07/06		
	$R_{D(\mathrm{LT})}$	0.067	0.166	0.096	0.033	0.004	11.40	0.043
	$R_{D(\mathrm{HL})}$	0.638	0.742	0.641	0.619	0.629		
Culex nigripalpus	Date	07/05	09/05	09/06	08/06	10/06		
	$R_{D(\mathrm{LT})}$	0.022	0.040	0.076	0.009	0.002	5.21	0.106
	$R_{D(\mathrm{HL})}$	0.299	0.463	0.611	0.291	0.433		
Culex quinquefasciatus	Date	08/04	03/05	04/05	05/05	05/06		
	$R_{D(\mathrm{LT})}$	0.160	0.235	0.122	0.134	0.230	3.15	0.174
	$R_{D(\mathrm{HL})}$	0.185	0.709	0.466	0.491	1.083		

* month/year of observation. Tabulated means based on $\log_{10}(nf_i + 1)$.

Table 5. Fitted linear model coefficients (±SE) for $R_{\mathrm{LT}} = R_{\mathrm{HL}}$.

Period(s)	β_0	$\beta_1(R_{\mathrm{HL}})$	$\beta_2(R^2_{\mathrm{HL}})$	$\beta_3(R^3_{\mathrm{HL}})$	P
Aedes albopictus			No significant fit		
Aedes triseriatus					
6–7–8–1	0.0081 (0.0109)	0.0015 (0.0802)	−0.0633 (0.1672)	−0.0714 (0.1020)	0.020
5–6–7–8–1	−0.0015 (0.0121)	0.1294 (0.0814)	−0.3726 (0.1578)	0.2707 (0.0893)	<0.001
Anopheles quadrimaculatus					
1–2–3–4–5	0.2029 (0.0848)	−1.4020 (0.5163)	3.2136 (0.9576)	−1.8802 (0.5269)	0.001
Culex nigripalpus					
1	−0.0965 (0.0387)	0.1960 (0.0447)			0.022
1–2	0.0178 (0.0375)	−0.0307 (0.1277)	0.1135 (.0917)		0.008
8–1–2	−0.0094 (0.0241)	0.0182 (0.0341)			0.033
1–2–3–4–5	0.0046 (0.0098)	0.1046 (0.0206)			<0.001
Culex quinquefasciatus					
6–7–8–1	0.3643 (0.2033)	−2.2273 (1.0925)	3.7427 (1.5430)	−1.4310 (0.6141)	0.001
5–6–7–8–1	0.0230 (0.0964)	0.0197 (0.3111)	0.2659 (0.2124)		0.001

time is constant), trap efficiency can be used to estimate absolute population density (Southwood & Henderson, 2000). Neither of the foregoing conditions was met in our study nor is either likely to be observed in nature. This fact notwithstanding, the crucial measure of mosquito availability for capture in a vector surveillance systems (using LT or any other device), and the response most relevant to disease agent transmission, is the number of female mosquitoes that land on a human/animal host per unit of time. LT efficiency determined on this basis was generally low in each period (e.g. 80% of CEI < 0.17), regardless of mosquito species. An exception to this pattern was for *Cx. quinquefasciatus* (average CEI = 0.43) in period 2, when capture rates using LT were 8% higher (more efficient) than those for HL.

Trap efficiency may also be considered in a relative sense as the ratio to one another of the numbers of each mosquito species captured using (in this case) LT compared with the same ratios as determined by HL. When we ranked the ratios of mean $R_{\mathrm{D(LT)}}$ to mean $R_{\mathrm{D(HL)}}$ observed for each species in this manner, the order of ranks was: *Cx. quinquefasciatus* (0.125:1) > *An. quadrimaculatus* (0.043:1) > *Cx. nigripalpus* (0.029:1) > *Ae. albopictus* (0.015:1) > *Ae. triseriatus* (0.011:1). Thus, in a hypothetical LT collection comprising these five species, *Ae. triseriatus* would be under-represented by 11% compared with *Cx. quinquefasciatus*, and *Ae. albopictus* would be under-represented by 3% compared with *An. quadrimaculatus*. And while these rankings clearly depend on the LT configuration and mosquito strains used in the present study, the relative efficiency of other trap designs has been compared in a similar manner in other studies (Kline *et al.*, 2006, 2007). Our findings suggest that the merits of any trapping technology being considered for use in a vector surveillance system should be ascertained via the comparison of mosquito capture rates using that technology with the concomitant rate of mosquito landing on human/animal subjects before such traps are deployed in the field.

The ideal vector surveillance system would enable early detection of mosquito vectors and the timely/accurate prediction of disease agent transmission. The effectiveness of any such system will depend on the estimation of critical population parameters in an unbiased manner (Morris, 1960; Bidlingmayer, 1985; Dye, 1986). This requires extraction of a sample of the habitat and enumeration of the target organisms contained in it (Southwood & Henderson, 2000) – an impractical approach for vector surveillance, given the mobility and constantly changing patterns of dispersion of adult mosquito populations. Nor are conventional mosquito traps, including recently developed mechanical and semiochemical-augmented trapping technologies (Kline, 2007), designed to acquire unbiased estimates of mosquito density. In the case of light traps, for example, their range of attractiveness to mosquitoes is unknown, as is the volume of habitat they sample. The interpretation of data obtained using light traps and other relative sampling methods (Southwood & Henderson, 2000) is thus limited by the confounding effects of trap location, by change in the density and behavior of mosquito populations in space and time, and by variations in trap efficiency caused by change in local weather conditions and/or other environmental factors (Bidlingmayer, 1985). A significant consequence of these sampling deficiencies is insensitivity of the vector surveillance system to arbovirus infection rates in the mosquito population (Reisen & Pfuntner, 1987).

An objective of this study was to identify strategies for LT operation that would provide field-testable hypotheses relative to the accurate identification of mosquito landing rates on a human host. This was not possible for *Ae. albopictus*, although more recently devised trap configurations for other aedine species (Kröckel *et al*., 2006; Chambers *et al*., 2009) may enable such comparisons in the future. For *An. quadrimaculatus*, a single plan of LT operation comprised trap operation from sunset through sunrise. Multiple schemes for LT operation were identified for *Cx. nigripalpus*, *Cx. quinquefasciatus* and *Ae. triseriatus*. For *Cx. nigripalpus*, the most precise index of the mosquito landing rate is from LT data collected between sunset and sunrise, even though LT data obtained for this species before, during and after sunset provide similar (albeit less precise) information. In this same context, optimal LT operation times for *Cx. quinquefasciatus* and *Ae. triseriatus* are sunrise through sunset. It is important to note that these strategies for LT operation are based on a correlation between mosquito responses to LT and HL for specific times of the diel period. These times may not be the same as those for maximum and/or minimum mosquito flight activity.

Evaluation of the results of this study under field conditions is an important next research step, particularly in cases where the objective of the monitoring/surveillance program is to understand, depict and/or accurately forecast the rate of human contact with pest and/or vector mosquito species. This may not be possible for *Ae. albopictus*, where LT responses do not accurately represent the timing or intensity of mosquito contact with humans. Actual measurement of the mosquito landing rate, in such cases, may be required. For other species, the results of our study suggest it is feasible to identify specific LT operating times and to interpret the resulting capture data in terms of the frequency of mosquito-human host contact. Under the conditions of this study, for example, LT operation between sunset and sunrise provided a reliable index of the mosquito landing rate on humans by *Cx. nigripalpus* and *An. quadrimaculatus*, whereas LT operation between sunrise and sunset or continuously for 24 h did not. Similarly, for *Cx. quinquefasciatus* and *Ae. triseriatus*, the times of LT operation for this purpose are best restricted to between sunrise and sunset.

Finally, in some operational venues, an index of mosquito activity such as provided by LT is considered as, or more, useful than obtained by other methods (including HL). This may be the case for pest mosquito species known to present little or no danger of disease agent transmission to humans or livestock. An important requirement, in such situations, is to obtain mosquito samples under the same conditions, keeping in mind that each trap location is unique and that microclimate, illumination levels and other local conditions profoundly influence mosquito flight (Bidlingmayer, 1985). Furthermore, in such cases, it may be prudent to develop a sampling plan that targets individual mosquito species, rather than the composite population of airborne mosquito species. This can be done using knowledge of the natural history of the target species and care in the selection of the habitat(s) in which traps are deployed and from which samples of the adult mosquito population are obtained.

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