A Comparison Of Two Commercial Mosquito Traps for the Capture Of Malaria Vectors In Northern Belize, Central America

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A COMPARISON OF TWO COMMERCIAL MOSQUITO TRAPS FOR THE CAPTURE OF MALARIA VECTORS IN NORTHERN BELIZE, CENTRAL AMERICA[1]

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ABSTRACT. To achieve maximum success from any vector control intervention, it is critical to identify the most efficacious tools available. The principal aim of this study was to evaluate the efficacy of 2 commercially available adult mosquito traps for capturing *Anopheles albimanus* and *An. vestitipennis*, 2 important malaria vectors in northern Belize, Central America. Additionally, the impact of outdoor baited traps on mosquito entry into experimental huts was assessed. When operated outside of human-occupied experimental huts, the Centers for Disease Control and Prevention (CDC) miniature light trap, baited with human foot odors, captured significantly greater numbers of female *An. albimanus* per night (5.1 ± 1.9) than the Biogents Sentinel™ trap baited with BG-Lure™ (1.0 ± 0.2). The 2 trap types captured equivalent numbers of female *An. vestitipennis* per night, 134.3 ± 45.6 in the CDC trap and 129.6 ± 25.4 in the Sentinel trap. When compared to a matched control hut using no intervention, the use of baited CDC light traps outside an experimental hut did not impact the entry of *An. vestitipennis* into window interception traps, 17.1 ± 1.3 females per hour in experimental huts vs. 17.2 ± 1.4 females per hour in control huts. However, the use of outdoor baited CDC traps did significantly decrease the entry of *An. albimanus* into window interception traps from 3.5 ± 0.5 females per hour to 1.9 ± 0.2 females per hour. These results support existing knowledge that the underlying ecological and behavioral tendencies of different *Anopheles* species can influence trap efficacy. Furthermore, these findings will be used to guide trap selection for future push–pull experiments to be conducted at the study site.

KEY WORDS Belize, push–pull strategy, *Anopheles albimanus*, *Anopheles vestitipennis*, outdoor traps

INTRODUCTION

Operational realities such as the management of insecticide resistance and the need to target the behavioral patterns (e.g., outdoor-, early evening– and/or day-biting) of a wide range of vector species are limiting the effectiveness of traditional vector control tools such as indoor residual spraying and long-lasting insecticide nets in many malaria endemic settings (Grieco et al. 2007, Achee et al. 2009, Enayatiand Hemingway 2010, Afrane et al. 2012, Mwangangi et al. 2013). It is not surprising, therefore, that the development of novel vector control strategies has been identified as a top priority within the global health community (WHO 2003, 2010; Townson et al. 2005; Enayati and Hemingway 2010; malERA 2011). One novel strategy under consideration is a push–pull method, whereby the complementary actions of spatial repellents (which ''push'' or deter vectors from entering treated spaces) and mosquito traps (which ''pull'' or remove vectors from a given outdoor area) are used simultaneously to decrease the probability of human exposure to mosquito bites. Such an outcome could serve to prevent pathogen transmission in a variety of settings (Cook et al. 2007, Kitau et al. 2010, Paz-Soldan et al. 2011, Achee et al. 2012).

As with all vector control interventions under development, identifying the most efficacious tools, and challenges to their implementation, is critical to achieving maximum success. This includes a thorough understanding of local disease transmission dynamics such as recognition of the primary vector species and target vector behavior patterns as well as, ideally, field evaluation to drive optimization. The current study represents one component of a larger field project focused on the evaluation of a push–pull strategy for the control of malaria vectors in northern Belize, Central America, where *Anopheles vestitipennis* Dyar and Knab and *An. albimanus* Wiedemman are both known to be regionally important vectors (Achee et al. 2000, Grieco et al. 2005a, Gaffigan et al. 2012, Sinka 2013, WRBU 2013). Importantly, each species has also been characterized, in Belize, to exhibit different behavioral profiles: *An. vestitipennis* is known to be more highly endophagic and anthropophagic, whereas *An. albimanus* tends to

[1] The opinions contained herein are the private views of the authors and are not to be construed as official or reflecting the views of the Department of Defense or the Uniformed Services University of the Health Sciences. The use of commercial names does not constitute product endorsement or recommendation.

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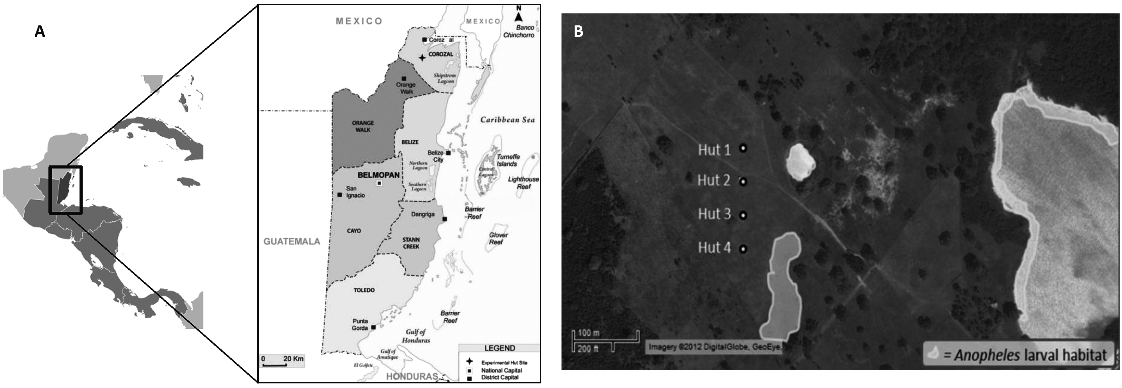

Fig. 1. (A) The study site in Corozal District, Belize. (B) Hut placement on-site, with known *Anopheles* spp. larval habitats highlighted.

be more highly exophagic and zoophagic (Bangs 1999, Grieco et al. 2002, Grieco et al. 2005a).

Trapping malaria vectors has often been described as difficult (Enserink 2002, Wong et al. 2013). However, several traps have been successfully integrated into research and routine surveillance activities. Among these established traps, the Centers for Disease Control and Prevention (CDC) Miniature Light Trap Model 512 (CDC LT) (John W. Hock Company, Gainesville, FL) and the BG Sentinel™ trap (BGS) (Biogents, A.G., Regensburg, Germany) were selected for evaluation in the current study. Both traps are widely available, field deployable, and easily baited. The CDC LTs remain an industry standard trap used to sample and capture *Anopheles* spp. for public health and vector research applications (Sikaala et al. 2013, WHO 2013). The BGS trap, though specifically designed to capture *Aedes* spp., has shown some efficacy at trapping *Anopheles* spp. and has been previously integrated into field experiments demonstrating proof of concept of a push–pull strategy for the control of dengue virus vectors (Schmied et al. 2008, Hiwat et al. 2011, Achee et al. 2012, Salazar et al. 2013).

Specific objectives of the experiments described here included quantifying 1) the efficacy of the CDC light trap and BG-Sentinel trap at capturing the target vectors *An. vestitipennis* and *An. albimanus* and 2) the effect of baited traps in the peridomestic area on mosquito entry into human-occupied experimental huts.

MATERIALS AND METHODS

Field site

The study site was located in an open pasture near Progresso village in the Corozal District of northern Belize (18°11′52″N, 88°26′18″W), surrounded by freshwater swamplands and several permanent lagoons (Fig. 1). The rainy season in northern Belize typically lasts from May to December, when the region experiences average rainfall of around 200 mm/month and *Anopheles* spp. densities are highest (Grieco et al. 2005b, Gonquez 2013).

Experimental huts

Two identical experimental huts, located 50 m apart along a straight north–south transect, were constructed on-site (Fig. 2). Based on a previously described portable hut design (Achee et al. 2005), huts were made in a style typical of regional homes using locally acquired materials. Briefly, each structure measured 3.6 m^2 and was constructed of an untreated pine lumber frame with plywood walls and flooring and a corrugated tin roof. Both huts were fashioned with one 182-cm by 76.2-cm door cut into the east-facing wall, and each of the 3 remaining walls contained one 76.2-cm^2 window built to accommodate interception traps.

Mosquito lures, trap placement and outdoor collections

Two types of mosquito lures were used in conjunction with the outdoor traps. First was the BG-Lure™ (Biogents, A.G.), a blend of components including lactic acid, ammonia, caproic acid, and other fatty acids (von Witzendorff et al. 2004). Although specifically intended to capture *Aedes* spp. in conjunction with the BGS trap, there is some evidence that the BG-Lure can attract *Anopheles* spp. as well (Mohammed and Smith 2011) and it is recommended by the manufacturer to increase BGS trap yields of other mosquitoes, including *Anopheles* spp. (Biogents 2012). The BG-Lure was handled and used according to manufacturer's instructions for use in tropical climates. The 2nd lure consisted of human foot residues emanating from worn cotton socks placed on top of the CDC LT rain guard

Fig. 2. (A) An experimental hut at the field site with an open window (portal for mosquito entry), with a window interception trap used to monitor mosquito entry (inset). (B) Baited Centers for Disease Control and Prevention light traps and (C) baited BG-Sentinel™ traps positioned outside each portal of entry.

(Njiru et al. 2006, Schmied et al. 2008). Prior to use in mosquito collections, sock lures were worn one pair at a time for 12 h by the same individual during periods of roughly equal activity (i.e., daily preparations at the field site or during the overnight collections). For use as mosquito lure, a randomly selected pair of worn socks aged between 24 and 72 h was placed at each CDC LT. Each pair of socks was used for 2 collections before replacement with a more recently worn pair. When not in use, sock lures were kept at ambient temperature in sealed plastic bags away from direct sunlight. Baited traps were positioned outside each window of an occupied experimental hut and operated according to manufacturer's instructions: CDC traps were hung 2 m above ground level while BGS traps were positioned parallel to the hut platform (1 m above ground). Both trap types were set at a 1-m distance from the exterior hut wall (Fig. 2B, 2C). Outdoor traps were positioned, baited, and switched on 30 min prior to sunset (approximately 1730 h) and operated continuously to just after sunrise (approximately 0600 h). Collection bags were removed and replaced every 60 min during each 12-h collection period.

Indoor collections

Window interception traps were based on the designs of Muirhead-Thomson (1950) and Grieco et al. (2000) and fitted onto each experimental hut window (Fig. 2A). Collections from interception traps were conducted every 30 min during a 12-h sampling period (1800 h to 0600 h). Collections were further divided into four 3-h shifts to allow for equal rotation of collectors. The door of each hut remained closed during the entire sampling period so that the windows, with attached interception traps, represented the only portals of hut entry for host-seeking mosquitoes. To facilitate mosquito collection, trap portals were temporarily closed with 3-inch polyurethane foam (Landy's and Sons, Ltd., Orange Walk Town, Belize) at the beginning of each collection interval and immediately reopened afterwards. Thirty minutes before dusk (approximately 1730 h), a 2-person collection team entered each hut to provide indoor host cues and prepare for mosquito collections. Starting at 1800 h, 1 collector in each hut aspirated all mosquitoes from the interception traps for a total of 15 min (i.e., 1 5-min interval per window), while the 2nd collector rested. Collectors rotated capture and resting activities at the conclusion of every 3-h shift. Collected mosquitoes were placed into plastic cups individually labeled by hut, time, and unique window code and killed using acetone vapors.

Study design

To control for bias in mosquito capture efficiency, collector attractiveness between teams, and/or mosquito abundance by hut locations, a Latin square study design was employed such that each trap type, lure, and collection team was rotated between each hut. Initial window interception trap collections conducted without any experimental interventions from September 28 to October 6, 2011, indicated high mosquito densities and excellent baseline comparability between huts, collection teams, and nights with no significant differences (ANOVA, $\alpha = 0.05$) observed in terms of mosquitoes collected (data not presented). Three separate experiments were then conducted. The 1st, from October 17 to October 27, 2011, was a head-to-head comparison of the efficacy of CDC LTs using the literature-recommended sock lure and BGS traps using the manufacturer's specified BG-Lure, operated simultaneously at different occupied huts over 4 nights (a total of 12 trap-nights for each outdoor trap type). The second experiment, also over 4 nights, was conducted November 3–19, 2011, and measured the impact of baited CDC LTs on mosquito hut entry compared to an untreated control (a total of 12 interception trap-nights each for light trap intervention and control). The final experiment was conducted January 10–28, 2012, to compare the efficacy of CDC LTs baited with either sock lure or

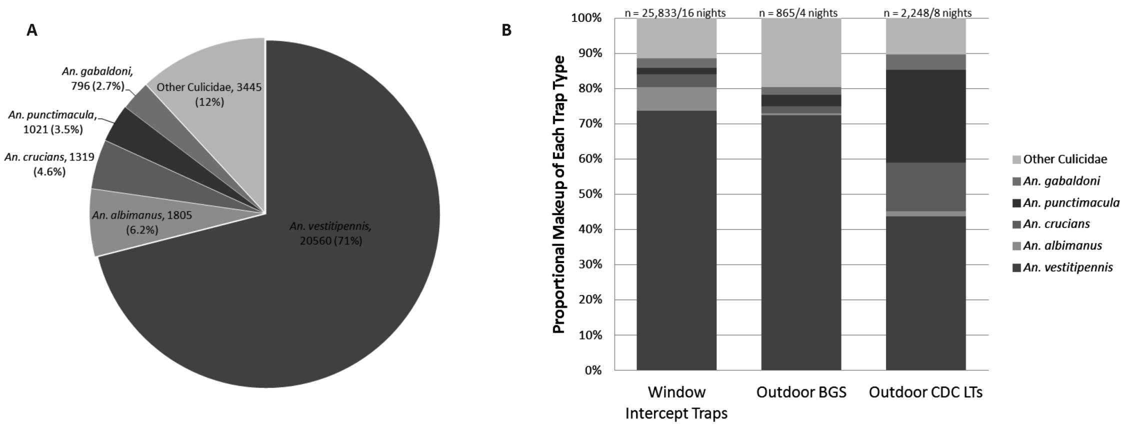

Fig. 3. (A) The total adult female mosquito composition at the field site during 16 overnight (12-h) collections from September to December 2011. A total of 28,946 specimens were collected by window interception traps, baited Centers for Disease Control and Prevention light traps (CDC LTs) and baited BG Sentinel™ traps (BGS). (B) The species composition, by proportion, of the mosquitoes collected using the 3 methods.

BG-Lure (6 trap-nights for each lure type). All mosquitoes captured in the study were identified to subfamily, with anopheline mosquitoes further identified to species using a site-appropriate morphological key (Wilkerson et al. 1990).

Data analysis

Unless otherwise stated, the geometric means of mosquitoes captured are presented with the standard error of the means. Raw data in the form of total numbers of mosquitoes captured were $\log_e$ transformed and means, standard errors of the means, and confidence interval endpoints were calculated and then back-transformed to obtain the geometric means. Means were compared via Student's t-test ($\alpha = 0.05$) using Excel 2007 (Microsoft, Redmond, WA) and SPSS Statistics 20 (IBM, Armonk, NY). For the 1st experiment, the difference in the mean number of mosquitoes collected from CDC LTs and BGS traps was calculated. In the 2nd experiment, the impact of the presence of outdoor CDC LTs on mosquito entry into experimental huts was evaluated by comparing the mean number of mosquitoes collected from interception traps in control huts (no CDC LTs) and from interception traps in huts with baited CDC LTs hanging outside the windows. Lastly, a head-to-head comparison of lure types used in conjunction with CDC LTs calculated the difference in mean number of mosquitoes collected from traps using human foot odors and traps using the BG-Lure. Weather data, including indoor and outdoor temperature, humidity, and wind speed and direction, were recorded using HOBO® Pro Series Weatherproof Data Loggers (Forestry Suppliers Inc., Jackson, MS) and a DiC-3 handheld anemometer (Maximum Weather Instruments, New Bedford, MA).

RESULTS

A total of 28,946 mosquitoes were collected during 16 all-night (12-h) collections. In all, 5 *Anopheles* species were identified: 20,560 *An. vestitipennis* (71.0%), 1,805 *An. albimanus* (6.2%), 1,319 *An. crucians* Wiedemman (4.6%), 1,021 *An. punctimacula* Dyar and Knab (3.5%), and 796 *An. gabaldoni* Vargus (2.7%) (Fig. 3A). Culicine mosquitoes, which were not able to be identified to species level, accounted for the remaining 11.9% of the total caught and included predominately *Culex* spp., *Psorophora* spp., and *Mansonia* spp. (Fig. 3A). The proportional abundance of each species captured in the 3 trap types differed: *An vestitipennis* made up 73.6% (19,025/25,833), 72.4% (627/865), and 43.6% (774/1,773) of all mosquitoes collected from window interception traps, BGS traps, and CDC LTs, respectively (Fig. 3B). Conversely, *An. albimanus* accounted for 6.7% (1,754/25,833) and 1.5% (28/1,773) of all mosquitoes from window interception traps and CDC LTs, respectively, and less than 0.5% (4/865) of all mosquitoes captured in BGS traps (Fig. 3B).

Results from trials comparing the efficacy of CDC LTs and BGS traps indicate that there were no significant differences in the nightly average numbers of mosquitoes entering the experimental huts based on outdoor trap type (Table 1). Outdoors, the nightly mean *An. vestitipennis* captured in CDC LTs baited with human foot odors (134.3 ± 45.6) did not significantly differ from the mean captured in BGS traps baited with the BG-Lure (129.6 ± 25.4) over the 4-night trial (Fig. 4A). However, the CDC LTs did capture a greater number of *An. vestitipennis* than the BGS traps on 3 of the 4 nights. For *An. albimanus*, the CDC LTs captured an average of 5.1 ± 1.9 mosquitoes per night, significantly more ($P < 0.05$) than the BGS traps, which captured 1.0 ±

Table 1. Nightly average mosquitoes collected during 4 overnight (12-h) collections comparing the efficacy of Centers for Disease Control and Prevention light traps and BG Sentinel™ traps.[1]

	Mean ± SEM window intercept traps: CDC LT[2] hut	Mean ± SEM window intercept traps: BGS hut	Mean ± SEM outdoor CDC LT	Mean ± SEM outdoor BGS
Anopheles vestitipennis	1138 ± 102	1160 ± 105	134 ± 46	130 ± 25
An. albimanus	71 ± 11	75 ± 9	5 ± 2*	1 ± 0*
An. crucians	38 ± 7	39 ± 10	44 ± 41*	4 ± 1*
An. punctimacula	21 ± 5	40 ± 14	77 ± 80*	7 ± 1*
An. gabaldoni	45 ± 8	57 ± 15	10 ± 14	3 ± 3
Other Culicidae	112 ± 15	165 ± 5	38 ± 7	38 ± 5

[1] Outdoor traps were hung near the windows of experimental huts, which were fitted with interception traps and occupied by 2 collectors. Geometric means are presented.
[2] SEM, standard error of the mean; CDC LT, Centers for Disease Control and Prevention miniature light trap; BGS = BioGents Sentinel™ trap.
* indicates significant difference between trap type, Student's t-test $P < 0.05$.

0.2 mosquito per night (Fig. 4B). CDC LTs also captured greater nightly averages of *An. crucians* (43.9 ± 40.7 vs. 3.8 ± 0.9) and *An. punctimacula* (77.3 ± 79.8 vs. 6.9 ± 1.0) than did BGS traps (Table 1). When CDC LTs were used in conjunction with 2 different lure types, data showed human foot residues on socks to attract significantly more *An. vestitipennis* (2.6 ± 2.4 per night) than the BG-Lure did (0.0 per night) (Table 2). There were no *An. albimanus* collected during this particular experiment.

Results from the evaluation of sock-baited CDC LTs on hourly mosquito hut entry suggest that the presence of baited CDC LTs outside of windows did not significantly reduce the numbers of *An. vestitipennis* collected per hour in window interception traps compared to control huts with no outdoor trap treatment (17.1 ± 1.3 CDC LTs vs. 17.2 ± 1.4 control) (Fig. 5). Additionally, there was no impact on the time of peak entry, or general entry pattern, observed for *An. vestitipennis* (Fig. 6). On the other hand, significantly fewer *An. albimanus* were captured per hour from portals of entry when CDC LTs were positioned outdoors compared to a control (1.9 ± 0.2 vs. 3.5 ± 0.5) (Fig. 5). Further analysis based on time of collection showed that the reduction in *An. albimanus* entry was statistically significant ($\alpha < 0.05$) only during the early evening, within 3 h of sunset (Fig. 6).There was also a significant reduction in *An. punctimacula* entry in the presence of a baited CDC LT (0.3 ± 0.3 vs. 0.2 ± 0.0) (Table 3).

Meteorological data showed that winds were predominantly out of the northeast and generally calm with nightly maximum speeds consistently occurring during the first 3 h of the collection period, averaging 2.4 ± 0.75 km/h. Outdoors, the average temperature was 21.6 ± 3.1°C with a range from 28.3°C to 17.5°C while the average relative humidity was 94.7 ± 2.6%. Indoors, temperature and relative humidity measurements were not significantly different ($P = 0.05$) between huts and data points were therefore pooled from both structures: indoor temperatures averaged 23.4 ± 2.9°C, ranging from 29.9°C to

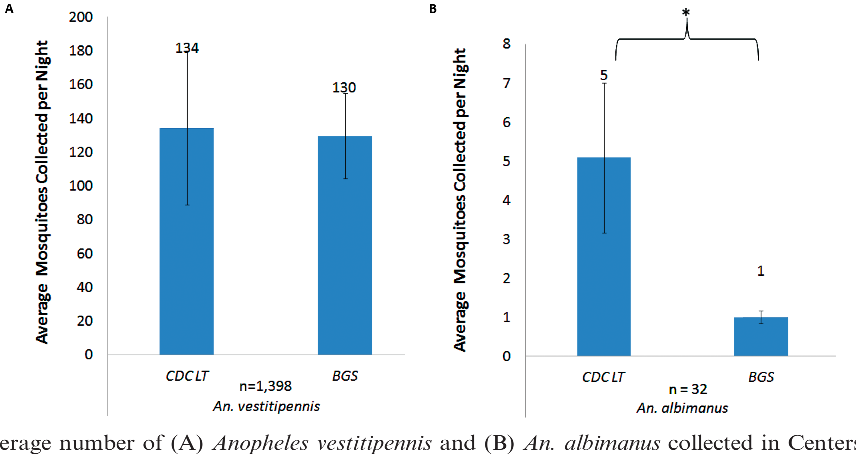

Fig. 4. Average number of (A) *Anopheles vestitipennis* and (B) *An. albimanus* collected in Centers for Disease Control and Prevention light traps (CDC LTs) baited with human foot odor and in BioGents BG-Sentinel™ (BGS) traps baited with the BG-Lure™ over 4 all-night (12-h) collections in northern Belize, Central America. Geometric means with standard error of the means are presented; * indicates a significant difference at $P < 0.05$.

Table 2. Efficacy of Centers for Disease Control and Prevention light traps baited with either human foot odor (sock lure) or BG-Lure™ over 4 all-night (12-h) collections.

	Sock lure		BG-Lure™	
	Total collected	Nightly mean ± SEM[1]	Total collected	Nightly mean ± SEM
Anopheles vestitipennis	18	2.6 ± 2.4*	0	0*
An. albimanus	0	0	0	0

[1] Geometric means are presented; SEM, standard error of the mean.
* indicates significant difference between trap type, Student's *t*-test $P < 0.05$.

19.1°C, and the average indoor relative humidity measured 81.6 ± 6.4%. Though rain showers were common during daytime hours, no rainfall occurred during these overnight collection periods.

DISCUSSION

The first objective of the current study was to quantify the efficacy of 2 outdoor traps, used in conjunction with different lures, in capturing 2 important malaria vectors in northern Belize, *An. vestitipennis* and *An. albimanus*. The goal was to determine which trap would be most appropriate for future use in combination with an indoor spatial repellent for the evaluation of a push–pull mosquito control strategy. Our findings show both the CDC LT and BGS trap captured target *Anopheles* species, but differences in density and proportion of each species existed by trap type. These results reflect similar findings from other studies that have also shown species composition, in terms of proportions of total catches, is dependent on the specific trap and lure used (Enserink 2002, Missawa et al. 2011, Obenauer et al. 2013, Wong et al. 2013). Although both CDC LTs and BGS traps collected equivalent numbers of *An. vestitipennis*, the CDC LT captured significantly more *An. albimanus* females. Additionally, during experiments performed at the end of the 2011 rainy season with lower overall mosquito densities, the use of the BG-Lure with CDC LTs failed to capture any *Anopheles* spp. mosquitoes and was clearly outperformed by CDC LTs using the human foot-odor bait. This suggests that the CDC LT baited with dirty socks is suitable for subsequent push–pull experiments at this study site. However, a more comprehensive investigation of lure and trap type might be warranted for providing valuable insight into lure efficacy across different mosquito species.

A 2nd objective was to quantify the effect of a baited CDC LT hanging in the peridomestic area on mosquito entry into an occupied experimental hut. Baited light traps are often used for sampling local mosquito populations (Kline 2006, Sikaala et al. 2013, WHO 2013) but are not generally effective as stand-alone interventions to control them (Kline 2006, Revay et al. 2013). Accordingly, it was somewhat unexpected that the use of CDC LTs outside experimental hut windows would have an impact on mosquito entry into the hut, as measured by collections from window interception traps. However, there was a significant reduction in early evening *An. albimanus* entry. Caution should be used not to over-interpret this reduction (an average of 8 fewer

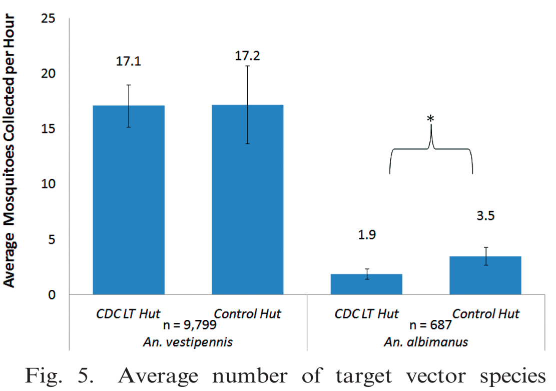

Fig. 5. Average number of target vector species collected entering experimental huts in the presence of Centers for Disease Control and Prevention light traps (CDC LTs) outside windows of experimental huts over 4 all-night (12-h) collections. Geometric means with standard error of the means are presented; * indicates a significant difference (Student's *t*-test) at $P < 0.05$.

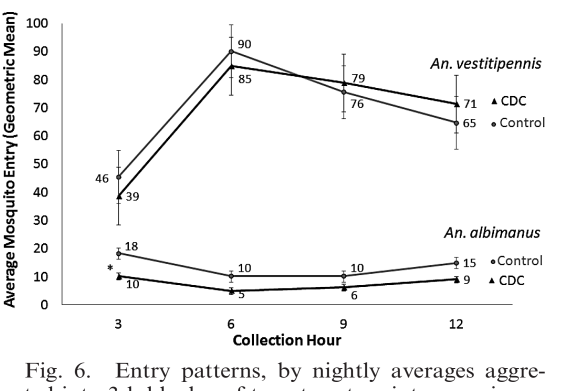

Fig. 6. Entry patterns, by nightly averages aggregated into 3-h blocks, of target vectors into experimental huts in the presence (CDC) and absence (control) of outdoor Centers for Disease Control and Prevention light traps baited with human foot odor. * Indicates a significant difference at $P < 0.05$. Error bars indicate the standard error of the mean.

Table 3. The impact of outdoor baited Centers for Disease Control and Prevention light traps on mosquito entry into an experimental hut during 4 all-night (12-h) collections.[1]

	Hourly mean ± SEM window intercept traps: control[2] hut	Hourly mean ± SEM window intercept traps: CDC LT hut	Hourly mean ± SEM outdoor CDC LT
Anopheles vestitipennis	17.2 ± 1.4	17.1 ± 1.3	7.6 ± 0.5
An. albimanus	3.5 ± 0.5*	1.9 ± 0.2*	1.1 ± 0.2
An. crucians	1.0 ± 0.2	1.4 ± 0.2	7.5 ± 0.8
An. punctimacula	0.33 ± 0.3*	0.17 ± 0.0*	3.9 ± 0.5
An. gabaldoni	0.44 ± 0.1	0.55 ± 0.1	1.0 ± 0.2
Other Culicidae	1.8 ± 0.4	1.4 ± 0.3	3.8 ± 0.7

[1] Outdoor traps were hung near the windows of experimental huts, which were fitted with window interception traps and occupied by 2 collectors. Geometric means are presented. CDC LT, Centers for Disease Control and Prevention miniature light trap.
[2] Paired control hut with no outdoor traps.
* indicates significant difference between trap type, Student's *t*-test $P < 0.05$.

mosquitoes during the first 3 h after sunset) as epidemiologically significant. It is, however, important to note that the decreased entry was most prominent during the time of night that corresponds to peak feeding behaviors previously observed for *An. albimanus* in Belize (Bangs 1999, Roberts et al. 2002, Achee et al. 2006, Achee et al. 2007). This suggests that an outdoor trap alone could negatively impact indoor densities of this important malaria vector, thereby disrupting human–vector contact. Interestingly, there was no corresponding effect of outdoor CDC LTs on *An. vestitipennis* entry, as the numbers of *An. vestitipennis* females collected from window interception traps was the same at control and experimental huts. These results may indicate that an outdoor baited trap more effectively targets the exophagic *An. albimanus*, whose outdoor host-seeking behaviors are more likely to be impacted by the outdoor lure, whereas the more endophagic *An. vestitipennis*, with a stronger affinity for feeding indoors, appears more likely to bypass an outdoor trap and proceed to enter an occupied structure to feed.

In conclusion, findings from the experiments described herein support existing knowledge that the underlying ecological and behavioral tendencies of different *Anopheles* species can profoundly influence trap efficacies. Such information underlies the importance of characterizing disease transmission dynamics at the local level in order to drive the development and optimization of vector control strategies. At this site in northern Belize, CDC LTs baited with human foot odors and BGS traps baited with BG-Lure were equally effective in collecting the malaria vector *An. vestitipennis* when deployed outside of occupied experimental huts, but the CDC LT was clearly more efficacious in collecting the sympatric vector *An. albimanus*. Accordingly, the CDC LT baited with foot odors is the better trap choice for further studies of malaria vector control interventions at the site.

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