Monitoring Malaria Vector Control Interventions: Effectiveness of Five Different Adult Mosquito Sampling Methods

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ABSTRACT Long-term success of ongoing malaria control efforts based on mosquito bed nets (long-lasting insecticidal net) and indoor residual spraying is dependent on continuous monitoring of mosquito vectors, and thus on effective mosquito sampling tools. The objective of our study was to identify the most efficient mosquito sampling tool(s) for routine vector surveillance for malaria and lymphatic filariasis transmission in coastal Kenya. We evaluated relative efficacy of five collection methods—light traps associated with a person sleeping under a net, pyrethrum spray catches, Prokopack aspirator, clay pots, and urine-baited traps—in four villages representing three ecological settings along the south coast of Kenya. Of the five methods, light traps were the most efficient for collecting female *Anopheles gambiae* s.l. (Giles) (Diptera: Culicidae) and *Anopheles funestus* (Giles) (Diptera: Culicidae) mosquitoes, whereas the Prokopack aspirator was most efficient in collecting *Culex quinquefasciatus* (Say) (Diptera: Culicidae) and other culicines. With the low vector densities here, and across much of sub-Saharan Africa, wherever malaria interventions, long-lasting insecticidal nets, and/or indoor residual spraying are in place, the use of a single mosquito collection method will not be sufficient to achieve a representative sample of mosquito population structure. Light traps will remain a relevant tool for host-seeking mosquitoes, especially in the absence of human landing catches. For a fair representation of the indoor mosquito population, light traps will have to be supplemented with aspirator use, which has potential for routine monitoring of indoor resting mosquitoes, and can substitute the more labor-intensive and intrusive pyrethrum spray catches. There are still no sufficiently efficient mosquito collection methods for sampling outdoor mosquitoes, particularly those that are bloodfed.

KEY WORDS mosquito surveillance, trapping method, low vector density, light trap, Prokopack

The current impetus for malaria control through reduced contact between vector and humans (long-lasting insecticidal nets [LLINs]), vector control (indoor residual spraying [IRS]), and prompt treatment with artemisinin-based combination therapy has resulted in dramatic declines in malaria vectors (Bayoh et al. 2010, Mutuku et al. 2011) and in the number of clinical malaria cases (Bhattarai et al. 2007, Ceesay et al. 2008, O'Meara et al. 2008, Lee et al. 2010). These successful results have rekindled the notion of malaria elimination, but many challenges remain. Key among them are the threats of insecticide resistance (Kawada et al. 2011, Mathias et al. 2011, Ranson et al. 2011, Trape et al. 2011) and drug resistance (Jambou et al. 2005, Dondorp et al. 2009). Other challenges include emergence of previously secondary vectors, which are less susceptible to LLINs and IRS, and vector behavioral plasticity, that is, changing host–species preferences (Lyimo and Ferguson 2009) and shifting peak feeding times from late evening and early morning, when most people are indoors and in bed, to early evening, when most people are still outdoors (Geissbühler et al. 2007, Russell et al. 2011, Yohannes and Boelee 2012). These challenges are confounded by other locality-specific obstacles that impede sustainable malaria reduction or elimination, including local heterogeneity in transmission patterns and variations in distribution and adoption of control measures (Tatem et al. 2010). Given these obstacles and lessons learnt from the Global Malaria Eradication Program, establishment of a sustainable system for monitoring vectors is a necessary long-term goal (Bockarie et al. 2009, Najera et al. 2011).

The overall reductions in mosquito population densities reported in the recent past, as the direct result

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of malaria control activities (Bayoh et al. 2010, Mutuku et al. 2011) or because of other anthropogenic changes (Jaenisch et al. 2010, Meyrowitsch et al. 2011), mean that current mosquito collection tools may not be sufficient for quantifying infection risk. With low vector densities and growing prominence of the highly adaptable *Anopheles arabiensis* (Patton) (Diptera: Culicidae) (Bayoh et al. 2010, Mutuku et al. 2011, Derua et al. 2012), efficient indoor and outdoor mosquito collection tools are required. In sub-Saharan Africa, where *Anopheles gambiae* s.l. (Giles) (Diptera: Culicidae) and *Anopheles funestus* (Giles) (Diptera: Culicidae) are the important malaria and lymphatic filariasis (LF) vectors, efficient mosquito collection tool(s) will be needed to be able to collect these vectors effectively, both indoors and outdoors, even at very low densities.

Human landing catch (HLC) remains the mosquito collection method of choice in many localities and for the majority of malaria and LF vectors. However, this method is expensive and labor intensive, and has raised ethical concerns about risks to HLC workers. Alternative safer mosquito trapping techniques are therefore encouraged, and HLC is recommended for use only when it is absolutely essential (World Health Organization 2003). The other most commonly used methods are light traps associated with a person sleeping under a net (LT/N), pyrethrum spray catches (PSCs), pit shelter, and manual aspiration. Other emerging mosquito trapping techniques include Prokopack aspirator (PP) (Vazquez–Prokopec et al. 2009, Maia et al. 2011), clay pots (CPs) (Odiere et al. 2007), and urine-baited traps (UBTs) (Kweka et al. 2009). For *An. gambiae* s.l., studies on suitability of Centers for Disease Control and Prevention (CDC) light traps as the unbiased alternative for HLC produced mixed results. Whereas some studies demonstrated comparable or higher relative efficiencies of LT/N compared with HLC (Lines et al. 1991, Davis et al. 1995, Costantini et al. 1998, Magbity et al. 2002, Mathenge et al. 2004, Govella et al. 2009, Fornadel et al. 2010), others reported lower efficiencies of LT/N (Mbogo et al. 1993, Mathenge et al. 2005, Okumu et al. 2008, Govella et al. 2011).

PSCs are commonly considered the gold-standard method for determining indoor resting mosquito density. Whereas PSC reduces the overall nuisance at least temporarily from mosquitoes and other unwanted organisms, it is inconvenient for residents of sprayed houses because furniture, food, cookware, animals, and water have to be removed from the dwellings in the early morning before the spraying. PSC samples mainly endophilic mosquitoes and is less sensitive where mosquito populations are more exophagic and exophilic (Mboera 2005). The new PP is an effective tool for collecting both indoor and outdoor resting mosquitoes (both female and male), and, in comparison with the CDC backpack aspirator, is also easier to use, is quicker, is cheap, and can be self-assembled by using easily obtainable parts (Vazquez–Prokopec et al. 2009, Maia et al. 2011). In a recent comparison of the two aspirators, their performance was similar (Maia et al. 2011).

For the outdoor fraction of malaria and LF vectors, a sensitive tool(s) for sampling is still lacking, despite various recent attempts to make improvements (Odiere et al. 2007; Govella et al. 2009, 2011; Sikulu et al. 2009). Although resting boxes were not useful for outdoor routine mosquito surveillance during their initial evaluation (Govella et al. 2009, 2011; Sikulu et al. 2009), they were more sensitive than HLC in collecting *An. arabiensis* when baited with cattle urine (Kweka et al. 2009), the more exophilic sibling species of the *An. gambiae* complex (White 1974). CPs were initially reported as effective in collecting the outdoor resting fraction of mosquitoes (Odiere et al. 2007), but their subsequent use in other ecological settings failed to confirm this finding (Bijllaardt et al. 2009, Mutuku et al. 2011).

Long-term success of the ongoing malaria control efforts based on mosquito bed nets and IRS is dependent on continuous monitoring of the mosquito vectors, and thus on effective mosquito sampling tools (The malERA Consultative Group on Vector Control 2011). The objective of our study was to identify the most efficient mosquito sampling tool(s) for routine surveillance of malaria and LF in coastal Kenya. We evaluated relative efficacy of five collection methods for anopheline and culicine mosquitoes, and also quantified their relative effectiveness for collecting infected and bloodfed mosquitoes: Light traps associated with a person sleeping under a net, PSCs, PP, CPs, and UBTs in four villages representing three ecological settings along the south coast of Kenya.

Materials and Methods

Our study was conducted in four villages along the south coast of Kenya in Msambweni and Kwale Districts, Kwale County (Fig. 1). Altitude ranges from 0 to 464 m above sea level. There are two rainy seasons: the long rains (April to June) and the short rains (October to December). July to September is cool and dry and January to March is hot and dry. Annual precipitation varies from 900 to 1,500 mm per annum along the coastal belt to 500–900 mm inland. Both malaria and LF are endemic. The predominant vectors for human malaria are *An. gambiae* s.l. and *An. funestus*, and they occur year-round with peaks of population abundance coinciding with seasonal rains (Mbogo et al. 2003, Mutuku et al. 2011). The same mosquitoes transmit LF with an added role for *Culex quinquefasciatus* (Say) (Diptera: Culicidae) (Bögh et al. 1998; Muturi et al. 2006a,b). The current estimated population for Msambweni and Kwale districts is 288,000 and 152,000, respectively, totaling ≈440,000 (Kenya National Central Bureau of Statistics 2009). Msambweni and Kwale districts are inhabited predominantly by the Digo and Duruma communities, with small proportions of Kamba and other communities, especially in urban areas. These communities practice livestock keeping, fishing, and subsistence farming. The main crops are cassava, cashew nuts, coconut, man-

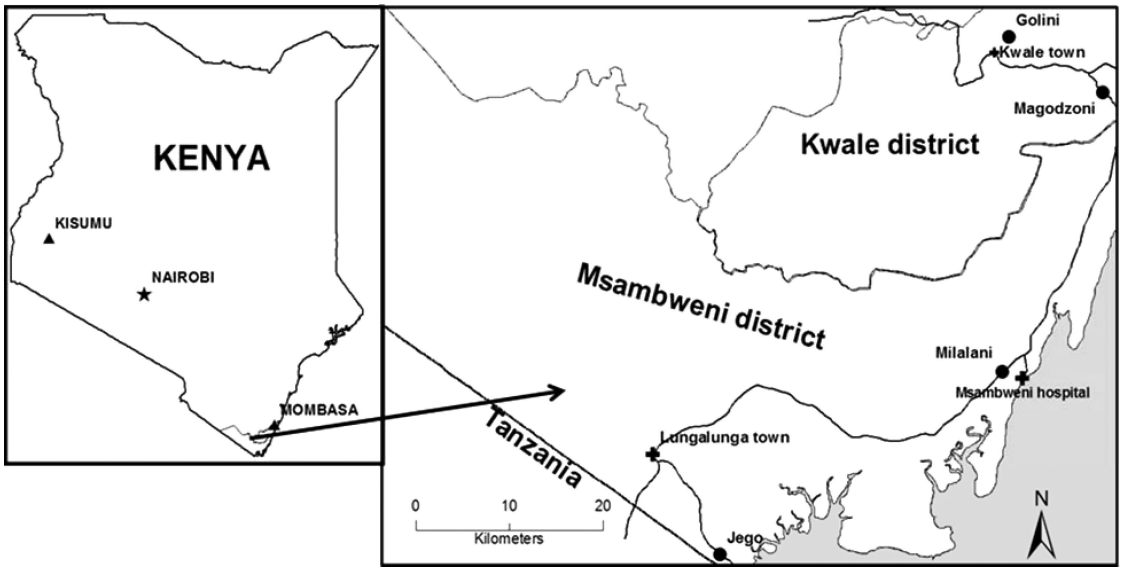


Fig. 1. Map of study villages. Inset: map of Kenya showing location of study area. (Online figure in color.)

goes, and maize. Houses are constructed by using sticks from coconut trees or bamboo as a frame, mud walls, and palm-thatched roofs.

Study Villages. The four villages represent three ecological settings as determined by rainfall, elevation, relative humidity, distance from the sea, and topography (Fig. 1; Table 1). Both Milalani and Jego villages have generally flat terrain, and at comparable distance from the sea (≈2 km), with Jego village characterized by aquatic habitats with higher salinity compared with other villages. Milalani village was assigned to the "coastal plain" environmental category, and Jego village to the "estuarine" category. The furthest village from the sea (Golini) has also the widest elevation range. Temperature and rainfall decreased with increasing elevation, whereas relative humidity increased with increasing elevation (Table 1). Because of the topography and elevation changes, for purposes of analysis, both Magodzoni and Golini villages were assigned to a "coastal slope" environmental category.

Study Design. Three dispersed mosquito collection clusters of 10 houses each were chosen in each of the four study villages. Mosquitoes were collected during three surveys that corresponded to the short rainy season (22 November to 17 December 2010), the end of the hot dry season (14 March to 8 April 2011), and the end of the long rainy season (15 June to 11 July 2011). During each biweekly survey, all five mosquito collection methods were deployed once in each sampling cluster (of two study villages). Table 2 shows a typical biweekly collection schedule for two hypothetical villages. A similar schedule was applied to the remaining two villages, with each mosquito collection survey lasting a month. CPs were paired with PSC, that is, the night before the mosquito collection morning, pots were deployed outside the 10 PSC houses (three pots per house during the first survey and one CP per house during the other two surveys). Similarly, UBTs were matched with the PP (three UBTs per house during the first survey and one UBT per house during the other two surveys).

Mosquito Sampling Methods. *CDC Miniature Light Traps (LT/N).* On every mosquito trapping night, mosquitoes were collected from five randomly selected houses within a single mosquito collection cluster. In each house, CDC light traps were hung at a distance of ≈150 cm from an occupied bed covered with either an insecticide-treated or an untreated bed net. The light traps were switched on at 1800 hours and switched off at 0600 hours the next morning.

Pyrethrum Spray Catches. PSCs were conducted in 10 houses (representing one mosquito collection cluster) during each mosquito collection night. White sheets were systematically laid on the entire floor and over the furniture within all the rooms. The house was

Table 1. Environment of the four villages: distance from shoreline, elevation, and average daily temperature, humidity, and rainfall for 2009–20011

Villages	Distance from shoreline (KM)	Elevation range (M)	Temp °C (range)	Relative humidity (%)	Rainfall (mm)
Jego	≈2.0	4–26	28.2 (23.4–34.1)	71.5 (54.8–92.7)	1,384
Milalani	≈1.9	20–28	27.8 (23.4–32.2)	78.9 (64.5–100.0)	1,214
Magodzoni	≈4.6	40–124	26.7 (22.1–30.5)	77.7 (62.1–95.2)	846
Golini	≈16.4	200–390	25.4 (21.2–29.2)	81.3 (66.0–94.5)	846

Table 2. Experimental schedule used for deploying mosquito collection tools

Week	Village	Week day	Cluster 1	Cluster 2	Cluster 3
One	A	Monday	LT/N	PP + UBT	PSC + CP
	B	Wednesday	PP + UBT	PSC + CP	LT/N
	A	Friday	PSC + CP	LT/N	PP + UBT
Two	B	Monday	LT/N	PP + UBT	PSC + CP
	A	Wednesday	PP + UBT	PSC + CP	LT/N
	B	Friday	PSC + CP	LT/N	PP + UBT

LT/N, CDC miniature light trap; PP, Prokopack aspirator; UBT, urine-baited trap; CP, clay pots; PSC, pyrethrum spray catch.

then sprayed with 10% pyrethrins dissolved in kerosene, by using the method described by Gimnig et al. (2003). The house was then closed for 10–15 min to knock down the endophilic mosquitoes, after which they were collected from the white sheets by using forceps and placed on moist filter paper inside labeled petri dishes. The same procedure was repeated for all the 10 houses.

Prokopack Aspirator. The PP is a relatively new mosquito sampling tool whose functional design is similar to the CDC backpack aspirator. The PP used here was powered by a 12V battery. Similar to PSC, indoor resting mosquitoes were collected by using the PP in one mosquito collection cluster (10 houses) per mosquito collection night. In each room of each house for all the 10 houses, walls and the area under the roof were systematically aspirated by using progressive downward and upward movements along the inside of the room, as described by Maia et al. (2011).

Clay Pots. The CPs used here were similar to those used by Odiere et al. (2007). Outdoor mosquito collections from CPs were matched with indoor collections by PSC. The pots were deployed at 1900 hours the night before the collection morning. Recovery of mosquitoes from the pots was conducted between 0600 and 0730 hours by using the procedure described by Odiere et al. (2007).

Urine-Baited Traps. During the short rainy season survey, three UBTs were deployed outdoor near each of the 10 mosquito collection cluster houses (30 traps per collection night). These 24-cm-wide and -long and 14-cm-high UBTs were constructed from cardboard completely covered with a polythene paper to prevent the trap from getting soaked in case of rain. A black cotton cloth soaked in 7-d-old cow urine covered the inner part of the trap. However, we determined that these UBTs were too small, and therefore replaced them with larger (≈50 cm in length and width and 60 cm in height) plastic-made buckets during the dry season and long rain season surveys. Only a single trap per house per mosquito collection night was deployed during the dry and long rain seasons (10 traps per collection night). Urine was consistently collected, from female Zebu cows (*Bos indicus*) of the same herd in the Msambweni area. Black cotton cloth was soaked in the fresh cattle urine daily for 7 d before deployment in the traps to aid in attracting the host-seeking mosquitoes. The soaked cotton clothing materials were placed at the bottom and held in place by a metal rod. The rest of the inside of the bucket was lined with a netting material that acted as a cage when recovering mosquitoes from the bucket. Mosquitoes were collected from the UBTs between 0600 and 0730 hours.

Mosquito Processing. All caught mosquitoes were taken to the laboratory at Msambweni District Hospital, where live mosquitoes were killed by using ethyl acetate, sorted, and counted. *An. gambiae* s.l., *An. funestus,* and *Cx. quinquefasciatus* were identified morphologically (Gillies and Coetzee 1987) and their abdominal condition scored as unfed, fed, or gravid. Other culicines were not identified to species, but their abdominal status was recorded. All mosquitoes were dried over silica gel and then stored at −20°C. The head and thorax of a portion of all female malaria vectors (*An. gambiae* s.l. and *An. funestus*) caught were tested for *Plasmodium falciparum* (Haemosporida: Plasmodiidae) circumsporozoite protein by enzyme-linked immunosorbent assay (ELISA) (Wirtz et al. 1987). To determine host bloodmeals, abdomens of bloodfed and half-fed mosquitoes were separated from the thorax and head, grounded in 50 μl of phosphate-buffered saline with subsequent addition of 950 μl of phosphate-buffered saline, and then stored at −20°C. Bloodmeal sources were identified by a direct ELISA by using anti-host (IgG) conjugates (Kirkegaard and Perry, Gaithersburg, MD) against human, bovines, and goats (Beier et al. 1988).

Data Analysis. Mosquito counts caught by different traps were compared between the different villages and seasons. Distribution of mosquito counts by sex and species fit a negative binominal frequency distribution. Consequently, a longitudinal regression analysis by using the generalized estimating equations procedure (GENMOD) in SAS, version 9.1 (SAS Institute 2000–2004), was used to compare mosquito counts of different categories (species and sex). "House" was treated as the relevant clustering level, because mosquito count data were amassed by household. Mosquito collection traps, village, and season were treated as both within-subject and between-subject factors. In the longitudinal regression analysis, the Wald statistic tests for the significance of the main effects were estimated first. The main effects here were sampling method (df = 4), season (df = 2), and village (df = 3).

If the Wald statistic was significant for sampling method, then comparisons of individual sampling methods were performed, so that the light trap method was the reference against which the four other methods were tested. There were no statistical outputs including parameter estimates for light trap. If the parameters were negative and carried a statistically significant Z-score, then the sampling method yielded fewer mosquitoes than did the light trap. Conversely, if the parameter was not significant, then there was no difference in yield of mosquitoes between that sampling method and the light trap. If the parameter value was positive and significant, then the sampling method yielded more mosquitoes than did the light trap method. Similar interpretation was performed for season, with the long rainy season as the reference season,

Table 3. Summary of mosquito collections by different sampling methods in south coastal Kenya, Nov. 2010 to July 2011

Sampling method	Trap night	*An. gambiae*	*An. funestus*	*Cx. quinquefasciatus*	*Culex* spp.	*Aedes* spp.	Total
Short rain season (Nov.–Dec. 2010)							
LT/N	54	3	126	0	333	10	472
PSC	120	0	14	19	772	0	805
PP	120	0	12	23	1,323	3	1,361
CP	360	1	32	1	44	4	82
UBT	360	0	20	0	5	1	26
Subtotal	1,014	4	204	43	2,477	18	2,746
Dry rain season (Mar–April 2011)							
LT/N	51	85	11	6	237	0	339
PSC	120	2	8	7	181	0	198
PP	120	4	11	33	324	0	372
CP	120	2	2	0	3	0	7
UBT	120	3	0	0	2	0	5
Subtotal	530	96	32	46	747	0	921
Long rain season (June–July 2011)							
LT/N	42	10	53	7	328	2	400
PSC	120	0	25	48	783	0	856
PP	120	0	14	79	737	0	830
CP	120	0	6	1	24	0	31
UBT	120	5	0	2	22	0	29
Subtotal	521	15	98	137	1,894	2	2,146
Total	2,065	115	334	226	5,118	20	5,813

LT/N, CDC light trap; PSC, pyrethrum spray catch; PP, Prokopack aspirator; UBT, urine-baited traps.

and for village, with Golini village as the reference village (Tables 4 and 5). The Kruskal–Wallis test was used to compare separately the performance of the paired sets of indoor and outdoor methods for each species, PP and UBT and PSC and CPs. Kruskal–Wallis test was also used to separately compare the relative efficiency of PP and PSC in collecting indoor resting mosquitoes.

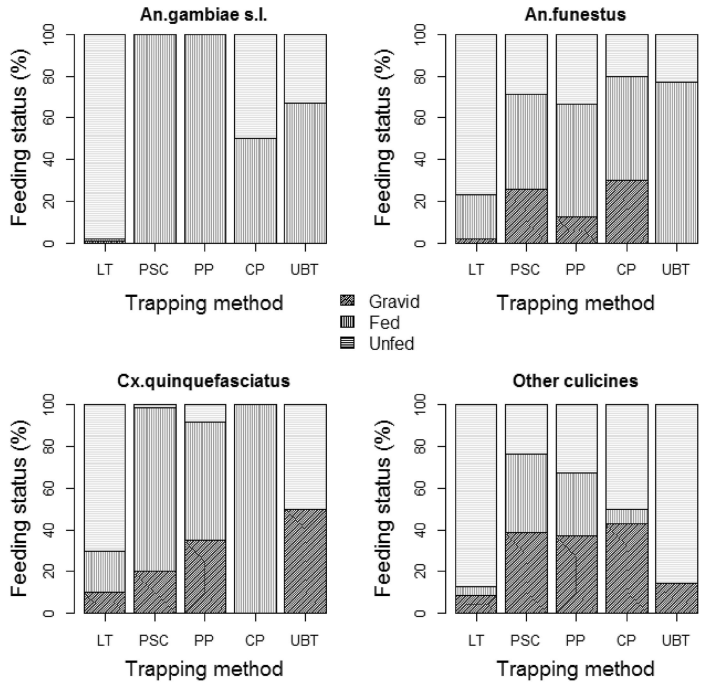
Results

Overall Performance of the Sampling Methods. In total, 5,856 mosquitoes were collected during the study period: 2,563 (43.8%) by PP, 1,254 (21.4%) by CDC light traps, 1,859 (31.8%) by PSC, 120 (2.1%) from CPs, and 60 (1.0%) from baited traps (Table 3). The majority of the mosquitoes were "other culicines" (87.4%), followed by *An. funestus* (5.7%), *Cx. quinquefasciatus* (3.9%), *An. gambiae* s.l. (2.0%), and *Aedes* spp. (0.3%). Other species that were collected from CDC light traps were *Mansonia* spp. (0.6%) and *Anopheles coustani* (0.1%). The mean mosquito density per house over the entire study duration was 0.06 for *An. gambiae* s.l., 0.17 for *An. funestus*, 0.12 for *Cx. quinquefasciatus*, and 1.45 for other culicines. Of the 1,587 houses in which mosquito collections were performed, at least one mosquito was collected in 1.4, 7.6, 6.7, and 32.0% of the houses for *An. gambiae* s.l., *An. funestus*, *Cx. quinquefasciatus*, and the other culicines, respectively.

The two paired sets of indoor and outdoor methods performed differently for the different mosquito species. The PP captured similar numbers of *An. gambiae* s.l. as UBT. Similarly, total PSC catches were comparable to those of CPs. For *An. funestus*, the indoor methods, PP (Kruskal–Wallis $\chi^2 = 8.28$; $P < 0.01$) and PSC (Kruskal–Wallis $\chi^2 = 3.87$; $P < 0.05$) captured significantly more female mosquitoes than their corresponding outdoor methods (UBT and CPs, respectively). The indoor methods sampled significantly higher numbers of *Cx. quinquefasciatus* (Kruskal–Wallis $\chi^2 = 58.30$; $P < 0.0001$) and other culicines (Kruskal–Wallis $\chi^2 = 228.89$; $P < 0.0001$) than the outdoor. Overall, whereas the PP was the best for collecting *Cx. quinquefasciatus* and other culicines, light traps outperformed all the other methods in sampling *An. funestus* and *An. gambiae* s.l. in all villages and across seasons.

Light traps were biased toward collecting females of all species, whereas PSC and UBTs mainly collected mostly female mosquitoes of all species, except for other culicines. The PP and CPs yielded most of the males collected. The abdominal status of collected females varied with species and trapping method (Fig. 2). For all species collected (*An. gambiae* s.l., *An. funestus*, *Cx. quinquefasciatus*, and other culicines), light traps consistently trapped mostly unfed mosquitoes, PSC and PP were consistent in mostly trapping fed and gravid mosquitoes, and UBT and CPs were not consistent in trapping a specific nutritional status (Fig. 2). For *An. gambiae* s.l., light traps collected almost exclusively unfed mosquitoes, whereas PSC and PP trapped only fed mosquitoes. Fed, unfed, and gravid females were evenly represented among the *An. funestus* mosquitoes recovered by all traps, with the exception of the UBTs.

Comparative Sampling Efficiency of Sampling Methods. In general, *An. gambiae* s.l. population levels were extremely low in most villages and across the three seasons. Unlike in the dry season, when all sampling methods recovered *An. gambiae* s.l., few or no *An. gambiae* s.l. were collected by most of the sampling methods during either the short or long rainy seasons (Table 3). The bulk (82/110) of *An. gambiae* s.l. were collected during a single trapping night by three CDC light traps in three different houses in one village



LT/N-CDC miniature light trap; PP-Prokopack aspirator; UBT-urine baited trap; CP-clay pots; PSC-pyrethrum spray catch

Fig. 2. Percentage of *An. gambiae* s.l., *An. funestus*, *Cx. Quinquefasciatus*, and other culicine females in three physiological categories (bloodfed, gravid, or unfed) collected by different mosquito trapping methods.

(Jego) during the dry season. Light traps were the most efficient trapping method for *An. gambiae* s.l. in all other nights (Fig. 3a and b). *An. gambiae* s.l. was slightly less abundant in the short rainy season (Fig. 3c and d) and evenly distributed in all villages, except Golini village, where only one mosquito was collected (Fig. 3f). We did not perform longitudinal regression analysis for either female or male *An. gambiae* s.l. because of the low numbers recovered.

Mosquito sampling method was significantly associated with efficacy in sampling female *An. funestus* ($P < 0.01$). CDC light traps were the most efficient mosquito trapping method for *An. funestus*, trapping four times as many *An. funestus* as the other four trapping methods combined (Fig. 3a and b; Table 4). PSC and PP were similar in sampling both *An. gambiae* s.l. and *An. funestus* (Fig. 3a and b; Table 4), but the PP was better in sampling *Cx. quinquefasciatus* and other culicines, especially males (Tables 4 and 5). Significantly more *An. funestus* and culicines were collected during the rainy seasons compared with the dry season, whereas significantly more *Cx. quinquefasciatus* were collected during the long rains in comparison with the dry and short rainy seasons (Fig. 3c and d; Tables 4 and 5). For almost all species, higher densities of mosquitoes were collected in Milalani village, followed by Jego village, Magodzoni, and Golini villages, in that order (Tables 4 and 5; Fig. 3).

A separate comparison of the PP and PSC showed that the PP and PSC collected similar numbers of *An. gambiae* s.l. and *An. funestus* in terms of diversity of mosquito sex and physiological status. However, the PP collected significantly higher numbers of males ($P < 0.01$), females ($P < 0.01$), and both sexes ($P < 0.01$) of *Cx. quinquefasciatus* compared with PSC. In addition, the PP collected significantly more males ($P < 0.001$) but not of females ($P > = 0.3$) of other culicines than PSC.

Infection Rates. Of the 274 and 95 female *An. funestus* and *An. gambiae* s.l. collected, respectively, 257 *An. funestus* and 79 *An. gambiae* s.l. were tested for *P. falciparum* circumsporozoite protein by ELISA. Overall, the infection rate was 1.78% (6/336)—by species, it was 1.94% (5/257) for *An. funestus* and 1.26% (1/79) for *An. gambiae* s.l.; χ^2 statistics did not show significant differences in infection rates by village, by collection method, by season, or by species. The CDC light trap caught most (4/6) of the infected mosquitoes, with PSC capturing the remaining two. The single infected *An. gambiae* s.l. was collected during the dry season in Jego village. Four of the five infected *An. funestus* were collected during the short rainy season, and only a single mosquito (*An. funestus*) was infected during the long rainy season. Infected mosquitoes were evenly distributed across Jego, Milalani, and Magodzoni villages (two infected mosquitoes in each village). No infected mosquitoes were collected from Golini village, and none of the eight tested *An. coustani* was infected.

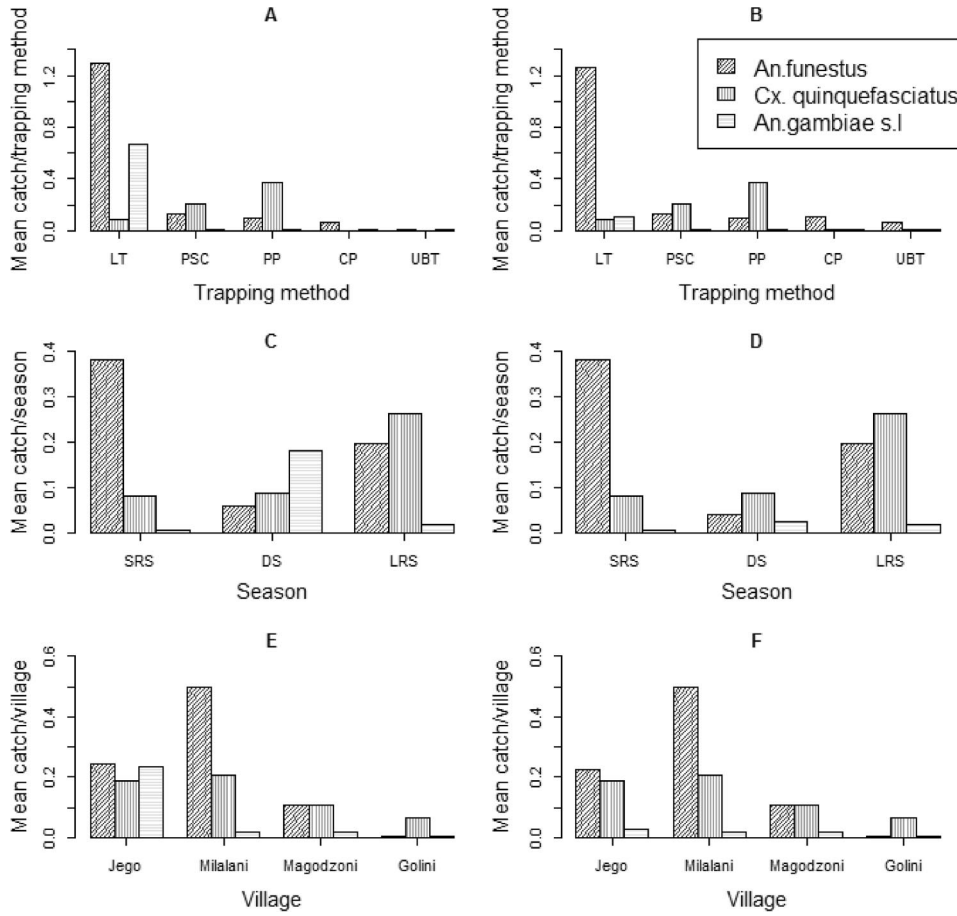


Fig. 3. Mean number of mosquitoes by trapping method (A, B), season (C, D), and village (E, F). B, D, and F, did not include the single trapping night by three CDC light traps in three different houses in Jego village during the dry season where the bulk (82/110) of *An. gambiae* s.l. were collected, but these houses are included in the outcomes shown in A, C, and E. SRS, short rainy season; DS, dry season; LRS, long rainy season; LT/N-CDC, miniature light trap; PP, Prokopack aspirator; UBT, urine baited trap; CP, clay pots; PSC, pyrethrum spray catch.

Bloodmeals. Of the 777 bloodfed mosquitoes of different species, 55.9% (434) were tested for host blood sources (Table 6). ELISA identified bloodmeal sources for 66.6% (289) of the tested mosquitoes. Overall, humans were the bloodmeal source for more than a half of the tested specimens (156, or 54%); with the exception of *An. funestus*, humans were the preferred bloodmeal source for all tested species (Table 6). Origins of 33% of the tested mosquitoes were unidentified, whereas nonhuman sources of bloodmeals (cattle and goats) represented 10%. The distribution of the tested *An. funestus* by village was 14 from Jego, 45 from Milalani, and 13 from Magodzoni. All tested *An. funestus* in Jego village had fed on cattle, with 43% coming from two adjacent houses near where relatively large herds of cattle were kept. In Milalani, 11 and19 *An. funestus* had cattle/goat and human bloodmeals, respectively, whereas feeds of 15 mosquitoes were not identified. On average, 7 and 11% households owned cattle or goats/sheep, respectively. The majority (9/11) of *An. funestus* that had fed on cattle/goat come from two houses also with herds of both cattle and goat. Except in UBT, where a half (5/11) of the tested bloodfed *An. funestus* had fed on cattle, there were no other obvious associations between host bloodmeal sources for any species with the different traps.

Discussion

In the absence of a better alternative to HLCs, utilization of CDC light traps and PSC has persisted for assessing the success of malaria and LF control interventions. Of the five mosquito sampling methods evaluated, light traps were the most efficient for collecting female *An. gambiae* s.l. and *An. funestus* mosquitoes, whereas the relatively new PP was the most efficient in collecting *Cx. quinquefasciatus* and other culicines. Besides mosquito collection method and the mosquito species, results also differed by season and location (village). Although Milalani village recorded slightly higher mosquito numbers than Jego village, the dif-

Table 4. Results of regression analysis using generalized estimating equations of mosquito collections by five sampling methods in four villages during three seasons in Kwale County, south coastal Kenya, Nov. 2010 to July 2011

Parameter	Sampling method	Wald statistic	Parameter estimate	Lower, upper CI	Z score
An. funestus females					
Sampling method	UBT	19.4***	−3.45	−4.30, −2.60	−7.93***
	CP		−3.73	−4.63, −2.83	−8.08***
	PP		−2.67	−3.42, −1.92	−6.98***
	PSC		−2.41	−3.14, −1.69	−6.52***
	Light trap			–	–
Season	Short rain season	16.3***	−0.15	−0.72, 0.41	−0.52
	Dry season		−1.41	−2.10, −0.72	−3.99***
	Long rain season				
Village	Jego	22.6***	4.33	2.17, 6.51	3.92***
	Milalani		5.06	2.90, 7.23	4.58***
	Magodzoni		3.43	1.12, 5.63	3.05**
	Golini			–	–
Cx. quinquefasciatus females					
Sampling method	UBT	35.3***	−2.68	−4.36, −0.99	−3.11**
	CP		−3.33	−5.51, −1.45	−2.99**
	PP		1.43	0.56, 2.31	3.21**
	PSC		0.76	−0.46, 1.65	1.66^{ns}
	Light trap			–	–
Season	Short rain season	11.3**	−1.11	−1.71, −0.50	−3.61***
	Dry season		−1.13	−1.73, −0.52	−3.65***
	Long rain season			–	–
Village	Jego	10.6*	1.23	0.47, 1.99	3.19**
	Milalani		1.22	0.47, 1.98	3.18**
	Magodzoni		0.37	−0.44, 1.18	0.89^{ns}
	Golini			–	–

Analysis for both male and female *An. gambiae* s.l. and *An. funestus* and *Cx. quinquefasciatus* males was not performed due to small sample.
ns, not significant.
*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

ferences were not significant. Hence, the commonly observed trend (Bødker et al. 2003, Kulkarni et al. 2006, Ndenga et al. 2006) of a decrease in the number of mosquitoes collected with increasing altitude was observed for all species and collection methods, a phenomenon that was also observed with increasing distance from the sea (Table 1). Higher mosquito densities corresponded to the rainy seasons for *An. funestus*, *Cx. quinquefasciatus*, and other culicines, but not for *An. gambiae* s.l. In addition, mosquito densities were highly aggregated in space and time, especially for *An. gambiae* s.l. and *Cx. quinquefasciatus.*

Our intensive trapping effort across three seasons, by using five different traps, demonstrated that malaria and LF vectors densities remained very low in all the four villages. The anopheline densities reported here were lower than those reported by a recent study (Mutuku et al. 2011). These low mosquito counts and sparse distribution prevented meaningful comparisons between and among the several trapping methods, especially for the two outdoor methods. Nonetheless, our results suggest that light traps will continue to play an important role in mosquito surveillance for monitoring the malaria and LF vectors, *An. gambiae* s.l. and *An. funestus. An. arabiensis*, which comprised the larger proportion of the *An. gambiae* s.l. population (86%), is known to be highly anthropophilic (Mutuku et al. 2011). The observation that light traps alone sampled 92% of the total *An. gambiae* s.l. is consistent with previous reports from locations with comparable ecological conditions (Mathenge et al. 2004, Fornadel et al. 2010). Similar sensitivity of light traps was recorded for *An. funestus* that accounted for 56% of the total catch, with the remaining 44% shared almost equally between the other four collection methods.

The PP was shown to be a useful tool for sampling *Cx. quinquefasciatus*, an important vector for LF in our study area (Pedersen and Mukoko 2002, Rwegoshora et al. 2005). The PP was not only sensitive, but also collected a representative female population structure of *Cx. quinquefasciatus* and other culicines. In addition, the PP recorded relatively higher efficiencies for male *Cx. quinquefasciatus* and other culicines and is suitable for vector control programs involving larval control and/or use of sterile male mosquitoes. Unlike light traps, which typically sample unfed mosquitoes, the PP is likely to be more appropriate for arbovirus surveillance because it mainly captures the fraction of the mosquito population (bloodfed and gravid mosquitoes) likely to harbor these viruses. Our results suggest that the PP can substitute for the commonly used, but cumbersome, PSC in collecting the indoor resting fraction of *An. gambiae* s.l. and *An. funestus.* However, these results require further validation through longitudinal comparisons of the PP with PSC (Maia et al. 2011).

Similar to other mosquito collection method evaluation studies, collection method did not affect the observed sporozoite rates (Mathenge et al. 2004, Ndiath et al. 2011). Other recent studies have either reported levels of infection too low for a meaningful analysis (Sikulu et al. 2009) or no infection at all (Fornadel et al. 2010). The low infection rates in the

Table 5. Results of regression analysis using generalized estimating equations of culicine mosquito collections by five sampling methods in four villages during three seasons in Kwale County, south coastal Kenya, Nov. 2010 to July 2011

Parameter	Sampling method	Wald statistic	Parameter estimate	Lower, upper CI	Z score
Culex spp. Males + females					
Sampling method	UBT	63.7	−4.78	−5.46, −4.09	−13.68***
	CP		−3.84	−4.41, −3.27	−13.21***
	PP		−0.30	−0.74, 0.15	−1.30^{ns}
	PSC		−0.69	−1.14, 0.24	−2.99**
	Light trap			–	–
Season	Short rain season	28.9	0.11	−0.23, 0.45	0.64^{ns}
	Dry season		−1.29	−1.66, −0.92	−6.84***
	Long rain season			–	–
Village	Jego	37.34	1.64	1.20, 2.08	7.25***
	Milalani		2.11	1.67, 2.54	9.48***
	Magodzoni		−0.70	−1.20, −0.19	−2.70**
	Golini			–	–
Culex spp. males					
Sampling method	UBT	44.0***	−4.01	−4.89, −3.14	−8.98***
	CP		−3.22	−3.97, −2.47	−8.41***
	PP		0.41	−0.17, 1.00	1.38***
	PSC		−0.22	−0.81, 0.37	0.73^{ns}
	Light trap			–	–
Season	Short rain season	23.5***	0.02	−0.41, 0.44	0.08^{ns}
	Dry season		−1.63	−2.12, −1.14	−6.57***
	Long rain season				
Village	Jego	26.8***	1.86	1.30, −2.44	6.44***
	Milalani		2.37	1.81, 2.93	8.31***
	Magodzoni		−0.23	−1.87, 0.41	−0.71^{ns}
	Golini			–	–
Culex spp. females					
Sampling method	UBT	66.1***	−5.52	−6.45, −4.58	−11.59***
	CP		−4.24	−4.87, −3.62	−14.35***
	PP		−0.90	−1.32, −0.47	−4.14***
	PSC		−0.95	−1.37, −0.52	−4.37***
	Light trap			–	–
Season	Short rain season	21.8***	0.30	−0.04, 0.64	1.71^{ns}
	Dry season		−0.80	−1.17, −0.43	−4.24***
	Long rain season			–	–
Village	Jego	44.6***	1.30	0.87, 1.74	5.92***
	Milalani		1.89	1.47, 2.31	8.77***
	Magodzoni		−1.17	−1.70, −0.64	−4.25***
	Golini			–	–

ns, not significant.
*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

mosquitoes and the absence of association with sampling method are in contrast to previous studies (Mbogo et al. 1993, Davis et al. 1995) and are likely because of insecticidal intervention (Bayoh et al. 2010, Mutuku et al. 2011). It reinforces the need for more efficient mosquito sampling tools.

Although the identified blood source for *An. gambiae* s.l. was solely humans, this observation should be considered with caution because of the small number of specimens tested. *An. funestus* human blood index (HBI) 2 years before the current collections was 0.94 (Mutuku et al. 2011), thus the dramatic decline in HBI to 0.24, as reported here, was surprising. Reduced human–vector contact because of intervention pressure from insecticide-treated bed nets is likely forcing this species to look for alternative bloodmeal hosts, and hence the high number of cattle/goat feeds. The differences in HBI between the two studies may also be attributed, at least in part, to differences in experimental designs. The few and unevenly distributed alternative hosts may explain the concentration of nonhuman vertebrate

Table 6. Bloodmeal sources for collected mosquitoes

Species	No. collected	No. tested	Human (%[a])	Cattle + goats (%[a])	Mixed[b] (%[a])	Unknown (%[a])
An. gambiae s.l.	7	4	2 (50)	0 (0)	0 (0)	2 (50)
An. funestus	83	72	17 (24)	25 (35)	3 (4)	27 (37)
Cx. quinquefasciatus	116	101	68 (67)	2 (2)	0 (0)	31 (31)
Other culicines	571	257	147 (57)	14 (6)	11 (4)	85 (33)
Total	777	434	234 (54)	41 (10)	14 (3)	145 (33)

[a] Percentage of total number of tested specimens.
[b] Mixed bloodmeals included human blood mixed with either that of cattle or goat.

feeds in few houses. Lack of anti-chicken conjugate in the current study may explain the large proportion of unidentified bloodmeal hosts in all the species collected (*An. funestus*, *An. gambiae* s.l., *Cx. quinquefasciatus*, and other culicines) (Bögh et al. 1998). It is also possible that some of the unidentified specimens were feeds from other avian and nonhuman vertebrate hosts.

Although light traps outperformed all the other methods in this study with regard to total number of anophelines, they are likely not the best tool for collecting host-seeking mosquitoes (Overgaard et al. 2012). In Dar es Salaam, Tanzania, where mosquito densities are comparable with those in the study area, light traps were inferior to HLC (Govella et al. 2011), but they were highly sensitive in Macha, Zambia (Fornadel et al. 2010). Furthermore, the performance of light traps has been reported to be dependent on mosquito abundance, especially at low densities (Mbogo et al. 1993, Overgaard et al. 2012). Like many other studies in Africa (Lines et al. 1991; Magbity et al. 2002; Mathenge et al. 2004, 2005; Fornadel et al. 2010), we did not find the performance of light traps to be a function of mosquito density. Overall, despite their shortcomings, HLCs may still be the most suitable method for estimating human biting rates in the study area. A follow-up study with both indoor and outdoor HLC is called for. Our study design was such that PSC (indoor) was paired with CPs (outdoor) and PP was paired with UBT. Although, it is possible that the performance of the indoor methods was impacted by the corresponding outdoor method, this impact, if any, was minimal, given the very low numbers of mosquitoes collected outdoors, and could not be assessed. Future study can be designed to assess the extent of such influence.

Given various potential aims of mosquito collections (e.g., feeding preference, infection rate, blood-questing mosquitoes), no single method for mosquito collection can provide a representative sample of the mosquito population structure, and this is especially true when densities of mosquitoes are low, as is the case at present for much of sub-Saharan Africa where malaria interventions (LLINs and/or IRS) are in place. Light traps will remain a relevant tool for host-seeking mosquitoes in the absence of HLC. For a fair representation of the indoor mosquito population, they will need to be combined with the use of light-weight aspirators. This has potential for routine monitoring of indoor resting mosquitoes, and may be substituted for the more labor-intensive and intrusive PSC. There remains an obvious lack of an efficient mosquito collection method for sampling the outdoor mosquitoes, and hence intensified efforts for development of a safe, sensitive, cheap, practical, and affordable alternative to HLC are necessary.

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