

COMPARATIVE FIELD EVALUATION OF THE MBITA TRAP, THE CENTERS FOR DISEASE CONTROL LIGHT TRAP, AND THE HUMAN LANDING CATCH FOR SAMPLING OF MALARIA VECTORS IN WESTERN KENYA

EVAN M. MATHENGE, GEORGE O. OMWERI, LUCY W. IRUNGU, PAUL N. NDEGWA, ELIZABETH WALCZAK, TOM A. SMITH, GERRY F. KILLEEN, AND BART G. J. KNOLS

International Centre of Insect Physiology and Ecology, Mbita Point Research and Training Centre, Mbita Point, Kenya; Department of Zoology, University of Nairobi, Nairobi, Kenya; Vector Biology Research and Control Centre, Centers for Disease Control and Prevention/Kenya Medical Research Institute, Kisumu, Kenya; Department of Public Health and Epidemiology, Swiss Tropical Institute, Basel, Switzerland; Laboratory of Entomology, Wageningen University and Research Centre, Wageningen, The Netherlands

Abstract. The mosquito sampling efficiency of a new bed net trap (the Mbita trap) was compared with that of the Centers for Disease Control miniature light trap (hung adjacent to an occupied bed net) and the human landing catch in western Kenya. Overall, the Mbita trap caught $48.7 \pm 4.8\%$ (mean \pm SEM) the number of *Anopheles gambiae* Giles sensu lato caught in the human landing catch and $27.4 \pm 8.2\%$ of the number caught by the light trap. The corresponding figures for *Anopheles funestus* Giles were $74.6 \pm 1.3\%$ and $39.2 \pm 1.9\%$, respectively. Despite the clear differences in the numbers of mosquitoes caught by each method, both the Mbita trap and light trap catches were directly proportional to human landing catches regardless of mosquito density. No significant differences in parity or sporozoite incidence were observed between mosquitoes caught by the three methods for either *An. gambiae* s.l. or *An. funestus*. Identification of the sibling species of the *An. gambiae* complex by a polymerase chain reaction indicated that the ratio of *An. gambiae* Giles sensu stricto to *An. arabiensis* Patton did not vary according to the sampling method used. It is concluded that the Mbita trap is a promising tool for sampling malaria vector populations since its catch can be readily converted into equivalent human biting catch, it can be applied more intensively, it requires neither expensive equipment nor skilled personnel, and it samples mosquitoes in an exposure-free manner. Such intensive sampling capability will allow cost-effective surveillance of malaria transmission at much finer spatial and temporal resolution than has been previously possible.

INTRODUCTION

The use of human landing catch for sampling malaria vectors remains the most direct and reliable method of monitoring the human-biting mosquito population relevant to transmission and control of malaria.¹ However, due to ethical and logistical shortcomings associated with this method,^{2,3} a viable alternative is needed. Many sampling methods have been evaluated as alternatives to human landing catch⁴ with varying degrees of success. For the estimation of malaria transmission intensity, however, it is an important prerequisite that the sampling methods used are calibrated against the human landing catch.⁵ This is because human landing catches translates directly into human biting rates, which serve as an essential parameter in the estimation of both the entomologic inoculation rate and vectorial capacity.^{6,7}

Some studies have attempted to compare light trap catches to human landing catch^{3,8} with differing conclusions. Discrepancies in these studies may be attributed partly to the different experimental designs used, or differences in mosquito species' composition and densities in the study areas. Nevertheless, there is evidence to suggest that these differences arise largely due to the statistical methods applied in data analysis.⁹

Here, we report the consistent proportionality of anopheline mosquitoes caught by a new bed net trap design, the Mbita trap,¹⁰ relative to human landing catch and Centers for Disease Control (CDC) miniature light trap catch. Since the Mbita trap was developed under semi-field conditions, primarily using colony specimens of *Anopheles gambiae* s.s., our aim was to assess its performance under field conditions for the sampling of anopheline population in western Kenya.

MATERIALS AND METHODS

Study area. The studies were carried out in Lwanda village in the Suba District of western Kenya. Details of location and site characteristics have been described elsewhere.^{10–13}

Sampling. In Lwanda, three houses were selected upon receiving consent from the household heads. Occupants were given a non-impregnated bed net per sleeping space and instructed on their correct use. With informed consent, three young men were recruited and trained in the three alternative mosquito collection methods. On each experimental night, one of the three subjects slept in the Mbita trap, another slept in a bed net with a CDC light trap suspended beside it, and the third conducted a human landing catch.⁴ Both the Mbita trap and the CDC light trap-bed net system were set on mattresses placed on mats laid on the floor and not on beds. In all the experiments we used a standard miniature CDC light trap (Model 512; John W. Hock Company, Gainesville, FL) with an incandescent light bulb. The trap was hung beside the bed net on the foot side of the sleeping person with its shield touching the side of the net and its inlet about 25 cm above the sleeping person. The Mbita trap is a modified conical bed net made of light cotton cloth instead of netting and its working principle uses mosquito host-seeking behavior.¹⁰ Each of the three sampling methods was allocated to one of the three houses on a given night in a 3×3 randomized Latin square experimental design replicated 18 times. The human baits did not move around the sites so that the effects of a particular site and the attractiveness of the human bait associated with it were combined for simplified statistical analysis. Sampling was carried out from 8:00 PM to 6:00 AM between January and July 2002.

Ethical considerations. Informed consent was obtained from all the participants. Thick and thin blood smears were

regularly taken from the participants to examine for the presence of malaria parasites and, when found positive, they were treated with pyrimethamine-sulfadoxine (Fansidar®; F. Hoffmann-La Roche, Basel, Switzerland). A follow-up was made to ensure that all the parasitemic episodes were fully cleared. If the episodes did not clear, the participants were referred to the hospital for further treatment with second-line drugs. The Kenya Medical Research Institute (KEMRI) through the KEMRI/National Ethical Review Committee granted ethical approval (KEMRI/7/3/1) for this study.

Mosquito processing. Mosquitoes were taken to the laboratory and killed either by freezing or by suffocation with chloroform vapor. They were counted and identified morphologically using taxonomic keys.^{14,15} A sub-sample of mosquitoes caught by each method was dissected and parity was determined by examining the coiling or uncoiling of their ovarian tracheoles.¹⁶ All mosquitoes were then desiccated over silica gel and kept at room temperature until further processed. The heads and thoraces of silica-dried female anopheline mosquitoes were tested by an enzyme-linked immunosorbent assay using the 2A10 monoclonal antibodies to detect circumsporozoite protein of *Plasmodium falciparum*.¹⁷ Abdomens of *An. gambiae* s.l. were analyzed by a polymerase chain reaction (PCR) for sibling species identification.¹⁸

Statistical methods. We used the WINBUGS¹⁹ software package to fit models assuming Poisson-distributed sampling variation in all measurements as described previously.²⁰ This approach avoids biases that arise with the use of Williams' mean transformations and linear regression, when some of the mosquito counts are equal to zero.^{9,20} The relationship between catches by the Mbita trap or CDC light trap-bed net system and human landing catch on the same night (i) was analyzed by fitting either a model which assumes simple proportionality or an alternative non-linear model representing the possibility of non-proportional sampling.²⁰ In the simpler proportional model, the expected catch with either Mbita trap or the CDC light trap ($E(y_i)$) are assumed to be directly related to that expected from the human landing catch ($E(x_i)$) and their relative sampling efficiency is expressed by the coefficient β_0 . The only difference between this analysis and that described previously²⁰ is that variations in attractiveness of the catchers and the mosquito densities in their associated houses were also modeled and expressed as β_c :

$$E(y_i) = \beta_0 \beta_c E(x_i) \quad (1)$$

To test for non-proportionality, a non-linear model was also fitted, using a similar form but with the sampling efficiency either increasing or decreasing with increasing mosquito density as determined by the human landing catch reference method.²⁰ In this model, the baseline sampling efficiency of the tested method at low densities ($x_i \approx 0$) is reflected by β_1 and the change in sampling efficiency in response to higher densities (increasing x_i) is expressed as β_2 , as follows:

$$E(y_i) = \beta_1 \beta_c E(x_i)^{\beta_2} \quad (2)$$

Thus, non-proportionality between two sampling methods can be identified by examining the estimate for parameter β_2 of the fitted model described by equation 2. If the estimate for β_2 differs significantly from 1, non-proportionality and density-dependent sampling efficiency is indicated. Also, Poisson-distributed sampling variation is accounted for in all mea-

surements and density-dependent sampling can be tested for directly. Such analysis was used for comparison of the CDC light trap-bed net system and human landing catch in Papua New Guinea and allowed conclusive demonstration that the CDC light trap-bed net system is not only of poor sampling efficiency, but is also misrepresentative because it is density-dependent for a number of important vector species in that setting.²⁰

For comparison of the species composition, parity rates and sporozoite incidence of the mosquitoes sampled by the different methods, we used SPSS version 11.01 (SPSS, Inc., Chicago, IL) to carry out Pearson chi-square tests.

RESULTS

Over the 54 nights of the trial, the Mbita trap, human landing collection, and the CDC light trap-bed net system caught 592, 1,215, and 2,162 *An. gambiae* s.l. and 291, 390, and 742 *An. funestus*, respectively. No density-dependence for the relative sampling efficiency of the Mbita trap relative to the human landing catch for sampling *An. gambiae* s.l. or *An. funestus* was detected using the model described in equation 2 since confidence intervals for both the estimates of β_2 overlapped with 1 (Table 1). In contrast, the CDC light trap showed some deviation from linear proportionality with increasing efficiency at high densities as reflected by β_2 values which exceeded 1, and in the case of *An. gambiae*, this deviation was significant (Table 1). Nevertheless, examination of both model fits to the data reveals that the deviations of the CDC light trap-human landing catch relationships from proportionality were minor (Figure 1). We therefore consider β_0 from the simple proportional model expressed in equation 1 as the most appropriate and direct estimate of the relative sampling efficiency of the bed net trap and light trap, compared with human landing catch. Based upon these estimates

TABLE 1
Parameter estimates from hierarchical models*

Model and parameter	Parameter estimate (95% CI)	
	<i>Anopheles gambiae</i>	<i>Anopheles funestus</i>
Simple proportionality (equation 1)		
β_0		
Human landing catch	1.00 (NA)	1.00 (NA)
Bed net trap	0.50 (0.45–0.56)	0.70 (0.59–0.83)
CDC light trap	1.86 (1.73–2.00)	1.91 (1.66–2.19)
β_c		
Catcher 1	1.0 (NA)	1.0 (NA)
Catcher 2	0.46 (0.42–0.50)	0.32 (0.28–0.37)
Catcher 3	0.45 (0.41–0.48)	0.50 (0.44–0.56)
Density-dependent sampling efficiency (equation 2)		
β_1		
Human landing catch	1.00 (NA)	1.00 (NA)
Bed net trap	0.68 (0.40–1.08)	1.45 (0.17–3.44)
CDC light trap	0.35 (0.20–0.53)	1.43 (0.31–3.20)
β_2		
Human landing catch	1.00 (NA)	1.00 (NA)
Bed net trap	0.93 (0.80–1.06)	0.81 (0.40–1.54)
CDC light trap	1.43 (1.32–1.56)	1.17 (0.80–1.69)
β_c		
Catcher 1	1.0 (NA)	1.0 (NA)
Catcher 2	0.46 (0.42–0.50)	0.34 (0.28–0.38)
Catcher 3	0.43 (0.39–0.47)	0.48 (0.41–0.55)

* CI = confidence interval; NA = not applicable; CDC = Centers for Disease Control.

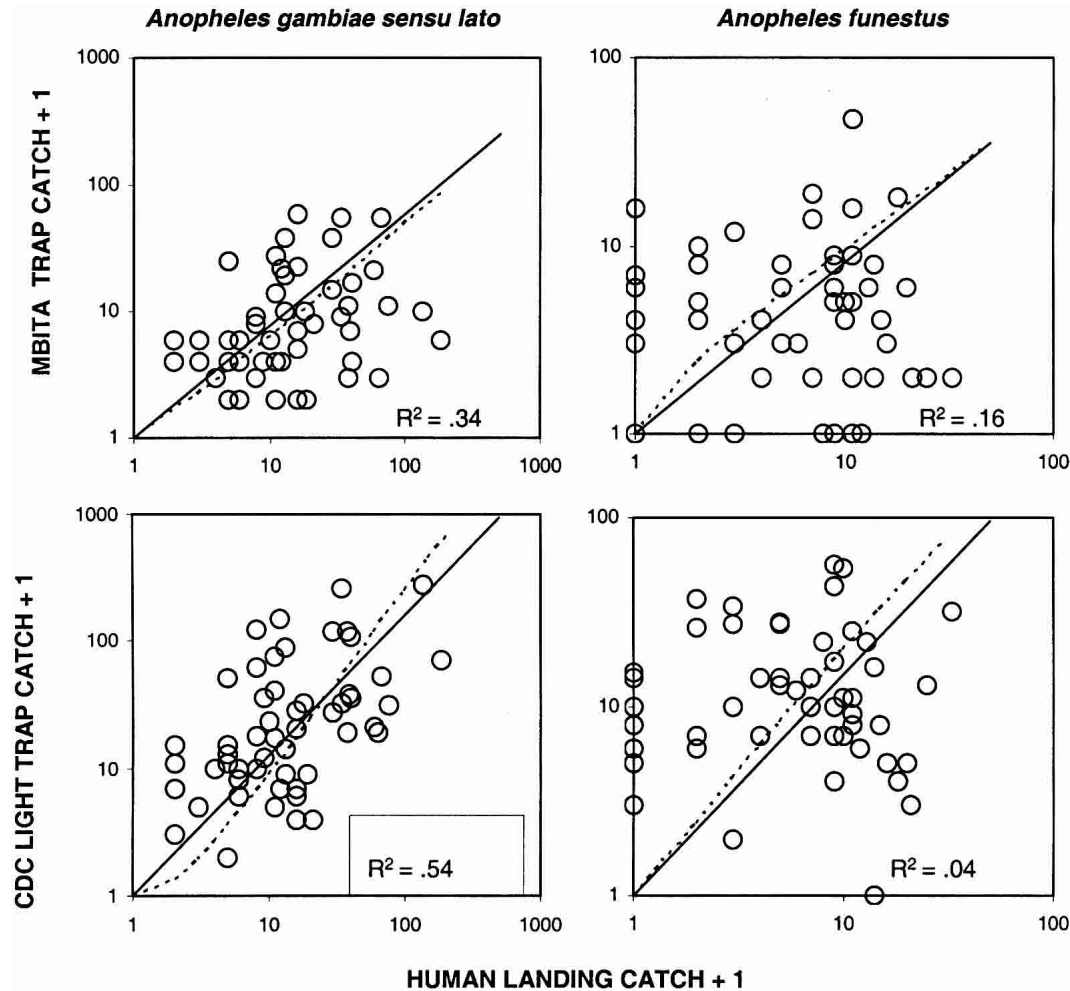


FIGURE 1. Numbers of female mosquitoes caught by the three sampling methods in 54 nights in western Kenya. Regression lines (solid lines) depict the fitted simple proportionality model (equation 1) and the non-proportional lines (dotted lines) depict the density-dependent sampling efficiency model (equation 2). CDC = Centers for Disease Control.

(Table 1), the Mbita trap caught approximately 50% and 70% the number of *An. gambiae* s.l. and *An. funestus* caught in the human landing collections, respectively. The CDC light trap-bed net system caught almost twice the number of *An. gambiae* s.l. and *An. funestus* caught in the human landing collection (Table 1).

Identification of *An. gambiae* s.l. by PCR showed that $55.1 \pm 1.4\%$ (mean \pm SEM) (162 of 294) of the successfully amplified specimens were *An. gambiae* s.s., with the remainder being *An. arabiensis*. The species composition of *An. gambiae* s.l. did not vary according to the sampling method used ($\chi^2 = 0.9$, degrees of freedom [df] = 2, $P = 0.6$; $n = 35, 159$, and 100 for the Mbita trap, the CDC light trap-bed net system, and human landing collection, respectively). Sporozoite incidence did not vary according to species ($\chi^2 = 5.9$, df = 3, $P = 0.1$; $n = 158, 162, 132$, and 98 for unidentified, *An. gambiae*, *An. arabiensis*, and *An. funestus* specimens, respectively). Therefore, in subsequent analyses all mosquito samples were pooled to determine whether important traits of the vector population were sampled representatively by the Mbita trap and the CDC light trap-bed net system when compared with human landing collections. The overall sporozoite prevalence was $3.5 \pm 1.4\%$ (19 of 550) and this was not depen-

dent upon the sampling method used ($\chi^2 = 4.1$, df = 2, $P = 0.1$; $n = 116, 266$, and 168). Similarly, parity prevalence of *An. gambiae* s.l. was estimated to be $78.0 \pm 1.2\%$ (170 of 218) and was independent of the sampling method used ($\chi^2 = 3.4$, df = 2, $P = 0.1$, $n = 68, 88$, and 62 for the Mbita trap, the CDC light trap-bed net system, and human landing collection, respectively).

DISCUSSION

Consistent with our previous semi-field evaluation,¹⁰ the Mbita trap caught approximately half the number *An. gambiae* s.l. caught in the human landing catch. Note that because houses where sampling was done were occupied, this field evaluation is comparable with the competitive rather than non-competitive experimental set up in the semi-field system.¹⁰ The bed net trap proved to be better for *An. funestus*, catching approximately 75% of the number caught in the human landing catch. In contrast with the semi-field experimental set up,¹⁰ the catch size for the CDC light trap-bed net system was almost twice that in human landing collections for both species, which contrasts sharply with previous reports in

which it was typically catching less mosquitoes than human landing collections.^{3,8,21} The proportion of the mosquitoes caught by the CDC light trap relative to those caught in the human landing collections was even higher than the highest reported for this method.² The mosquito host-seeking process and the relative availability of the human baits in the three sampling methods used can possibly explain these observations. When all people in a house are protected by bed nets, mosquito-feeding success is greatly reduced. The main stimuli available to the host-seeking mosquitoes in such a scenario are the human-specific cues emanating from the people sleeping under the nets. After the mosquitoes are within close vicinity of the host, an additional stimulus such as light might be more important to the host-seeking mosquitoes than the already existing and saturated ones. Therefore, mosquitoes that otherwise would be equally distributed among the house occupants may tend to cluster around the light from the CDC light trap, thus increasing their chance of being trapped.

It has been shown that light from incandescent bulbs increased the catch size of *An. gambiae* s.l. and *An. funestus* by about 2.5 times as compared with traps whose light bulbs were removed.²² On the contrary, a human performing a landing catch does not constitute an additional close-range stimulus to the host-seeking mosquitoes, as does the light from the CDC light trap. However, a fully exposed human is relatively more available to the mosquitoes than either a person sleeping in the Mbita trap or under an ordinary bed net. Conversely, since the use of the Mbita trap is very similar to that of non-impregnated nets, the mosquitoes are likely to be distributed almost equally among the house occupants. Consequently, the number trapped by the Mbita trap could most likely represent those attracted to a single person in a particular night.

Perhaps the most important finding in this study was the fact that the numbers of mosquitoes sampled by the three methods were more or less consistently proportional to each other. We are therefore able to predict what the human landing catch would have been on the night of a given Mbita trap or CDC light trap-bed net system catch. It has been recommended that human landing collections be used to calibrate other sampling methods⁵ and our study has shown that this is possible. We can consequently conclude that both the Mbita trap and CDC light trap-bed net system are representative sampling methods in this setting, although the slight density dependence that was demonstrated for the CDC light trap could be important in some epidemiologic settings. However, even after such a calibration, it is important to perform a limited number of human landing collections to re-check whether a substitute sampling method can be relied upon to provide an unbiased measure of density and infection rates of human-biting mosquitoes at each new location.³ Since the Mbita trap is based primarily on mosquito host-seeking behavior rather than on other secondary stimuli as light, it is likely to work quite well under different epidemiologic settings unlike mechanical devices, although it will be important to confirm that its sampling efficiency is reasonably constant.

The three methods sampled mosquitoes with similar parity rates and sporozoite incidence. Similar results have been obtained for *An. gambiae* s.l.³ and *An. funestus*²³ sampled by either the CDC light trap or by the human landing collection. Other studies have shown higher sporozoite incidence in *An. gambiae* s.l.^{2,8} and *An. arabiensis*²³ collected by light traps

than in those from the human landing collections. It has been shown that the location of the light trap relative to the human sleeping under a net greatly influences both the number and parity rates of mosquitoes caught,²¹ and this might account for the different results obtained from the various studies. To date, there is no clear consensus on the best position to place the CDC light trap relative to the person occupying the net. There is therefore a need to standardize the use of this method to enable valid comparisons of results from the various studies in different epidemiologic settings.

From this first field trial of the Mbita trap, it is clear that it does indeed have great potential for both research and routine surveillance in operational settings. Additional field trials in other African settings with, for example, different vector species composition, lower densities, and different house designs will be necessary so that its flexibility and consistency can be fully explored prior to wider application. Nevertheless, at this stage it can be argued that the Mbita trap can be applied more intensively, requiring neither expensive equipment nor skilled labor¹⁰ and its catch can be converted into equivalent human biting intensities. We therefore conclude that the Mbita trap is a promising tool for sampling vector populations because for any given degree of sampling effort and expense, far more person-sampling nights can be carried out, resulting in more sensitive measurements of biting density. Such intensive sampling capability allows much finer spatial and temporal resolution in measurements of malaria transmission intensity, a particularly important advantage as it is highly aggregated in these dimensions.^{24–26}

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Authors' addresses: Evan M. Mathenge, International Centre of Insect Physiology and Ecology, Mbita Point Research and Training Centre, PO Box 30, Mbita Point, Kenya and Department of Zoology, University of Nairobi, PO Box 30197, Nairobi, Kenya, E-mail: emathenge@mbita.mimcom.net. George O. Omweri, International Centre of Insect Physiology and Ecology, Mbita Point Research and Training Centre, PO Box 30, Mbita Point, Kenya. Lucy W. Irungu and Paul N. Ndegwa, Department of Zoology, University of Nairobi, PO Box 30197, Nairobi, Kenya. Elizabeth Walczak, Vector Biology Research and Control Centre, Centers for Disease Control and Prevention/Kenya Medical Research Institute, PO Box 1578, Kisumu, Kenya. Tom A. Smith, Department of Public Health and Epidemiology, Swiss Tropical Institute, Socinstrasse 57, CH-4002, Basel, Switzerland. Gerry F. Killeen, Department of Public Health and Epidemiology, Swiss Tropical Institute, Socinstrasse 57, CH-4002, Basel, Switzerland and Ifakara Health Research and Development Centre, PO Box 53, Ifakara, Kilombero District, Tanzania. Bart G. J. Knols, Laboratory of Entomology, Wageningen University and Research Centre, PO Box 8031, 6700 EH, Wageningen, The Netherlands.

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