STUDIES ON THE RESTING BEHAVIOUR AND HOST CHOICE OF
*Anopheles gambiae* and *An. arabiensis* FROM MULEBA,
TANZANIA

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Running Head. *Resting and feeding in Anopheles*

KEY WORDS
*Anopheles*; *gambiae*; *arabiensis*; resting; indoors; outdoors; host choice;
prokopack

ABSTRACT
An understanding of the resting and feeding habits of malaria vectors can inform control decisions. We compared the behaviour of *An. gambiae* before, and *An. arabiensis* 11 months after, an intervention of long lasting insecticide treated nets and indoor residual spraying with insecticide. The relative efficacy of a mechanical (Prokopack) collection versus manual aspiration was evaluated. Sequential removal samples from three sites indicated that a single sample with the Prokopack collected more than a third of the available insects. Prior to the intervention *An. gambiae* constituted 97.7% of the 312 *An. gambiae* complex identified, with 2.3% being *An. arabiensis*. After the intervention *An. arabiensis* constituted 83% of the 183 specimens identified.

In both species gonotrophic development was estimated to take two days. In grass roofed, smoke free houses, mosquitoes were collected from both roof and walls. In smoky houses there was a preponderance of mosquitoes on the walls. Whilst *An. gambiae* was endophilic, completing gonotrophic development inside houses *An. arabiensis* completed egg development in outdoor sites. From one of these sites unfed, part fed and engorged insects were dissected for age determination. With the exception of two parous mosquitoes unfed mosquitoes were virgins whilst the majority of engorged insects were parous (with well contracted sacs). Most (81%) of the 191 engorged, outdoor resting *An. arabiensis* tested had fed on cows and only 9% had fed on humans. It is concluded that the Prokopack sampler is better than manual collection of resting mosquitoes and that the reduction in malaria prevalence seen in the village following the intervention was due to a change in dominant member of the *An. gambiae* complex in the village, from the anthropophilic/endophilic *An. gambiae* to the catholic/exophilic *An. arabiensis*. Monitoring the proportions of the two species may be a sign of impending breakdown in control.

**INTRODUCTION**

The principal malaria vectors in Africa, *Anopheles gambiae*, *An. coluzzii* and *An. funestus*, tend to feed and rest inside houses whilst the other vector, *An. arabiensis*, is more plastic in its, feeding and resting behaviour (Charlwood et al.,
1995; Charlwood et al., 1999). Indoor resting mosquitoes are vulnerable to current chemical interventions, which is one of the main reasons for the recent decline in malaria transmission in Africa (Bhatt et al., 2015). Long lasting insecticide treated mosquito nets (LLIN’s) prevent feeding, whilst spraying the interior walls of houses with a residual insecticide (IRS) kills and repels mosquitoes that attempt to rest indoors.

In some IRS campaigns, it is only the walls that are treated but if the roof is made of grass, and should the insects predominately rest there, then these also need to be sprayed when undertaking such control (WHO 2015). Where the mosquito rests may depend on whether the house is smoky due to people cooking inside (Bockarie et al., 1994). Whether the numbers of mosquitoes successfully obtaining a blood meal, and resting indoors differs between traditional homes without separate kitchens as compared to modern homes with separate kitchens may, therefore, also impact the efficacy of IRS programs, with considerable cost and logistic implications. Information on the favoured resting site(s) of malaria vectors can, therefore, help guide the implementation of potential control measures.

In many cases resting mosquitoes are blood fed (Silver, 2008). Knowledge of the feeding preference also inform models of transmission because host choice is an important component of vectorial capacity (Dye 1992). Anthropophagic behaviour appears to be responsible for An. dirus being a vector in Cambodia (Charlwood et al., 2016) whilst reduced feeding on humans was responsible for a reduced transmission by An. coluzzii on the archipelago of São Tomé and Príncipe (Sousa et al., 1998). Endophilic mosquitoes collected inside houses, that
do not contain domestic animals, have primarily fed on humans (Charlwood et al., 1995; Gillies & De Meillon, 1968 – and references therein). When people use LLIN’s the mosquitoes are more likely to feed outside on a variety of hosts, including man (Charlwood and Graves, 1987; Lefevre et al., 2009). Feeding outside may also mean that they rest outside depending upon the availability of suitable outdoor shelters (White, 1974; Charlwood and Graves, 1987; Githeko et al., 1994). The analysis of the stomach contents of mosquitoes resting outdoors provides information on the host range (and possibly host choice) of the mosquito population. Determining the range of preferred hosts also becomes important when undertaking control, since a propensity to obtain a blood meal from alternative hosts to humans may enable mosquito populations to be sustained until the efficacy of indoor control decreases and transmission indoors is resumed.

In order to undertake assessments of feeding and resting behaviour suitable collection methods are required. The methods include manual aspiration, spray collections, CDC light-trap collection and Prokopack sampler (Silver, 2008; Vazques-Prokopek et al., 2009; Maia et al. 2011). Manual aspiration (using a sucking tube and torch) is a common way of collecting mosquitoes resting indoors. Although it may depend on the proficiency of the collector and type of resting site (Charlwood et al., 1995). The Prokopack sampler is efficient and less bulky compared to CDC backpack (CDC-BP) sampler and is much easier to use (Maia et al., 2011). It is a better method than pyrethrum spray catches, clay pots or urine baited traps (Onyango et al., 2013). Although it is becoming a standard
method for collection of resting mosquitoes (Killeen et al., 2017) its efficacy at collecting anophelines has yet to be assessed.

In a series of collections in Tanzania Maia et al., (2011) found that CDC-BP and Prokopack aspirators were equivalent in efficiency for collecting mosquitoes in general but that the Prokopack, being less bulky, was much easier to use. Moreover, there was increased consistency between the numbers of mosquitoes collected between four different collectors operating the Prokopack compared to the CDC-BP. Among their samples, however, only 46 (2%) of the 2000+ mosquitoes collected were anophelines and only a small proportion were collected inside houses. Similarly, Onyango et al. (2013) found that the Prokopack was the most suitable method for the collection of indoor resting *Culex quinquefasciatus* compared to pyrethrum spray catches, clay pots or urine baited traps. Like Maia et al., (2011) they caught too few anophelines to undertake such a comparison for this genus. Thus, although it is becoming a standard method for collection of resting mosquitoes (Killeen et al., 2017) its efficacy at collecting anophelines has yet to be assessed.

Manual aspiration (using a sucking tube and torch) is a common way of collecting mosquitoes resting indoors. It may depend on the proficiency of the collector and type of resting site, although Charlwood et al., (1995) in a series of collections from Tanzania, found that there was little variation in the efficiency of different collectors.

Here we provide information on the host range and age among vectors collected from a village in Northern Tanzania, after an intervention (of LLIN's with a synergist and IRS) was introduced into the village. We also provide comparisons
between the Prokopack collection and manual aspiration of resting mosquitoes inside houses undertaken before the intervention was introduced into the village.

**METHODS**

**STUDY AREA**

The village of Kakindo/Kyamyorwa B in Muleba District, Kagera Region in northwest Tanzania (02°04′27.5˝S, 31°34′10.8˝E), described by LeClair et al. (2017), was used for the study. The village is separated by a floodplain, used for agricultural purposes, from an inlet of Lake Victoria where the majority of villagers’ farm. Most of the houses in the village are mud-walled and thatched roofed, although corrugated iron roofs are becoming more common, and a number of houses are made of brick. The region has two rainy seasons: the main rains occur between March-May (average monthly rainfall 300 mm) and the short rains in October–December (average monthly rainfall 160 mm). Malaria is endemic with peaks of transmission at the end of the rainy seasons. At the start of the study a pyrethroid resistant population of *An. gambiae* was the primary vector in the area (Protopopoff et al., 2013; West et al., 2014) although *An. arabiensis* was also present. In January of 2015 the interior walls of all houses in the village were sprayed with the residual insecticide pirimiphos-methyl (Actelic 300CS) and villagers were given LLIN’s impregnated with a pyrethroid insecticide and PBO (Olyset Plus©).

**COLLECTION METHODS**

Host seeking mosquitoes were collected indoors using CDC light-traps hung close to an occupied mosquito bednet and outdoors with a Furvela tent-trap (Charlwood et al., 2017).
Resting mosquitoes were collected with a Prokopack sampler and by manual aspiration. Collections took place between 7 and 11am. The Prokopack was systematically passed in an up and down direction over the walls or a side to side direction close to the roof, at approximately 1m per second, so that all of the surface of each was sampled once for each replicate collection. Manual collection was performed by experienced collectors with the aid of a torch and an aspirator. As with the Prokopack collection all surfaces to be collected in the sampled room were carefully scanned once for each replicate sample.

Multiple samples were taken from roof and walls. In order to compare the two collection methods; manual aspiration and Prokopack were alternated. Thus, if the roof was sampled initially with the Prokopack a second sample was taken immediately afterwards manually, and if in that house, on that day, the roof had been sampled initially with the Prokopack then the first collection off the walls was undertaken manually to be followed by a sample with the Prokopack.

The efficiency of the Prokopack sampler was determined by removal sampling, as described by Southwood (1978), from three outdoor sites in 2017. In this technique, the rate at which collections decline is directly related to the size of the total population and the number removed. For removal sampling to function adequately a number of assumptions must be met: the catching procedure must not affect the probability of an animal being caught; the population must remain stable during the catching period and, most importantly, the chance of being caught must be equal for all animals. A relatively large proportion of the population must also be caught to obtain reasonably precise estimates. Numbers collected on each trapping interval must decline for estimates to be meaningful.
(Charlwood et al., 1995). Zippin’s (1958) method, based on maximum likelihood, which provides an estimate of the standard error, was used to estimate the total population in each site. As described by Southwood (1978) the total catch \( T = n_1 + n_2 + n_3 + n_4 + n_5 \) where \( n_1..n_5 \) are the number of insects caught on each respective round of sampling. Then the value of \( \sum_{i=1}^{k} (i-1)y_i \) is found, where \( k \) is the number of samples and \( i = 1 \) and \( y_i = \) the catch on the \( i \)th occasion.

Following this the ratio \( R \) is determined where

\[
R = \sum_{i=1}^{k} (i-1)y_i
\]

and

\[
R = \frac{q}{p} - kq^k / (1-q^k)
\]

\( p = \) the probability of capture on a single occasion and \( q=1-p \) and the estimated size of the total population is

\[
\bar{N} = T / (1-q^k)
\]

RESTING SITES

INDOOR COLLECTIONS

Indoor collections largely took place before the interventions were introduced into the village. Indoor sites consisted of village houses that either had an outdoor kitchen or where cooking, with a wood fire, took place indoors. Collections from the roof and from the walls and by collection method were kept and scored separately. Collections from the roof were made to the height of the first two cross-beams rather than to the apex of the roof.
OUTDOOR COLLECTIONS

A variety of outdoor sites including vegetation, were searched prior to the introduction of the intervention. A pit-shelter (WHO, 1965) was dug close to a house known to have a high density of mosquitoes (in light-trap collections), and two cardboard boxes (50cm on a side) with their interior walls painted black and openings partially obscured were placed close to a corral where five cows spent the night. After the intervention a latrine, with walls of dried banana leaves and a roof of grass, was sampled for 19 days, only with the Prokopack collector. Subsequently an abandoned mud-walled house, a cow shed and a further latrine were sampled in March 2017. The species, age and blood meal source of mosquitoes collected from these outdoor resting sites were determined.

MOSQUITO PROCESSING

Collected anophelines were identified to species or species group using the keys of Gillies and DeMeillon (1968) and Gillies and Coetzee (1987). Samples of the An. gambiae s.l. from both collection periods collected indoors with CDC light-traps, were identified to species by PCR following the protocols of Bass et al., (2008).

AGE DETERMINATION

The ovaries of samples of unfed, blood-fed and semi-gravid female An. gambiae s.l. collected outdoor resting were dissected according to the protocols described by Charlwood et al., (2003) and daily survival rates (p) determined according to the formula:

\[ p = \sqrt[3]{\text{ovarian count}} \]
where \( m \) is the parous rate and \( \mu \) is the duration of the oviposition cycle.

Following dissection, the abdomens of part-fed, engorged and semi-gravid females were squashed onto filter papers and preserved in a sealed plastic bag containing silica gel.

**Blood meal analysis**

Blood meal analysis of the sample of fed mosquitoes was done by direct ELISA, using a technique based on that of Beier *et al.* (1998). Filter paper blood spots were cut using an 8mm diameter hole punch and eluted with 600 μl phosphate buffered saline (PBS), centrifuged for 5–10 seconds and incubated at 4°C overnight. The following morning samples were centrifuged again and a 50 μl aliquot dispensed into each micro-plate well, covered, and incubated at room temperature for 3 hours. Each well was washed twice with washing buffer (PBS/Tween 0.5%), filled with blocking buffer (PBS/Casein in NaOH; 200 μl) and incubated for 1 hour. Wells were washed twice with washing buffer and a host-specific conjugate added. Conjugates were: Goat anti-human IgG H&L (50 μl) diluted 1:4000; Goat anti-dog IgG H-L (50 μl) diluted 1:2000; Goat anti-bovine IgG H-L (50 μl) diluted 1:2000, and Rabbit anti-goat IgG H-L (50 μl) diluted 1:2000 (Kirkegaard and Perry Laboratories). After 1 hour, wells were emptied and washed four times with washing buffer, and ABTS peroxidase substrate (100 μl; Kirkegaard and Perry Laboratories) was added to each well. Initially thirty minutes after addition of the substrate absorbance was read at 405 nm in an ELISA reader (Multiskan FC® Thermoscientific). Each sample was run in duplicate and was tested against two antibodies per run (initially anti-human paired with anti-dog). All samples with negative results were tested using anti-
bovine and anti-goat. Plates included two positive and four negative controls, hence a maximum of 16 samples were run per plate. Subsequently plates were scored visually by two independent readers (in the manner described by Charlwood et al., 2014).

DATA ANALYSIS

Data was entered into Excel ® spreadsheets and analysed using Stata 12 (Statacorp, 2011).

ETHICS

The study was conducted as a component of the Pan African Malaria Vector Research Consortium project ‘Evaluation of a novel long lasting insecticidal net and indoor residual spray product, separately and together, against malaria transmitted by pyrethroid resistant mosquitoes’ which received ethical clearance from the ethics review committees of the Kilimanjaro Christian Medical College (certificate number 781 on 16/09/2014), the Tanzanian National Institute for Medical Research (20/08/2014), and the London School of Hygiene and Tropical Medicine (reference 6551 on 24/07/2014). The trial is registered with ClinicalTrials.gov (registration number NCT02288637) on 11/7/2014. Prior to beginning collections, informal sensitization sessions were conducted with village members to explain sampling-related activities. Written and verbal informed consent was obtained from all participants who could withdraw from the study at any time should they wish to do so.

RESULTS

No mosquitoes were collected from the outdoor sites prior to the intervention and it was only from samples made 11 and 13 months’ post intervention that
outdoor resting mosquitoes were found. Eleven months after the intervention a member of the An. gambiae complex, could still be collected inside bedrooms using CDC light-traps when these were hung next to an occupied bednet but the mosquito did not rest indoors. With the exception of a small number of insects collected indoors twelve months after the intervention no insects were collected from a series of ad hoc collections from inside houses after the application of the insecticide, including those sampled prior to the IRS. In 2014 (before the intervention) 303 (97.7%) of the 310 of the An. gambiae s.l. identified to species, collected from CDC light-traps in the village, were An. gambiae. Hence, we assume that our results from this period apply to this species.

Eleven months’ post intervention the species ratio had changed. Only 26 (17%) of the 135 An. gambiae s.l identified to species from light-traps between January and February 2016 were An. gambiae. A similar proportion was identified from the 48 insects identified from Furvela tent-traps from this period. The other 83% of the insects were An. arabiensis. Thus, following the intervention this member of the complex had apparently supplanted the original An. gambiae.

**INDOOR COLLECTIONS**

Prior to the intervention collections were made on 25 days between the 5th June and 16th October 2014. Two hundred and seventy-seven samples from 20 houses were collected in this time, most from just three of the houses. A total of 893 female and 392 male An. gambiae s.l., 101 female and 13 male An. funestus, three female An. zeimanni as well as 36 female Culex sp. and 18 female Mansonia sp. were collected. Most of the An. gambiae s.l. and the An. funestus females collected
were blood-fed or semi-gravid although unfed, semi-gravid and gravid females as well as males were also collected (Table 1). The proportion of the collection that were unfed, part-fed and gravid were similar between manual aspirator and Prokopack collections (Table 2) although more were considered to be engorged and fewer semi-gravid in Prokopack collections than in the manual collections. The two categories combined were, however, identical (comprising 61% of the sample in both collections).

Negative binomial regression demonstrated that overall the Prokopack was significantly more efficient at collecting the insects than were the manual collectors \([\text{DRR} 1.42, 95\% \text{ CI}(1.0,2.0) \ p=0.05]\) (Table 3).

Surprisingly, a Kruskal-Wallis test showed that the number of anopheline females collected from smoky houses was not significantly different from smoke-free houses \( [X^2(1) = 0.027, p=0.87] \) but the numbers of anopheline females collected from the walls and the roof in smoky houses differed significantly \([\text{Kruskal-Wallis test} \ X^2(3) = 8.295, p=0.04]\) (Table 4)

### Outdoor Collections

The decline in number of mosquitoes collected by sequential removal sampling from the three outdoor sites sampled in March 2017 are shown in Figure 1. The totals collected from each site, differentiated by sex and abdominal condition are given in Table 5, along with the estimated total number of mosquitoes (with standard errors). The initial sample collected 44.2, 45.5 and 48.4% of the total collected and 37.6, 32.0 and 38.8% of the estimated total population in each site.
A total of 654 female and 804 *An. gambiae* s.l. and four female and one male *An. funestus* group were collected from 33 collections from the latrine in December 2016-January 2017. The high proportion (55%) of male *An. gambiae* s.l. collected from the latrine contrasts with the 31% of *An. gambiae* males (283 of 901 collected) in, earlier, indoor collections ($X^2 = 126, p<0.001$).

Sixty-one of the 68 unfed female mosquitoes dissected from these collections were virgins, five had a mating plug whilst the two remaining unfed mosquitoes were parous with D sacs (Table 4). Most (64%) of the 83 blood-fed insects dissected were parous and most of these (66%) had D sacs (Table 4). This was significantly different to the part fed and unfed insects ($X^2 = 87.75, p<0.001$). Assuming that the engorged females were the equivalent of the biting population (therefore discounting the unfed insects) the estimated parous rate was 0.64. Assuming a two-day gonotrophic cycle the estimated daily survival rate was 80%.

**Blood meal source**

The stomach contents of 272 of the mosquitoes from outdoor, and 41 from indoor collections made eleven months post-spray, were identified by ELISA. At the initial dilutions used a cross-reaction between anti-dog and anti-human samples and a cross-reaction between anti-bovine and anti-goat samples was observed but not vice versa. Dilutions were changed to 1:4000 for anti-human and 1:2000 for the rest of the three antibodies. At these dilutions, the cross-reactions were much weaker compared to the true positives reactions. In order to avoid false positives from the plate reader samples were subsequently scored visually by two independent observers. Given the possibility of false positives
potential mixed feeds were not assessed. This means that potential feeds on humans may have been emphasized compared to other blood-meal sources.

Twenty three of the 272 samples tested failed to produce a reaction. Among the remaining samples most of those from the latrine and cow shed collections were positive for either cows or goats (Table 5). Even among the small sample collected indoors at this time, cows constituted 53% of the positive samples with human fed mosquitoes making up the remaining 47%. From outdoor collections, human fed mosquitoes constituted only 9% of the positive samples.

**DISCUSSION**

The propensity to feed inside houses and to rest in them once they have done so may be independent adaptions. For example, Hayes and Charlwood, (1977) describe how, in Brazil *An. darlingi* came into the open houses used on the newly opened Manaus-Caracari highway, fed and left when engorged without touching a single surface in the room, other than the underside of people’s hammocks.

Consideration of the pattern of indoor resting behaviour of the malaria vector(s) is an important component of IRS programmes (WHO, 2015). An environmental stimulus, such as surface temperature, has previously been shown to modulate the indoor resting behaviour of *An. gambiae* (Smith et al., 1966).

Before the intervention the resting site of the *An. gambiae* differed between smoke-free and smoky houses. In the former the roof appeared to be the favoured site whilst in the latter it was the walls. This is not surprising (smoke after all rises) and previous studies reported that wood smoke, or environmental conditions associated with wood smoke (e.g. decreased humidity), may modify
the preferred resting location of *An. funestus* (Gibbons 1933) and induce an exophilic response in *An. gambiae* (Bockarie et al., 1994). Thus, the roof of smoky houses may not need to be sprayed in IRS campaigns. Interestingly, the numbers of *An. gambiae* collected from smoke-free and smoky households was not significantly different. The effect of smoke on the numbers of indoor *Anopheles* is inconclusive (Biran et al. 2007, McCann et al. 2017) and the variation observed between localities may be related to specific properties of the biofuel source (Debbun et al. 2006) as well as mosquito species. The results indicate, however, that in addition to achieving a high coverage of sprayable structures smoke-free thatched roofed houses, in areas such as the one under study, both walls and roof need to be sprayed for IRS to work most efficiently.

Indoor resting *An. gambiae* and *An. funestus* collected prior to the intervention showed similar semi-gravid and gravid rates to blood-feds indicating that both species completed egg development inside houses. The semi-gravid mosquitoes collected in the morning might all have become gravid by the afternoon, indicating that overall the mosquitoes had a two day gonotrophic cycle.

Among the small number of *An. arabiensis* collected indoors after the intervention there were significantly fewer gravid and semi-gravid insects compared to those collected from the latrine. This indicates that the insects completed gonotrophic development outside, rather than indoors, but overall the duration of egg development would also have been two days. Estimated daily survival rates of 80% among the engorged mosquitoes were lower than the 84%
obtained from more extensive studies elsewhere in Tanzania (Gillies & Wilkes, 1965, Charlwood et al., 1994, 1995).

The excess of male and unfed *An. arabiensis* collected from the latrine indicates that it was acting as an ‘outdoor’ shelter hence the blood meals obtained from these mosquitoes should reflect the whole range of hosts used rather than just those inside houses. Human, dog, cattle, and goat blood sources were identified from the outdoor resting collections (Table 6). This substantiates the catholic feeding behaviour and utilization of outdoor resting sites typical of *An. arabiensis*. Moreover, the high proportion blood meals of bovine origin, even given the potential emphasis on human feeds due to the order in which the blood meal tests were carried out, support previous studies describing *An. arabiensis* blood meal sources in rural Tanzanian villages (Kweka et al., 2008). The high proportion of blood meals of bovine origin in the study area may be explained by high community LLIN coverage after the intervention in concert with the presence of alternative hosts and suitable outdoor resting sites.

The deficit of males of *An. gambiae* seen in the the earlier indoor collections is not due to an excess of females (fed-gravid ratios being similar between indoor and outdoor collections \(X^2=0.48, p = 0.49, \text{n.s.}\) indicating that male *An. gambiae* may also rest outside. The deficit of unfed (i.e. newly emerged) female *An. funestus* as well as the deficit of males of this species implies that samples were being taken some distance from the breeding site of this, normally endophilic, mosquito. The great majority of unfed female *An. arabiensis* collected from the latrine were unfed virgins. The small number of unfed parous mosquitoes collected may have been insects that just arrived too late at the feeding site to
obtain a blood meal. Most of the parous mosquitoes were collected engorged. An
engorged virgin mosquito will not develop eggs (Charlwood et al., 2003). Virgin,
engorged mosquitoes would therefore have been pre-gravid. In São Tomé, fed
female An. coluzzii collected from swarms were all considered to be ‘part-fed’
rather than engorged (Charlwood et al. 2000). Virgins may, therefore, excrete
most of the meal (which impedes flying ability) before joining a swarm in the
evening. Whether or not pre-gravid feeds are sufficient for a malaria infection to
develop is not known.

Following the intervention in 2015 CDC light-trap collections demonstrated a
decline in the An. gambiae population and shift in the species ratio, with An.
arabiensis predominating in 2016. A shift in the relative abundance of
sympatrically occurring members of the An. gambiae complex has been reported
from Kenya (Bayouh et al., 2010) and rural Tanzania following universal
distribution of LLINs (Russell et al., 2011, Lwetoijera et al., 2014). The authors
attributed this shift to direct mortality and blood feeding inhibition in An.
gambiae. This may have contributed to fewer egg-laying An. gambiae females and
presumably reduced An. gambiae larval habitat occupancy. Laboratory and semi-
field studies (Kirby et al., 2008, Paaijmans et al., 2008, Schnider et al. 2000)
suggest that in the presence of mixed-species larval environments the survival of
An. gambiae is higher than An. arabiensis. The reduced number of An. gambiae
eggs laid following the intervention may have facilitated the rise of An.
arabiensis.
The change of hosts and feeding sites (cows, outdoors) rather than just a reduction in survival would appear to be responsible for a reduction in vectorial capacity leading, presumably, to a reduction in malaria transmission.

Although the Prokopack and manual aspiration sampled the different gonotrophic stages and sexes equally, the Prokopack was considerably more efficient than manual collections. During the removal sampling the initial collection with the Prokopack sampled more than a third of the estimated total from each site. This is a measure of the efficiency of the unit and reinforces its suitability for the collection of resting malaria vectors.

CONCLUSION

The replacement of An. gambiae by An. arabiensis following IRS and distribution of LLINs may be due to the latter species having a propensity to more readily feed on animals outside houses than on humans inside them. The Prokopack is a useful tool for the collection of resting mosquitoes.

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insecticides and reduced susceptibility to bendiocarb in north-western Tanzania.

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Table 1. Total number (and proportion of the total), by abdominal condition, of *An. gambiae* s.l. and *An. funestus* females and males (and total female/male ratio) collected indoor resting from 25 days of collection between 5th June and 16th October 2014, Kyamyorwa, Muleba, Tanzania.

<table>
<thead>
<tr>
<th>Abdominal condition</th>
<th>Unfed</th>
<th>Part-fed</th>
<th>Engorged</th>
<th>Semi-gravid</th>
<th>Gravid</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>An. gambiae</em> s.l.</td>
<td>96</td>
<td>58</td>
<td>409</td>
<td>143</td>
<td>187</td>
<td>392</td>
</tr>
<tr>
<td>Proportion</td>
<td>0.11</td>
<td>0.06</td>
<td>0.46</td>
<td>0.16</td>
<td>0.21</td>
<td>1/0.44</td>
</tr>
<tr>
<td><em>An. funestus</em></td>
<td>8</td>
<td>2</td>
<td>52</td>
<td>18</td>
<td>21</td>
<td>13</td>
</tr>
<tr>
<td>Proportion</td>
<td>0.08</td>
<td>0.02</td>
<td>0.51</td>
<td>0.18</td>
<td>0.21</td>
<td>1/0.13</td>
</tr>
</tbody>
</table>
Table 2. Proportion of the total collection by abdominal condition and ratio of males to females according to collection type (Manual aspiration or Prokopack), Kyamyorwa, Muleba, Tanzania.

<table>
<thead>
<tr>
<th>Abdominal condition</th>
<th>Unfed</th>
<th>Part-fed</th>
<th>Engorged</th>
<th>Semi-gravid</th>
<th>Gravid</th>
<th>male/female ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirator</td>
<td>0.13</td>
<td>0.08</td>
<td>0.38</td>
<td>0.23</td>
<td>0.18</td>
<td>0.68</td>
</tr>
<tr>
<td>Prokopack</td>
<td>0.11</td>
<td>0.06</td>
<td>0.51</td>
<td>0.10</td>
<td>0.23</td>
<td>0.68</td>
</tr>
</tbody>
</table>

Table 3. Density rate ratio (and 95% confidence interval) of the number of anophelines collected according to the method used, manual aspirator or Prokopack aspirator.

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<thead>
<tr>
<th>Method</th>
<th>n</th>
<th>N</th>
<th>Median</th>
<th>IQR</th>
<th>DRR [95% C.I.]</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirator</td>
<td>113</td>
<td>349</td>
<td>1</td>
<td>0-5</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Prokopack</td>
<td>122</td>
<td>534</td>
<td>3</td>
<td>1-6</td>
<td>1.48 [1.03, 2.12]</td>
<td>0.03</td>
</tr>
</tbody>
</table>

n – number of collections; N – total number of collected anophelines; DRR – density rate ratio; IQR – inter-quartile range
Table 4. Mean number (and standard deviation) of female and male *An. gambiae* collected from smoke-free houses by manual aspiration or Prokopack aspirator according to the order in which the collection was undertaken, Kyamyorwa B, Muleba, Tanzania.

<table>
<thead>
<tr>
<th>Order of collection</th>
<th>Females</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aspirator</td>
<td></td>
<td>Prokopack</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Roof</td>
<td>2.36 (1.5)</td>
<td>1.09 (0.9)</td>
<td>3.49 (3.3)</td>
</tr>
<tr>
<td>Walls</td>
<td>4.76 (2.2)</td>
<td>0.94 (0.6)</td>
<td>6.71 (4.0)</td>
</tr>
<tr>
<td>Males</td>
<td>0.93 (1.3)</td>
<td>0.50 (0.5)</td>
<td>1.70 (1.4)</td>
</tr>
<tr>
<td>Roof</td>
<td>1.97 (2.6)</td>
<td>0.67 (1.2)</td>
<td>4.89 (5.0)</td>
</tr>
</tbody>
</table>
Table 5. Mean numbers (with 95% confidence intervals) of *Anopheles gambiae* collected with the Prokopack mechanical aspirator in smoke-free and smoky houses.

<table>
<thead>
<tr>
<th></th>
<th>Number of collections</th>
<th>Mean</th>
<th>95% C.I.</th>
<th>Wall/Roof ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>No smoke</td>
<td>Wall</td>
<td>25</td>
<td>3.4</td>
<td>2.2-4.6</td>
</tr>
<tr>
<td></td>
<td>Roof</td>
<td>39</td>
<td>5.0</td>
<td>2.9-7.1</td>
</tr>
<tr>
<td>Smoke</td>
<td>Wall</td>
<td>29</td>
<td>3.1</td>
<td>1.2-4.8</td>
</tr>
<tr>
<td></td>
<td>Roof</td>
<td>29</td>
<td>1.2</td>
<td>0.5-1.9</td>
</tr>
</tbody>
</table>
Table 6. Number of *Anopheles gambiae* s.l. collected and estimated total population (based on Zippin's (1958) method, as described by Southwood (1978)), during removal samples. (S.E. – standard error)

<table>
<thead>
<tr>
<th>Site</th>
<th>Males</th>
<th>Females</th>
<th>Total Collected</th>
<th>Estimated Total</th>
<th>S. E.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>unfed</td>
<td>Fed/gravid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>70*</td>
<td>33*</td>
<td>10</td>
<td>113</td>
<td>3.26</td>
</tr>
<tr>
<td>2</td>
<td>93</td>
<td>40</td>
<td>23</td>
<td>156</td>
<td>8.28</td>
</tr>
<tr>
<td>3</td>
<td>81</td>
<td>56</td>
<td>24</td>
<td>161</td>
<td>13.85</td>
</tr>
</tbody>
</table>

* - includes 1 *Culex* sp.
Table 7. Gonotrophic age, according to abdominal condition, of *An. arabiensis* females collected resting outdoors, January–February 2016, Kyamyorwa, Tanzania.

<table>
<thead>
<tr>
<th>Gonotrophic Age</th>
<th>Nulliparous</th>
<th>Parous</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Virgin</td>
<td>Plug</td>
</tr>
<tr>
<td>Unfed</td>
<td>61</td>
<td>5</td>
</tr>
<tr>
<td>Part fed</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Engorged</td>
<td>6</td>
<td>19</td>
</tr>
</tbody>
</table>
Table 8. Host source of resting *Anopheles arabiensis*, determined by ELISA, collected indoors, outdoors (from a latrine with banana leaf roof and walls) and a cow shed, 2016, Kyamyorwa, Tanzania with percentage of the total analysed. (C.I. - adjusted Wald confidence intervals).

<table>
<thead>
<tr>
<th>Collection</th>
<th>Human</th>
<th>% (C.I.)</th>
<th>Dog</th>
<th>% (C.I.)</th>
<th>Cow</th>
<th>% (C.I.)</th>
<th>Goat</th>
<th>% (C.I.)</th>
<th>No result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indoor</td>
<td>16</td>
<td>47 (34-69)</td>
<td>0</td>
<td>0</td>
<td>18</td>
<td>53 (37-69)</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Cow shed</td>
<td>1</td>
<td>4 (1-7)</td>
<td>0</td>
<td>0</td>
<td>19</td>
<td>79 (59-91)</td>
<td>4</td>
<td>17 (6-36)</td>
<td>1</td>
</tr>
<tr>
<td>Outdoor</td>
<td>18</td>
<td>9 (6-14)</td>
<td>4</td>
<td>2 (1-7)</td>
<td>154</td>
<td>81 (74-86)</td>
<td>15</td>
<td>8 (5-13)</td>
<td>15</td>
</tr>
</tbody>
</table>

697

698
Figure 1. Decline in the number of mosquitoes collected with the Prokopack sampler from three sites in Kyamyorwa village, Tanzania, March 2017. The error bars are the standard deviations derived from the second site.