

1 **Biting patterns of malaria vectors of the lower Shire valley, southern Malawi.**

2 Monicah M. Mburu^{1,2*}, Themba Mzilahowa^{1,3}, Benjamin Amoah⁴, Duster Chifundo¹, Kamija S. Phiri¹,
3 Henk van den Berg², Willem Takken² and Robert S. McCann^{1,2}.

4 **Affiliation**

5 1. School of Public Health and Family Medicine, College of Medicine, University of Malawi,
6 Malawi.

7 2. Laboratory of Entomology, Wageningen University and Research, The Netherlands.

8 3. MAC Communicable Diseases Action Centre, Blantyre, Malawi.

9 4. Centre for Health Informatics, Computing and Statistics (CHICAS), Lancaster Medical School,
10 Lancaster University, United Kingdom.

11 **Email addresses**

12 monicahmirai@yahoo.com

13 tmzilahowa@mac.medcol.mw

14 b.amoah@lancaster.ac.uk

15 dusterchifundo@gmail.com

16 kphiri@medcol.mw

17 henk.vandenberg@wur.nl

18 willem.takken@wur.nl

19 robert.mccann@wur.nl

20 *Corresponding author

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22

23 **Abstract**

24 Assessing the biting behaviour of malaria vectors plays an integral role in understanding the
25 dynamics of malaria transmission in a region. Biting times and preference for biting indoors or
26 outdoors varies among mosquito species and across regions. These behaviours may also change
27 over time in response to vector control measures such as long-lasting insecticidal nets (LLINs).
28 Data on these parameters can provide the sites and times at which different interventions would be
29 effective for vector control. This study assessed the biting patterns of malaria vectors in Chikwawa
30 district, southern Malawi.

31 The study was conducted during the dry and wet seasons in 2016 and 2017, respectively. In
32 each season, mosquitoes were collected indoors and outdoors for 24 nights in six houses per night
33 using the human landing catch. Volunteers were organized into six teams of two individuals,
34 whereby three teams collected mosquitoes indoors and the other three collected mosquitoes
35 outdoors each night, and the teams were rotated among twelve houses. All data were analyzed
36 using Poisson log-linear models.

37 The most abundant species were *Anopheles gambiae* sensu lato (primarily *An. arabiensis*)
38 and *An. funestus* s.l. (exclusively *An. funestus* s.s.). During the dry season, the biting activity of *An.*
39 *gambiae*s.l. was constant outdoors across the categorized hours (18:00 h to 08:45 h), but highest
40 in the late evening hours (21:00 h to 23:45 h) during the wet season. The biting activity of *An.*
41 *funestus* s.l. was highest in the late evening hours (21:00 h to 23:45 h) during the dry season and
42 in the late night hours (03:00 h to 05:45 h) during the wet season. Whereas the number of *An.*
43 *funestus*s.l. biting was constant ($P = 0.662$) in both seasons, that of *An. gambiae*s.l. was higher
44 during the wet season than in the dry season ($P = 0.001$). *Anopheles gambiae* s.l. was more likely
45 to bite outdoors than indoors in both seasons. During the wet season, *An. funestus* s.l. was more
46 likely to bite indoors than outdoors but during the dry season, the bites were similar both indoors
47 and outdoors.

48 The biting activity that occurred in the early and late evening hours, both indoors and outdoors
49 coincides with the times at which individuals may still be awake and physically active, and therefore

50 unprotected by LLINs. Additionally, a substantial number of anopheline bites occurred outdoors.
51 These findings imply that LLINs would only provide partial protection from malaria vectors, which
52 would affect malaria transmission in this area. Therefore, protection against bites by malaria
53 mosquitoes in the early and late evening hours is essential and can be achieved by designing
54 interventions that reduce vector-host contacts during this period.

55

56 **Keywords:** Anophelines; Culicines; HLC; Biting, Indoors; Outdoors; Malawi

57 **Highlights**

- 58 • *Anopheles arabiensis* was more likely to bite outdoors than indoors in our study
- 59 • *Anopheles funestus* biting occurred predominantly indoors
- 60 • Humans are at risk of being bitten by malaria mosquitoes before going to bed in the
61 evening
- 62 • Outdoor-biting anophelines constitute a considerable risk of malaria transmission

63 **1. Introduction**

64 Vector control remains the most effective measure to prevent malaria transmission (WHO 2006,
65 2017, 2018). The most common methods of malaria vector control in the last 20 years have been
66 the use of indoor residual spraying (IRS), conventional insecticide-treated nets and long-lasting
67 insecticidal nets (LLINs). These methods provide protection against mosquitoes that bite and rest
68 indoors. The effectiveness of LLINs and IRS in reducing malaria vectors relies on the ability of the
69 vectors coming into contact with the insecticides applied either on the nets or on the inner walls of
70 houses (Killeen and Moore 2012). However, some malaria vector species bite outdoors at least as
71 often as indoors (White et al. 1974, Joshi et al. 1975, Highton et al. 1979, Fornadel et al. 2010,
72 Kenea et al. 2016, Kenea et al. 2017). Additionally, prolonged use of LLINs may lead to changes in
73 the biting preferences of malaria vectors from indoors to outdoors (Reddy et al. 2011, Russell et al.

74 2011, Padonou et al. 2012, Meyers et al. 2016). In both cases, the vectors biting outdoors are less
75 vulnerable to the insecticides applied indoors (LLINs and IRS), and outdoor biting can sustain or
76 enhance the risk of malaria transmission (Gillies 1964, Antonio-Nkondjio et al. 2006, Killeen et al.
77 2013, Mwangangi et al. 2013, Killeen 2014).

78 Besides biting location in relation to indoors or outdoors, knowledge about the peak biting times of
79 malaria vectors is also critical for understanding the impact of LLIN use in a given region. It is evident
80 that the biting behaviour of malaria vectors varies across regions (Pates and Curtis 2005). Thus,
81 there is a need for assessing the biting behaviour of malaria vectors to assess the risk of malaria
82 transmission in a given region. Historically, the highest biting activity of primary malaria vectors in
83 Africa was reported to occur indoors from midnight to late night hours (Fontenille et al. 1990, Githeko
84 et al. 1996, Fontenille et al. 1997), and therefore, the use of bed nets gained interest because people
85 sleeping under LLINs would be protected from most potentially infectious bites. Furthermore, these
86 late-night biting mosquitoes would experience high mortality from the insecticide on the net,
87 reducing vector populations. More recently, shifts in the peak biting times of malaria vectors have
88 been reported following large-scale use of LLINs. For example, in Benin, the peak biting time of *An.*
89 *funestus* populations shifted from 02:00 h to the early morning hours (05:00 h) (Moiroux et al. 2012),
90 and in Senegal the peak biting time of *An. funestus* was observed in the later morning hours (07:00
91 h to 11:00 h) (Sougoufara et al. 2014). In Tanzania, the biting activity of *An. arabiensis* and *An.*
92 *funestus* s.s. was in the early night hours (20:00 h to 23:00 h) (Russell et al. 2011). These regions
93 had high LLIN coverage, suggesting that the malaria vectors sought hosts at times when people
94 were not protected by LLINs.

95 The most direct and favoured method of estimating malaria transmission entomologically is the
96 human landing catch (HLC) (Lines et al. 1991, Service 1993, Davis et al. 1995, Beier 1998, Kline
97 2006, Govella et al. 2010, Lima et al. 2014). The HLC estimates the peak biting times for vectors,
98 the vectors' indoor/outdoor biting preferences and the number of infectious bites that a single
99 individual can receive per unit time (Charlwood and Graves 1987, Bockarie et al. 1996, Mboera

100 2005, Pates and Curtis 2005, Oyewole et al. 2007, Bayoh et al. 2014, Sougoufara et al. 2014). Data
101 on these parameters can provide the times at which different interventions would be effective for
102 vector control. In Malawi, the main malaria vectors are *An. gambiae* sensu stricto (s.s.), *An.*
103 *arabiensis* and *An. funestus* (Spiers et al. 2002, Mzilahowa et al. 2012), but little is known about the
104 biting behaviour of these vectors in the country. This study assessed the vectors' indoor/outdoor
105 biting preferences and the peaks in their biting activities.

106

107 **2. Methods**

108 **2.1. Study site**

109 The study was conducted in two neighbouring villages, Mwalija (-15.96, 34.78) and Njereza (-15.96,
110 34.77), in Chikwawa District, southern Malawi. The villages are along the low-lying regions that are
111 categorized as hot, wet and humid with high rates of malaria transmission (Kazembe et al. 2006,
112 Kabaghe et al. 2018). Most houses are made of sun-dried or fire-baked bricks with grass-thatched
113 or corrugated iron-sheet roofs. Residents of this region engage mostly in subsistence farming with
114 maize and millet as main crops. The National Malaria Control Programme implemented IRS in
115 Chikwawa District in 2010 and 2012 with alphacypermethrin, and mass distributions of LLINs
116 were conducted in 2012 and April 2016.

117 **2.2. Selection of households**

118 The two villages in this study were part of a cluster-randomised control trial assessing the effects of
119 larval source management and house improvement on malaria transmission (McCann et al. 2017).
120 The villages fell under the control arms of the trial (i.e. no larval source management or house
121 improvement were implemented in these two villages).

122 Inclusion criteria were applied to ensure a degree of uniformity across the houses and these were:
123 houses with grass thatched roofs and open eaves, that were $\geq 25\text{m}$ apart and $\geq 100\text{m}$ away from
124 any mosquito breeding habitat. Houses that were participating in other mosquito sampling efforts at
125 the time of the current study as part of the cluster-randomised trial referenced above were excluded
126 from the current study. A complete list of households in the two villages was used to randomly select
127 twelve households for the study.

128 **2.3. Mosquito sampling**

129 Mosquito sampling was done during the early months of the dry season (May-June 2016) and
130 following the peak of the rainy season (March-April 2017) using the HLC method (Fig. 1). In each
131 season, the sampling was conducted for 24 nights in 6 of the 12 houses each night. The same
132 houses were used in both seasons. Human volunteers from the study houses were organized into
133 six teams of two individuals. A pair of individuals collected mosquitoes in six houses each night,
134 whereby three teams of HLC volunteers collected mosquitoes indoors, and the other three teams
135 collected mosquitoes outdoors. The collections were from 17:00 h to 09:45 h and were divided into
136 two shifts. The first volunteer in each team sampled mosquitoes from 17:00 h to 01:45 h and the
137 second volunteer sampled from 02:00 h to 09:45 h. Each volunteer was provided with a headlight,
138 wristwatch, pencil, mouth aspirator and mosquito holding containers. Prior to the study, all
139 volunteers were trained in the HLC technique. The volunteers sat on stools exposing the lower part
140 of their legs and collected mosquitoes that landed on their legs. The mosquitoes were placed in
141 holding cups that had been pre-labeled with the house number, hour of collection and location
142 (indoors or outdoors). The volunteers collected mosquitoes for 45 min. and had a 15 min. break
143 within every hour. A research nurse screened the volunteers for malaria on a weekly basis using a
144 malariarapid diagnostic test (mRDT; SDBioline malaria Ag Pf HRP-2; Standard Diagnostics Inc,
145 Korea). Additionally, all volunteers were provided with doxycycline daily as malaria prophylaxis from
146 one week before the start of the study to one week after the end of the study.

147 Spot checks were conducted on random days and at random times by the research team and
148 members from a local community watch group. Likewise, sporadic phone calls were made to
149 volunteers' team leaders to check whether there were any challenges.

150 **2.4. Identification of mosquitoes and detection of *Plasmodium falciparum* DNA.**

151 In the laboratory, all mosquitoes were identified morphologically using the protocol by Gillies and
152 Coetzee (1987). All anophelines were classified as *An. gambiae* s.l., *An. funestus* s.l. or *An.*
153 *tenebrous*. There was no further classification of the culicines beyond the subfamily level.
154 Females from the *An. gambiae* species complex and the *An. funestus* species group were further
155 identified to species level using polymerase chain reaction (PCR) (Scott et al. 1993, Koekemoer
156 et al. 2002, Cohuet et al. 2003), respectively. For the *An. gambiae* species complex, the PCR
157 included species-specific primers for *An. gambiae* s.s., *An. arabiensis*, and *An. quadriannulatus*.
158 For the *An. funestus* species group, the PCR included species-specific primers for *An. funestus*
159 s.s., *An. vandeeni*, *An. rivulorum*, *An. rivulorum-like*, *An. parensis*, and *An. lesoni*. The heads
160 and thoraces of all female *An. gambiae* s.l. and *An. funestus* s.l. were tested for the presence of
161 *P. falciparum* DNA using real-time polymerase chain reaction (RT-PCR) (Perandin et al.
162 2004) with a Ct value ≤ 37.0 as the cut-off for *P. falciparum* positive.

163 **2.5. Data analysis**

164 Assuming the Poisson distribution for the count of mosquitoes and applying the log link function to
165 the Poisson rate parameter, generalized linear models were fitted to assess differences: a) in the
166 biting times of mosquitoes, b) in vectors' indoor/outdoor biting preference and c) in the abundance
167 of mosquitoes between seasons. Generalized estimating equations were used to account for
168 repeated measures by house. Each of the differences was assessed in a separate model for each
169 taxonomic group and, subsequently, for the pooled counts of all malaria vectors. The cooking
170 locations, number of people that slept in the house during the night of data collection, use of bed-

171 net and kind of livestock that stayed within 20m of the house during the night of data collection were
172 included as covariates in each of the models. Door and roof types were not included in the analysis
173 because all the doors were made of wood and all roofs were grass-thatched. Cooking locations
174 included: inside the house, on the veranda, outside the house but within 2m, and outdoors at more
175 than 2m from the house. Livestock categories were comprised of cattle, goats, and chickens. As
176 the human volunteers worked for 45 min within every hour, the average bites by mosquitoes were
177 divided by 0.75 to obtain the hourly catch rate. The hourly bites were further categorized as early
178 evening (18:00 h to 20:45 h), late evening (21:00 h to 23:45 h), early night (24:00 h to 02:45 h), late
179 night (03:00 h to 05:45 h) and early morning (06:00 h to 08:45 h). Hourly collections at 17:00 h
180 to 17:45 h and at 09:00 h to 09:45 h were low and were not considered in the analysis with the
181 categorical hours. All data were analysed using SPSS Version 20.0. Entomological inoculations
182 rates (EIRs) were estimated by pooling all the catches in all the locations (indoors and outdoors)
183 and calculating the average bites. The averages were divided by 0.75 as earlier explained. This
184 was then multiplied by the sporozoite rate that was estimated using RT-PCR.

185

186 **3. Results**

187 **3.1. Abundance of mosquitoes**

188 **3.1. 1. Abundance of mosquitoes during the dry season**

189 Combined across all locations, a total of 1,032 mosquitoes was collected during the dry season. Of
190 these, 25 were males (2 anophelines indoors and 4 outdoors; 11 culicines indoors and 8 outdoors)
191 and 1007 were females. Of the 1007 females, 917 (91%) were culicines (400 indoors, 517
192 outdoors), 43 (4.3%) were *An. tenebrosus* (25 indoors, 18 outdoors) and 47 (4.7%) were malaria
193 vector species. Of the 47 malaria vectors, 22 (46.8%) were *An. gambiae* s.l. (5 indoors and 17
194 outdoors) and 25 (53.2%) were *An. funestus* s.l. (16 indoors and 9 outdoors; Table 1). Of the 21

195 malaria vectors caught indoors, 14 were identified by PCR as *An. arabiensis* (n=4) and *An. funestus*
196 s.s. (n=10). DNA of seven of the twenty-one malaria vectors caught indoors failed to amplify (6 *An.*
197 *funestus* s.l. and 1 *An. gambiae* s.l.). Of the 26 caught outdoors, 23 were identified by PCR as *An.*
198 *arabiensis* (n=13), *An. gambiae* s.s. (n=1) and *An. funestus* s.s. (n=9). DNA of three of the twenty-
199 six vectors caught outdoors failed to amplify (3 *An. gambiae* s.l.).

200 Of the 47 malaria vectors tested for the presence of *P. falciparum* DNA, only one was positive for
201 *P. falciparum* (*An. funestus* s.s.). The sporozoite rate was 2.1% and the EIR was 3.4 infectious
202 bites/person /year

203 **3.1.2. Abundance of mosquitoes during the wet season.**

204 Combined across all locations, a total of 1,408 mosquitoes was collected during the wet season. Of
205 these, 18 were males (1 male anopheline outdoors, 10 culicines indoors and 7 outdoors) and 1390
206 were females. Of the 1,390 females, 1289 (92.7%) were culicines (568 indoors, 721 outdoors), 10
207 (1%) were *An. tenebrosus* (1 indoors, 9 outdoors) and 91 (6.5%) were malaria vector species. Of
208 the 91 malaria vectors, 69 (75.8%) were *An. gambiae* s.l. (25 indoors and 44 outdoors) and 22
209 (24.2%) were *An. funestus* s.l. (17 indoors and 5 outdoors; Table 1). Of the 42 caught indoors, 40
210 were identified by PCR as *An. arabiensis* (n=18), *An. gambiae* s.s. (n=6) and *An. funestus* s.s.
211 (n=16). DNA of two of the forty-two malaria vectors caught indoors failed to amplify (1 *An. funestus*
212 s.l. and 1 *An. gambiae* s.l.). Of the 49 outdoor malaria vectors, 46 were identified by PCR as *An.*
213 *arabiensis* (n=36), *An. gambiae* s.s. (n=4), *An. funestus* s.s. (n=5) and a hybrid of *An. arabiensis*
214 and *An. gambiae* s.s. (n=1). DNA of three of the forty-nine vectors caught outdoors failed to amplify
215 (3 *An. gambiae* s.l.).

216 Of the 91 malaria vectors tested for the presence of *P. falciparum* DNA, 4 were positive for *P.*
217 *falciparum* (3 *An. funestus* s.s. and 1 *An. gambiae* s.s.). The sporozoite rate was 4.4% and the EIR
218 was 13.5 infectious bites/person/year

219 The abundance of female *An. gambiae* s.l. was lower in the dry season than in the wet season (Risk
220 ratio (RR) = 0.32, 95% confidence intervals (CI) = [0.20-0.52], P = 0.001) but that of female *An.*
221 *funestus* s.l. did not differ between the two seasons (RR = 1.06, CI = [0.56-2.06], P = 0.854).

222 3.2. Biting times of mosquitoes

223 During the dry season, the indoor and outdoor biting by malaria vectors (combined across all
224 species) exhibited bi-modal and uni-modal peaks, respectively. For the indoor biting, the first peak
225 was observed between 21:00 h to 21:45 h and the second peak was at 23:00 h to 23:45 h. For the
226 outdoor biting, the peak was observed between 20:00 h to 20:45 h (Fig.2). Considering each
227 species complex/group separately, the biting activity of *An. gambiae* s.l. was lower indoors than
228 outdoors (RR = 0.29, CI = [0.11-0.80], P= 0.016). The biting activity of *An. gambiae* s.l., outdoors,
229 was constant across all the categorized hours (18:00 h to 08:45 h) (P ≥ 0.05). Whereas there was
230 no biting activity observed in the early morning hours, indoors, for *An. gambiae* s.l., the biting rates
231 of this species were constant from the late evening hours to the late night hours (21:00 h to 05:45
232 h) (P ≥ 0.05) (Fig. 3A). *Anopheles funestus* s.l. biting rates did not differ between indoors and
233 outdoors in the dry season (RR = 1.78, CI = [0.79-4.02], P = 0.167). The biting rate of *An. funestus*
234 s.l. indoors was highest during the late evening hours (21:00 h to 23:45 h) but absent in the early
235 morning hours. The outdoor biting rates of this species were constant from 18:00 h to 05:45 h (P ≥
236 0.05) (Fig. 3B).

237 During the wet season, the indoor and outdoor biting by malaria vectors (combined across all
238 species) exhibited uni-modal peaks. The highest activity of indoor biting was from 02:00 h to 04:00
239 h and that of outdoor biting was at 21:00 h (Fig. 2). Similar to the dry season, the biting activity of
240 *An. gambiae* s.l. in the wet season was lower indoors than outdoors (RR = 0.57, CI = [0.35-0.93], P
241 = 0.024). Outdoors, the peak biting time of *An. gambiae* s.l. occurred in the late evening hours
242 (21:00 h to 23:45 h) and this biting activity was higher than that observed in the early evening hours

243 (P = 0.001), early night hours (P = 0.037) and late night hours (P = 0.001). The indoor biting rates
244 of *An. gambiae* s.l. in the wet season were constant from 18:00 h to 05:45 h (P ≥ 0.05) (Fig. 3A).
245 *Anopheles funestus* s.l. was more likely to bite indoors than outdoors in the wet season (RR= 3.4,
246 CI = [1.25-9.22], P = 0.016). The peak biting time of *An. funestus* indoors in the wet season was in
247 the late night hours (03:00 h to 05:45 h) and was similar to the biting activity that was observed in
248 the early night hours (P = 0.317) but different from the biting activities in the early evening hours (P
249 = 0.021) and in the late evening hours (P = 0.021). The outdoor biting rates of *An. funestus* s.l. were
250 constant from 21:00 h to 05:45 h (P ≥ 0.05) (Fig. 3B).

251 The biting activity of female culicines was lower in the dry season than in the wet season (RR =
252 0.65, CI = [0.60-0.71], P = 0.001). Indoor culicine biting rates were lower than the outdoor biting
253 rates in the dry (RR = 0.85, CI = [0.74-0.97], P = 0.014) and wet (RR = 0.8, CI = [0.72-0.89], P =
254 0.001) seasons (Fig. 4).

255 The number of people that slept in the house each night, bed net use, cooking locations, presence
256 of cattle, goats and chicken did not influence the biting activity of *An. gambiae* s.l. indoors or
257 outdoors, during both seasons. This was the same for *An. funestus* s.l. with the exception that the
258 presence of chickens was positively associated with the biting activity of this species (Table 2).

259

260 **4. Discussion**

261 The malaria vectors identified in this study were *An. gambiae* s.l. (primarily *An. arabiensis*) and *An.*
262 *funestus* s.l. (exclusively *An. funestus* s.s.). Whereas the density of *An. funestus* s.s. was constant
263 in both seasons of this study, the density of *An. gambiae* s.l. was higher in the wet season than in
264 the dry season. In the dry season, the biting activity of *An. gambiae* s.l. was constant across the
265 categorized hours, outdoors, but highest in the late evening hours (21:00 h to 23:45 h) during the
266 wet season. During the dry season, the biting activity of *An. funestus* s.s. was highest in the late

267 evening hours, while in the wet season, the peak biting activity of this species was in the late night
268 hours (03:00 h to 05:45 h). Furthermore, *An. arabiensis* was more likely to bite outdoors than indoors
269 in both seasons, though some biting by this species also occurred indoors.

270 Previous studies in this region of Malawi conducted in the early 2000s identified three species of
271 malaria vectors: *An. funestus* s.s., *An. gambiae* s.s. and *An. arabiensis* (Spiers et al. 2002,
272 Mzilahowa et al. 2012). The current study identified these same three species, but *An. gambiae* s.s.
273 accounted for only 2% and 10% of the malaria vectors collected in the dry and wet seasons,
274 respectively. This low density of *An. gambiae* s.s. relative to that of *An. arabiensis* and *An. funestus*
275 s.s. agrees with other recent studies in this area (Kabaghe et al. 2018) and warrants further
276 investigation. Generally, similar to the present findings, the densities of malaria vectors in this region
277 have been low with *An. arabiensis* accounting for a sporozoite rate of 5.4% (Kabaghe et al. 2018).

278 The biting activity by *An. tenebrosus* in both seasons was surprising, as little is known about this
279 species. This species has not been incriminated as a malaria vector (Gillies and De Meillon 1968),
280 though it is closely related to *An. coustani* (Gillies and Coetzee 1987). However, in Tanzania, *An.*
281 *tenebrosus* was reported with infective larvae of *Dirofilaria immitis* (Gillies and Coetzee 1987), and
282 therefore, it may be a species of medical importance.

283 Currently, *An. arabiensis* and *An. funestus* s.s. may be considered the primary malaria vectors in
284 southern Malawi. Furthermore, the density of *An. gambiae* s.l. was higher during the wet season
285 than in the dry season, while that of *An. funestus* s.l. was constant in both seasons, similar to
286 previous studies from Mozambique, Malawi and Tanzania (Mendis et al. 2000, Mzilahowa et al.
287 2012, Finda et al. 2018), and highlighting the different impacts of seasonality on the abundance of
288 different mosquito species. In the case of *An. gambiae* s.l. and *An. funestus* s.s., this difference may
289 reflect differences in the preferred larval habitats of each species. While *An. funestus* s.s. typically
290 inhabits more permanent water bodies during its immature stages, *An. gambiae* s.l. is able to use

291 the more temporary larval habitats that occur more often in the wet season (Gimnig et al. 2001,
292 Mutuku et al. 2009).

293 *Anopheles arabiensis* was more likely to bite outdoors than indoors in this study, both in the dry and
294 wet season. This species is considered as a dominant malaria vector in neighbouring southern
295 Zambia (Kent et al. 2007, Fornadel et al. 2010) and has been associated with outdoor biting in other
296 regions (Mendis et al. 2000, Tirados et al. 2006, Geissbühler et al. 2007, Oyewole et al. 2007,
297 Russell et al. 2011). The biting densities of *An. funestus* s.s. were higher indoors than outdoors in
298 the wet season, confirming that this species is predominantly endophagic (Awolola et al. 2003,
299 Antonio-Nkondjio et al. 2006, Mwangangi et al. 2013). However, in the dry season, there was no
300 difference between the indoor and outdoor biting densities of *An. funestus* s.s. In other regions,
301 outdoor biting has been associated with the relative availability of hosts outdoors, when they were
302 sleeping in the courtyards or on the verandas of their houses (Faye et al. 1997). Although the current
303 study did not quantify host availability, some people in the region sleep outdoors during the dry
304 season because of higher temperatures as compared to the wet season. During the rainy season,
305 most people in this region sleep indoors, when many are protected by LLINs. Their exposure to
306 mosquito bites would, therefore, occur mostly at times when they are outdoors in the early evening
307 hours. In this context, outdoor biting activities by both *An. arabiensis* and *An. funestus* s.s. are
308 important factors to consider when selecting and planning malaria control interventions. Because
309 LLINs and IRS target indoor biting vectors, there is a need for additional tools that can provide
310 protection against outdoor biting (Govella and Ferguson 2012, Russell et al. 2013, Killeen et al.
311 2016).

312 Studies prior to the large-scale introduction of bed nets in Africa found that the major malaria
313 vectors, *An. gambiae* s.s., *An. arabiensis* and *An. funestus*, are nocturnal with peak biting activity
314 occurring in the late night hours (usually from 23:00 h or 24:00 h to 06:00 h) (Fontenille et al. 1990,
315 Githeko et al. 1996, Fontenille et al. 1997, Pates and Curtis 2005). We refer to this biting as the

316 historic biting time of malaria vectors. These historic biting times coincide with hours that people are
317 usually asleep, which is integral to the effectiveness of LLINs to protect sleepers from infectious
318 bites by malaria vectors. However, some studies have found peak biting activity of malaria vectors
319 outside of these historic biting times. For example, the peak biting activity of *An. arabiensis* in
320 Ethiopia was reported in the early evening hours (19:00 h to 20:00 h), both before and after the
321 implementation of LLINs (Yohannes et al. 2005, Yohannes and Boelee 2012). Such variation in the
322 historic biting times may be explained by regional differences. More recently, in some regions the
323 peak biting times of malaria vectors have been observed outside of the historic biting times, with
324 biting in the early evening (Reddy et al. 2011, Russell et al. 2011) or morning hours (Reddy et al.
325 2011, Moiroux et al. 2012, Sougoufara et al. 2014). Most of these studies lack data on the biting
326 times of malaria vectors in their specific study sites before the implementation of LLINs (Reddy et
327 al. 2011, Sougoufara et al. 2014) but the high levels of reported LLIN use support the hypothesis
328 that it is possible for malaria vector populations to shift peak biting times to avoid LLINs. In the
329 present study, the biting activities of *An. gambiae* s.l. in the early and late evening hours in the dry
330 and wet season, respectively, and *An. funestus* s.l. in the dry season, also differ from the historic
331 biting times of malaria vectors but are similar to results from studies in Ethiopia (Yohannes and
332 Boelee 2012), Mozambique and Tanzania (Mendis et al. 2000, Geissbühler et al. 2007, Russell et
333 al. 2011). One potential explanation for the observed peak biting time could be that the temperatures
334 are cooler in the late evening hours in this part of Malawi compared to regions closer to the equator,
335 resulting in the activation of the mosquitoes' host-seeking behaviour (Silver 2008). On the other
336 hand, it could be that *An. gambiae* s.l. had limited access to humans at times when people are
337 protected by LLINs as observed in other regions (Charlwood and Graves 1987, Yohannes and
338 Boelee 2012). Regardless of the explanation, our finding of outdoor biting has implications for
339 malaria control in the region because the biting coincides with the times at which many individuals
340 may still be active and therefore unprotected by LLINs. While the observed biting activity of *An.*
341 *funestus* s.l. in the early night hours during the wet season suggests that LLIN use still provides

342 significant protection from malaria transmission, the reported levels of insecticide resistance in *An.*
343 *funestus* populations in Malawi (Riveron et al. 2015, Mzilahowa et al. 2016) raises further concerns
344 about the long-term effectiveness of LLINs as an intervention.

345 The biting activity of female culicines was constant from the early evening hours to the late night
346 hours both indoors and outdoors. These mosquitoes are a nuisance and have been implicated as
347 vectors of other diseases. In the present study area, filariasis is prevalent (Nielsen et al. 2002,
348 Ngwira et al. 2007) and culicine species have been reported with infective filarial larvae (Merelo-
349 Lobo et al. 2003) highlighting the need for vector control tools that can also target these mosquitoes.

350 The use of LLINs is effective against indoor biting in the early and late night hours when many
351 individuals are likely to be asleep. However, the observed biting in the early and the late evening
352 hours before people would be under LLINs, both indoors and outdoors, is a major concern. Future
353 research should incorporate the behaviour of people when assessing the biting patterns of
354 mosquitoes (Monroe et al. 2019). Measuring the behaviour of people alongside mosquito biting
355 behaviour would allow quantification of when and where the two behaviours actually overlap in time
356 and space, and would provide a better understanding of the gap in protection left by current vector
357 control tools. In addition to identifying when and where human-vector contact occurs, it is also
358 important to understand who and why, as the proportion of people spending time indoors or
359 outdoors varies across regions, seasons and economic and social activities (Monroe et al. 2019).
360 Identifying these risk factors is critical for closing any gap in protection against malaria
361 transmission. Potential complementary tools to tackle early biting both indoors and outdoors have
362 been highlighted by Ferguson et al. (2010) and Williams et al. (2018). For instance, house
363 improvement protects all individuals in a house equally. This is being assessed in a number of
364 regions (Killeen et al. 2017b, McCann et al. 2017) as well as the use of insecticide-impregnated
365 tubes along the eaves, which are the preferred entry points for mosquitoes (Knols et al. 2016,
366 Sternberg et al. 2016, Oumbouke et al. 2018). The development of protective measures that divert

367 malaria vectors from human beings to alternative hosts like cattle is important, especially for species
368 with an opportunistic host-feeding behaviour such as *An. arabiensis*. However, such measures
369 would still sustain the densities of biting malaria vectors and therefore, as suggested by Killeen et
370 al. (2017a), the use of insecticide-treated cattle could be more effective in reducing the density of
371 biting malaria vectors. Other complementary measures that would reduce the densities of biting
372 malaria vectors significantly include the use of insecticide-treated clothes (Kimani et al. 2006, Banks
373 et al. 2014), larval source management and the 'push-pull' approach, which is directed at adult
374 vectors and can be implemented either by the use of attractive toxic sugar baits (Müller et al.
375 2010, Beier et al. 2012) or by use of attractants and repellents in traps (Menger et al. (2014), Menger
376 et al. 2016).

377

378 **5. Conclusion**

379 A considerable proportion of the biting by malaria vectors in this study, both indoors and outdoors,
380 occurred at times in the evening when many people are likely still active and not protected by bed
381 nets. This behaviour is likely to enhance malaria transmission. The development of vector control
382 tools that can tackle the biting activity in the early and late evening hours, both indoors and outdoors,
383 is highly recommended because the current, mostly indoor-based tools provide only partial
384 protection against bites by malaria vectors. (Govella and Ferguson, 2012; Killeen et al., 2016;
385 Russell et al., 2013).

386

387 **Abbreviations**

388 HLC: Human landing catch; LLINs: Long-lasting insecticidal nets; IRS: Indoor residual spraying;
389 PCR: Polymerase chain reaction; mRDT: malaria rapid diagnostic test.

390

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398

399 **Declarations**

400 **Ethical approval and consent to participate**

401 This study was approved by the College of Medicine Research and Ethics Committee in Malawi
402 (proposal number P.03/16/1901). Written permission to conduct the study was provided by the
403 District Health Officer of Chikwawa District, southern Malawi. The purpose and procedures of the
404 study were explained in the local language, Chichewa, to local leaders, community watch-team,
405 participating community members, and HLC volunteers. Heads of households and HLC
406 volunteers were only enrolled in the study after providing written consent prior to the start of the
407 study. An impartial witness was present in cases where the head of the household was illiterate.

408 **Consent for publication**

409 Not applicable.

410 **Availability of data and materials**

411 The datasets for this study are available upon a reasonable request.

412 **Competing interests**

413 The authors declare that they have no competing interests.

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417 **Authors' contributions**

418 MM, RM, WT conceived the study design. MM, HvdB, TM, RM, and WT were involved in the
419 design and implementation of the study. MM and DC did the molecular work. MM, BA, RM and
420 WT contributed to data analysis. MM wrote the first draft of the manuscript. All authors contributed
421 to the writing of the final manuscript. All authors read and approved the final manuscript.

422

423 **References**

- 424 Antonio-Nkondjio, C., C. H. Kerah, F. Simard, P. Awono-Ambene, M. Chouaibou, T. Tchuinkam, and D.
425 Fontenille. 2006. Complexity of the malaria vectorial system in Cameroon: contribution of
426 secondary vectors to malaria transmission. *Journal of Medical Entomology* **43**:1215-1221.
- 427 Awolola, T., K. Ibrahim, T. Okorie, L. Koekemoer, R. Hunt, and M. Coetzee. 2003. Species composition and
428 biting activities of anthropophilic Anopheles mosquitoes and their role in malaria transmission in
429 a holo-endemic area of southwestern Nigeria. *African Entomology* **11**:227-232.
- 430 Banks, S. D., N. Murray, A. Wilder-Smith, and J. G. Logan. 2014. Insecticide-treated clothes for the control
431 of vector-borne diseases: a review on effectiveness and safety. *Medical and Veterinary
432 Entomology* **28**:14-25.
- 433 Bayoh, M. N., E. D. Walker, J. Kosgei, M. Ombok, G. B. Olang, A. K. Githeko, G. F. Killeen, P. Otieno, M.
434 Desai, N. F. Lobo, J. M. Vulule, M. J. Hamel, S. Kariuki, and J. E. Gimnig. 2014. Persistently high
435 estimates of late night, indoor exposure to malaria vectors despite high coverage of insecticide
436 treated nets. *Parasites & Vectors* **7**:380.
- 437 Beier, J. C. 1998. Malaria parasite development in mosquitoes. *Annual Review of Entomology* **43**:519-543.
- 438 Beier, J. C., G. C. Müller, W. Gu, K. L. Arheart, and Y. Schlein. 2012. Attractive toxic sugar bait (ATSB)
439 methods decimate populations of Anopheles malaria vectors in arid environments regardless of
440 the local availability of favoured sugar-source blossoms. *Malaria Journal* **11**:31.
- 441 Binka, F. N., and P. Adongo. 1997. Acceptability and use of insecticide impregnated bednets in northern
442 Ghana. *Tropical Medicine & International Health* **2**:499-507.
- 443 Bockarie, M., N. Alexander, F. Bockarie, E. Ibam, G. Barnish, and M. Alpers. 1996. The late biting habit of
444 parous Anopheles mosquitoes and pre-bedtime exposure of humans to infective female
445 mosquitoes. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **90**:23-25.
- 446 Charlwood, J., and P. Graves. 1987. The effect of permethrin-impregnated bednets on a population of
447 Anopheles farauti in coastal Papua New Guinea. *Medical and Veterinary Entomology* **1**:319-327.
- 448 Cohuet, A., F. Simard, J.-C. Toto, P. Kengne, M. Coetzee, and D. Fontenille. 2003. Species identification
449 within the Anopheles funestus group of malaria vectors in Cameroon and evidence of a new
450 species. *The American Journal of Tropical Medicine and Hygiene* **69**:200-205.
- 451 Davis, J. R., T. Hall, E. M. Chee, A. Majala, J. Minjas, and C. J. Shiff. 1995. Comparison of sampling
452 anopheline mosquitoes by light-trap and human-bait collections indoors at Bagamoyo, Tanzania.
453 *Medical and Veterinary Entomology* **9**:249-255.
- 454 Faye, O., L. Konate, J. Mouchet, D. Fontenille, N. Sy, G. Hebrard, and J. P. Herve. 1997. Indoor Resting by
455 Outdoor Biting Females of Anopheles gambiae Complex (Diptera: Culicidae) in the Sahel of
456 Northern Senegal. *Journal of Medical Entomology* **34**:285-289.

457 Ferguson, H. M., A. Dornhaus, A. Beeche, C. Borgemeister, M. Gottlieb, M. S. Mulla, J. E. Gimnig, D. Fish,
458 and G. F. Killeen. 2010. Ecology: A Prerequisite for Malaria Elimination and Eradication. *PLoS*
459 *Medicine* **7**:e1000303.

460 Finda, M. F., A. J. Limwagu, H. S. Ngowo, N. S. Matowo, J. K. Swai, E. Kaindoa, and F. O. Okumu. 2018.
461 Dramatic decreases of malaria transmission intensities in Ifakara, south-eastern Tanzania since
462 early 2000s. *Malaria Journal* **17**:362.

463 Fontenille, D., J. P. Lepers, G. H. Campbell, M. Coluzzi, I. Rakotoarivony, and P. Coulanges. 1990. Malaria
464 Transmission and Vector Biology in Manarintsoa, High Plateaux of Madagascar. *The American*
465 *Journal of Tropical Medicine and Hygiene* **43**:107-115.

466 Fontenille, D., L. Lochouarn, M. Diatta, C. Sokhna, I. Dia, N. Diagne, J.-J. Lemasson, K. Ba, A. Tall, C. Rogier,
467 and J.-F. Trape. 1997. Four years' entomological study of the transmission of seasonal malaria in
468 Senegal and the bionomics of *Anopheles gambiae* and *A. arabiensis*. *Transactions of the Royal*
469 *Society of Tropical Medicine and Hygiene* **91**:647-652.

470 Fornadel, C. M., L. C. Norris, G. E. Glass, and D. E. Norris. 2010. Analysis of *Anopheles arabiensis* Blood
471 Feeding Behavior in Southern Zambia during the Two Years after Introduction of Insecticide-
472 Treated Bed Nets. *The American Journal of Tropical Medicine and Hygiene* **83**:848-853.

473 Frey, C., C. Traoré, M. De Allegri, B. Kouyaté, and O. Müller. 2006. Compliance of young children with ITN
474 protection in rural Burkina Faso. *Malaria Journal* **5**:70.

475 Geissbühler, Y., P. Chaki, B. Emidi, N. J. Govella, R. Shirima, V. Mayagaya, D. Mtasiwa, H. Mshinda, U.
476 Fillinger, S. W. Lindsay, K. Kannady, M. C. de Castro, M. Tanner, and G. F. Killeen. 2007.
477 Interdependence of domestic malaria prevention measures and mosquito-human interactions in
478 urban Dar es Salaam, Tanzania. *Malaria Journal* **6**:126.

479 Gillies, M. 1964. The role of secondary vectors of malaria in north-east Tanganyika. *Transactions of the*
480 *Royal Society of Tropical Medicine and Hygiene* **58**:154-158.

481 Gillies, M., and M. Coetzee. 1987. A Supplement to the Anophelinae of Africa South of the Sahara.
482 Publications of the South African Institute for Medical Research **55**:1-143.

483 Gillies, M. T., and B. De Meillon. 1968. The Anophelinae of Africa south of the Sahara (Ethiopian
484 zoogeographical region). The Anophelinae of Africa south of the Sahara (Ethiopian
485 Zoogeographical Region).

486 Gimnig, J. E., M. Ombok, L. Kamau, and W. A. Hawley. 2001. Characteristics of Larval Anopheline (Diptera:
487 Culicidae) Habitats in Western Kenya. *Journal of Medical Entomology* **38**:282-288.

488 Githeko, A. K., N. I. Adungo, D. M. Karanja, W. A. Hawley, J. M. Vulule, I. K. Seroney, A. V. Ofulla, F. K. Atieli,
489 S. O. Ondijo, and I. O. Genga. 1996. Some Observations on the Biting Behavior of *Anopheles*
490 *gambiae* ss, *Anopheles arabiensis*, and *Anopheles funestus* and Their Implications for Malaria
491 Control. *Experimental Parasitology* **82**:30 -315.

492 Govella, N. J., and H. H. Ferguson. 2012. Why use of interventions targeting outdoor biting mosquitoes
493 will be necessary to achieve malaria elimination. *Frontiers in Physiology* **3**.

494 Govella, N. J., J. D. Moore, and G. F. Killeen. 2010. An Exposure-Free Tool for Monitoring Adult Malaria
495 Mosquito Populations. *The American Journal of Tropical Medicine and Hygiene* **83**:596 - 600.

496 Highton, R., J. H. Bryan, P. Boreham, and J. Chandler. 1979. Studies on the sibling species *Anopheles*
497 *gambiae* Giles and *Anopheles arabiensis* Patton (Diptera: Culicidae) in the Kisumu area, Kenya.
498 *Bulletin of Entomological Research* **69**:43-53.

499 Joshi, G. P., M. W. Service, and G. D. Pradhan. 1975. A survey of species A and B of the *Anopheles gambiae*
500 Giles complex in the Kisumu area of Kenya prior to insecticidal spraying with OMS-43
501 (Fenitrothion). *Annals of Tropical Medicine and Parasitology* **69**:91-104.

502 Kabaghe, A. N., M. G. Chipeta, S. Gowelo, M. Mburu, Z. Truwah, R. S. McCann, M. van Vugt, M. P.
503 Grobusch, and K. S. Phiri. 2018. Fine-scale spatial and temporal variation of clinical malaria
504 incidence and associated factors in children in rural Malawi: a longitudinal study. *Parasites &*
505 *Vectors* **11**:129.

506 Kazembe, L. N., I. Kleinschmidt, T. H. Holtz, and B. L. Sharp. 2006. Spatial analysis and mapping of malaria
507 risk in Malawi using point-referenced prevalence of infection data. *International Journal of Health*
508 *Geographics* **5**:41.

509 Kenea, O., M. Balkew, H. Tekie, T. Gebre-Michael, W. Deressa, E. Loha, B. Lindtjorn, and H. J. Overgaard.
510 2016. Human-biting activities of *Anopheles* species in south-central Ethiopia. *Parasites & Vectors*
511 **9**.

512 Kenea, O., M. Balkew, H. Tekie, T. Gebre-Michael, W. Deressa, E. Loha, B. Lindtjorn, and H. J. Overgaard.
513 2017. Comparison of two adult mosquito sampling methods with human landing catches in south-
514 central Ethiopia. *Malaria Journal* **16**:30.

515 Kent, R. J., P. E. Thuma, S. Mharakurwa, and D. E. Norris. 2007. Seasonality, blood feeding behavior, and
516 transmission of *Plasmodium falciparum* by *Anopheles arabiensis* after an extended drought in
517 southern Zambia. *The American Journal of Tropical Medicine and Hygiene* **76**:267-274.

518 Killeen, G. F. 2014. Characterizing, controlling and eliminating residual malaria transmission. *Malaria*
519 *Journal* **13**:330.

520 Killeen, G. F., N. J. Govella, D. W. Lwetoijera, and F. O. Okumu. 2016. Most outdoor malaria transmission
521 by behaviourally-resistant *Anopheles arabiensis* is mediated by mosquitoes that have previously
522 been inside houses. *Malaria Journal* **15**.

523 Killeen, G. F., S. S. Kiware, F. O. Okumu, M. E. Sinka, C. L. Moyes, N. C. Massey, P. W. Gething, J. M. Marshall,
524 C. J. Chaccour, and L. S. Tusting. 2017a. Going beyond personal protection against mosquito bites
525 to eliminate malaria transmission: population suppression of malaria vectors that exploit both
526 human and animal blood. *BMJ Global Health* **2**.

527 Killeen, G. F., J. P. Masalu, D. Chinula, E. A. Fotakis, D. R. Kavishe, D. Malone, and F. Okumu. 2017b. Control
528 of malaria vector mosquitoes by insecticide-treated combinations of window screens and eave
529 baffles. *Emerging Infectious Diseases* **23**:782.

530 Killeen, G. F., and S. J. Moore. 2012. Target product profiles for protecting against outdoor malaria
531 transmission. *Malaria Journal* **11**:17.

532 Killeen, G. F., A. Seyoum, C. Sikaala, A. S. Zomboko, J. E. Gimnig, N. J. Govella, and M. T. White. 2013.
533 Eliminating malaria vectors. *Parasites & Vectors* **6**:1.

534 Kimani, E. W., J. M. Vulule, I. W. Kuria, and F. Mugisha. 2006. Use of insecticide-treated clothes for
535 personal protection against malaria: a community trial. *Malaria Journal* **5**:63.

536 Kline, D. L. 2006. Traps and trapping techniques for adult mosquito control. *Journal of American Mosquito*
537 *Control Association* **22**:490-496.

538 Knols, B. G., M. Farenhorst, R. Andriessen, J. Snetselaar, R. A. Suer, A. J. Osinga, J. M. Knols, J. Deschietere,
539 K. R. Ng'habi, and I. N. Lyimo. 2016. Eave tubes for malaria control in Africa: an introduction.
540 *Malaria Journal* **15**:404.

541 Koekemoer, L., L. Kamau, R. Hunt, and M. Coetzee. 2002. A cocktail polymerase chain reaction assay to
542 identify members of the *Anopheles funestus* (Diptera: Culicidae) group. *The American Journal of*
543 *Tropical Medicine and Hygiene* **66**:804-811.

544 Lima, J. B. P., M. G. Rosa-Freitas, C. M. Rodovalho, F. Santos, and R. Lourenço-de-Oliveira. 2014. Is there
545 an efficient trap or collection method for sampling *Anopheles darlingi* and other malaria vectors
546 that can describe the essential parameters affecting transmission dynamics as effectively as
547 human landing catches? - A Review. *Memórias do Instituto Oswaldo Cruz* **109**:685-705.

548 Lines, J. D., C. F. Curtis, T. J. Wilkes, and K. J. Njunwa. 1991. Monitoring human-biting mosquitoes (Diptera:
549 Culicidae) in Tanzania with light-traps hung beside mosquito nets. *Bulletin of Entomological*
550 *Research* **81**:77-84.

551 Mboera, L. E. 2005. Sampling techniques for adult Afrotropical malaria vectors and their reliability in the
552 estimation of entomological inoculation rate. *Tanzania Health Research Bulletin* **7**:117-124.

553 McCann, R. S., H. van den Berg, P. J. Diggle, M. van Vugt, D. J. Terlouw, K. S. Phiri, A. Di Pasquale, N. Maire,
554 S. Gowelo, M. M. Mburu, A. N. Kabaghe, T. Mzilahowa, M. G. Chipeta, and W. Takken. 2017.
555 Assessment of the effect of larval source management and house improvement on malaria
556 transmission when added to standard malaria control strategies in southern Malawi: study
557 protocol for a cluster-randomised controlled trial. *BMC Infectious Diseases* **17**:639.

558 Mendis, C., J. L. Jacobsen, A. Gamage-Mendis, E. Bule, M. Dgedge, R. Thompson, N. Cuamba, J. Barreto, K.
559 Begtrup, R. E. Sinden, and B. Høgh. 2000. *Anopheles arabiensis* and *An. funestus* are equally
560 important vectors of malaria in Matola coastal suburb of Maputo, southern Mozambique. *Medical*
561 *and Veterinary Entomology* **14**:171-180.

562 Menger, D. J., P. Omusula, K. Wouters, C. Oketch, A. S. Carreira, and M. Durka. 2016. Eave screening and
563 push-pull tactics to reduce house entry by vectors of malaria. *The American Journal of Tropical*
564 *Medicine and Hygiene* **94**.

565 Menger, D. J., B. Otieno, M. de Rijk, W. R. Mukabana, J. J. van Loon, and W. Takken. 2014. A push-pull
566 system to reduce house entry of malaria mosquitoes. *Malaria Journal* **13**:119.

567 Merelo-Lobo, A. R., P. J. McCall, M. A. Perez, A. A. Spiers, T. Mzilahowa, B. Ngwira, D. H. Molyneux, and
568 M. J. Donnelly. 2003. Identification of the vectors of lymphatic filariasis in the Lower Shire Valley,
569 southern Malawi. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **97**:299-301.

570 Meyers, J. I., S. Pathikonda, Z. R. Popkin-Hall, M. C. Medeiros, G. Fuseini, A. Matias, G. Garcia, H. J.
571 Overgaard, V. Kulkarni, and V. P. Reddy. 2016. Increasing outdoor host-seeking in *Anopheles*
572 *gambiae* over 6 years of vector control on Bioko Island. *Malaria Journal* **15**:239.

573 Moiroux, N., M. B. Gomez, C. Penetier, E. Elanga, A. Djenontin, F. Chandre, I. Djegbe, H. Guis, and V.
574 Corbel. 2012. Changes in *Anopheles funestus* Biting Behavior Following Universal Coverage of
575 Long-Lasting Insecticidal Nets in Benin. *Journal of Infectious Diseases* **206**:1622-1629.

576 Müller, G. C., J. C. Beier, S. F. Traore, M. B. Toure, M. M. Traore, S. Bah, S. Doumbia, and Y. Schlein. 2010.
577 Successful field trial of attractive toxic sugar bait (ATSB) plant-spraying methods against malaria
578 vectors in the *Anopheles gambiae* complex in Mali, West Africa. *Malaria Journal* **9**:210.

579 Mutuku, F., M. Bayoh, A. Hightower, J. Vulule, J. Gimnig, J. Mueke, F. Amimo, and E. Walker. 2009. A
580 supervised land cover classification of a western Kenya lowland endemic for human malaria:
581 associations of land cover with larval *Anopheles* habitats. *International Journal of Health*
582 *Geographics* **8**:19.

583 Mwangangi, J. M., E. J. Muturi, S. M. Muriu, J. Nzovu, J. T. Midega, and C. M. Mbogo. 2013. The role of
584 *Anopheles arabiensis* and *Anopheles coustani* in indoor and outdoor malaria transmission in
585 Taveta District, Kenya. *Parasit & Vectors* **6**.

586 Mzilahowa, T., M. Chiumia, R. B. Mbewe, V. T. Uzalili, M. Luka-Banda, and A. Kutengule. 2016. Increasing
587 insecticide resistance in *Anopheles funestus* and *Anopheles arabiensis* in Malawi, 2011–2015.
588 *Malaria Journal* **15**.

589 Mzilahowa, T., I. M. Hastings, M. E. Molyneux, and P. J. McCall. 2012. Entomological indices of malaria
590 transmission in Chikhwawa district, Southern Malawi. *Malaria Journal* **11**:380.

591 Ngwira, B. M., P. Tambala, A. M. Perez, C. Bowie, and D. H. Molyneux. 2007. The geographical distribution
592 of lymphatic filariasis infection in Malawi. *Filaria Journal* **6**:12.

593 Nielsen, N. O., P. Makaula, D. Nyakuipa, P. Bloch, Y. Nyasulu, and P. E. Simonsen. 2002. Lymphatic filariasis
594 in Lower Shire, southern Malawi. *Transactions of the Royal Society of Tropical Medicine and*
595 *Hygiene* **96**:133-138.

596 Oumbouke, W. A., I. Z. Tia, A. M. G. Barreaux, A. A. Koffi, E. D. Sternberg, M. B. Thomas, and R. N'Guessan.
597 2018. Screening and field performance of powder-formulated insecticides on eave tube inserts
598 against pyrethroid resistant *Anopheles gambiae* s.l.: an investigation into 'actives' prior to a
599 randomized controlled trial in Côte d'Ivoire. *Malaria Journal* **17**:374.

600 Oyewole, I. O., T. S. Awolola, C. A. Ibidapo, A. O. Oduola, O. O. Okwa, and J. Obansa. 2007. Behaviour and
601 population dynamics of the major anopheline vectors in a malaria endemic area in southern
602 Nigeria *Journal of Vector Borne Diseases* **44**:56 - 64.

603 Padonou, G. G., G. Gbedjissi, A. Yadouleton, R. Azondekon, O. Razack, O. Oussou, V. Gnanguenon, A. Rock,
604 M. Sezonlin, and M. Akogbeto. 2012. Decreased proportions of indoor feeding and endophily in
605 *Anopheles gambiae* sl populations following the indoor residual spraying and insecticide-treated
606 net interventions in Benin (West Africa). *Parasites & Vectors* **5**:262.

607 Pates, H., and C. Curtis. 2005. Mosquito behavior and vector control. *Annu. Rev. Entomol.* **50**:53-70.

608 Perandin, F., N. Manca, A. Calderaro, G. Piccolo, L. Galati, L. Ricci, M. C. Medici, M. C. Arcangeletti, G.
609 Snounou, G. Dettori, and C. Chezzi. 2004. Development of a Real-Time PCR Assay for Detection of
610 *Plasmodium falciparum*, *Plasmodium vivax*, and *Plasmodium ovale* for Routine Clinical Diagnosis.
611 *Journal of Clinical Microbiology* **42**:1214-1219.

612 Pulford, J., M. W. Hetzel, M. Bryant, P. M. Siba, and I. Mueller. 2011. Reported reasons for not using a
613 mosquito net when one is available: a review of the published literature. *Malaria Journal* **10**:83-
614 83.

615 Rajagopalan, P. K., P. Jambulingam, S. Sabesan, K. Krishnamoorthy, S. Rajendran, K. Gunasekaran, N. P.
616 Kumar, and R. M. Prothero. 1986. Population movement and malaria persistence in Rameswaram
617 Island: Foreword. *Social Science & Medicine* **22**:879-886.

618 Reddy, M. R., H. J. Overgaard, S. Abaga, V. P. Reddy, A. Caccone, A. E. Kiszewski, and M. A. Slotman. 2011.
619 Outdoor host seeking behaviour of *Anopheles gambiae* mosquitoes following initiation of malaria
620 vector control on Bioko Island, Equatorial Guinea. *Malaria Journal* **10**:184.

621 Ritmeijer, K., C. Davies, R. Van Zorge, S.-J. Wang, J. Schorscher, S. I. Dongu'du, and R. N. Davidson. 2007.
622 Evaluation of a mass distribution programme for fine-mesh impregnated bednets against visceral
623 leishmaniasis in eastern Sudan. *Tropical Medicine & International Health* **12**:404-414.

624 Riveron, J. M., M. Chiumia, B. D. Menze, K. G. Barnes, H. Irving, S. S. Ibrahim, G. D. Weedall, T. Mzilahowa,
625 and C. S. Wondji. 2015. Rise of multiple insecticide resistance in *Anopheles funestus* in Malawi: a
626 major concern for malaria vector control. *Malaria Journal* **14**:344.

627 Russell, T. L., N. W. Beebe, R. D. Cooper, N. F. Lobo, and T. R. Burkot. 2013. Successful malaria elimination
628 strategies require interventions that target changing vector behaviours. *Malaria Journal* **12**.

629 Russell, T. L., N. J. Govella, S. Azizi, C. J. Drakeley, S. P. Kachur, and G. F. Killeen. 2011. Increased proportions
630 of outdoor feeding among residual malaria vector populations following increased use of
631 insecticide-treated nets in rural Tanzania. *Malaria Journal* **10**:80.

632 Scott, J. A., W. G. Brogdon, and F. H. Collins. 1993. Identification of single specimens of the *Anopheles*
633 *gambiae* complex by the polymerase chain reaction. *The American Journal of Tropical Medicine*
634 *and Hygiene* **49**:520-529.

635 Service, M. 1993. Mosquitoes (Culicidae). In: *Medical Insects and Arachnids*. Lane RP, Crosskey RW (eds.)
636 Chapman and Hall, London.

637 Silver, J. B. 2008. Sampling Adults by Animal Bait Catches and by Animal-Baited Traps. In: *Mosquito*
638 *Ecology*. Springer, Dordrecht. Springer, Dordrecht:493-675.

639 Sougoufara, S., S. M. Diédhiou, S. Doucouré, N. Diagne, P. M. Sembène, M. Harry, J.-F. Trape, C. Sokhna,
640 and M. O. Ndiath. 2014. Biting by *Anopheles funestus* in broad daylight after use of long-lasting
641 insecticidal nets: a new challenge to malaria elimination. *Malaria Journal* **13**:125.

- 642 Spiers, A., T. Mzilahowa, D. Atkinson, and P. McCall. 2002. The malaria vectors of the lower Shire Valley,
643 Malawi. *Malawi Medical Journal* **14**:4-7.
- 644 Sternberg, E. D., K. R. Ng'habi, I. N. Lyimo, S. T. Kessy, M. Farenhorst, M. B. Thomas, B. G. J. Knols, and L.
645 L. Mnyone. 2016. Eave tubes for malaria control in Africa: initial development and semi-field
646 evaluations in Tanzania. *Malaria Journal* **15**:447.
- 647 Tirados, I., C. Costantini, G. Gibson, and S. J. Torr. 2006. Blood-feeding behaviour of the malarial mosquito
648 *Anopheles arabiensis*: implications for vector control. *Medical and Veterinary Entomology* **20**.
- 649 White, G. B., S. A. Magayuka, and P. F. L. Boreham. 1974. Comparative studies on sibling species of the
650 *Anopheles gambiae* Giles complex (Diptera:Culicidae): bionomics and vectorial activity of species
651 A and species B at Segera, Tanzania. *Bulletin of Entomological Research* **62**:215-317.
- 652 WHO. 2006. Malaria vector control and personal protection. World Health Organization Technical Report
653 Series, 936 .http://www.who.int/malaria/publications/atoz/who_trs_936/en/.
- 654 WHO. 2017. World Malaria Report. <http://www.who.int/malaria/publications/world-malaria-report-2017/en/>.
- 656 WHO. 2018. World Health Organization malaria report. <http://www.who.int/malaria/publications/world-malaria-report-2018/report/en/>.
- 658 Williams, Y. A., L. S. Tusting, S. Hocini, P. M. Graves, G. F. Killeen, I. Kleinschmidt, F. O. Okumu, R. G. A.
659 Feachem, A. Tatarsky, and R. D. Gosling. 2018. Chapter Six - Expanding the Vector Control Toolbox
660 for Malaria Elimination: A Systematic Review of the Evidence. Pages 345-379 in D. Rollinson and
661 J. R. Stothard, editors. *Advances in parasitology*. Academic Press.
- 662 Yohannes, M., and E. Boelee. 2012. Early biting rhythm in the afro-tropical vector of malaria, *Anopheles*
663 *arabiensis*, and challenges for its control in Ethiopia. *Medical and Veterinary Entomology* **26**:103-
664 105.
- 665 Yohannes, M., M. Haile, T. A. Ghebreyesus, K. H. Witten, A. Getachew, P. Byass, and S. W. Lindsay. 2005.
666 Can source reduction of mosquito larval habitat reduce malaria transmission in Tigray, Ethiopia?
667 *Tropical Medicine and International Health* **10**.

668 **Table and Figure legends**

- 669 Table 1: Mosquito collection during the dry and wet seasons.
- 670 Table 2: Effect of covariates on the biting activity of *An. gambiae* s.l. and *An. funestus* s.l.
- 671 Fig. 1: Typical house in the present study region (a) and HLC method (b)
- 672 Fig 2: Mean number of bites per hour by female anophelines both indoors and outdoors
673 during the dry and wet seasons.
- 674 Fig. 3: Mean number of bites (95% CI) per category by female *An. gambiae* s.l. (A) and
675 *An. funestus* s.l. (B) both indoors and outdoors during the dry and wet seasons.

676 Fig 4: Mean number of bites (95% CI) per category by female culicines both indoors
 677 and outdoors during the dry and wet seasons.

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679 Table 1: Mosquito collection during the dry and wet seasons

**Mosquito
collection**

	Indoors		Outdoors		Totals	
	Dry season	Wet season	Dry season	Wet season	Dry season	Wet season
No. of nights	72	72	72	72	144	144
<i>An. arabiensis</i>	4	18	13	36	17	54
<i>An. gambiae</i> s.s.	0	6	1	4	1	10
<i>An. arabiensis</i> / <i>An. gambiae</i> s.s (Hybrid)	0	0	0	1	0	1
<i>An. gambiae</i> s.l (no amplification)	1	1	3	3	4	4
<i>An. funestus</i> s.s.	10	16	9	5	19	21
<i>An. funestus</i> s.l (no amplification)	6	1	0	0	6	1
<i>An. tenebrosus</i>	25	1	18	9	43	10
Female culicines	400	568	517	721	917	1289
Male Anophelines	2	0	4	1	6	1
Male culicines	11	10	8	7	19	17

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686 Table 2: Effect of covariates on the biting activity of *An. gambiae* s.l. and *An. funestus* s.l.

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Treatment	Dry season		Wet season	
	RR	95% CI	RR	95% CI
<i>An. gambiae</i> s.l.				
Indoors	0.29	0.11-0.80	0.57	0.35-0.93
Outdoors	Ref	–	–	–
People that slept in the house the previous night	0.86	0.61-1.21	1.05	0.83-1.32
Mosquito control-bed-net	1.83	0.4-8.43	0.82	0.43-1.57
Mosquito control-none	Ref	–	Ref	–
Cooking inside the house	-	-	2.27	0.63-8.25
Cooking on the veranda	0.83	0.23-2.97	1.89	0.70-5.08
Cooking outside, within 2m of the house	1.02	0.34-3.08	1.27	0.47-3.40
Cooking outside, more than 2m from the house	Ref	–	Ref	–

Cow	1.27	0.16-9.91	1.68	0.78-3.62
Goat	-	-	1.09	0.56-2.14
Chicken	1.77	0.66-4.8	1.19	0.71-2.01

***An. funestus* s.l.**

Indoors	1.78	0.79-4.02	3.4	1.25-9.22
Outdoors	Ref	–	Ref	–
People that slept in the house the previous night	0.47	0.30-0.74	0.57	0.32-1.02
Mosquito control-bed-net	1.40	0.31-6.44	1.39	0.33-5.85
Mosquito control-none	Ref	–	Ref	–
Cooking inside the house	2.44	0.56-10.69	0.21	0.02-2.23
Cooking on the veranda	0.63	0.12-3.41	0.48	0.15-1.55
Cooking outside, within 2m of the house	1.39	0.47-4.07	0.17	0.04-0.71
Cooking outside, away from 2m of the house	Ref	–	Ref	–
Cow	1.57	0.34-7.38	2.73	0.70-10.59
Goat	0.90	0.31-2.63	0.61	0.11-3.40
Chicken	7.42	2.61-21.11	3.85	1.43-10.34

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691 Kabaghe, A. N., M. G. Chipeta, S. Gowelo, M. Mburu, Z. Truwah, R. S. McCann, M. van Vugt, M. P.
692 Grobusch, and K. S. Phiri. 2018. Fine-scale spatial and temporal variation of clinical malaria
693 incidence and associated factors in children in rural Malawi: a longitudinal study. *Parasites &*
694 *vectors* **11**:129.

695 Monroe, A., S. Moore, H. Koenker, M. Lynch, and E. Ricotta. 2019. Measuring and characterizing night
696 time human behaviour as it relates to residual malaria transmission in sub-Saharan Africa: a
697 review of the published literature. *Malaria Journal* **18**:6.

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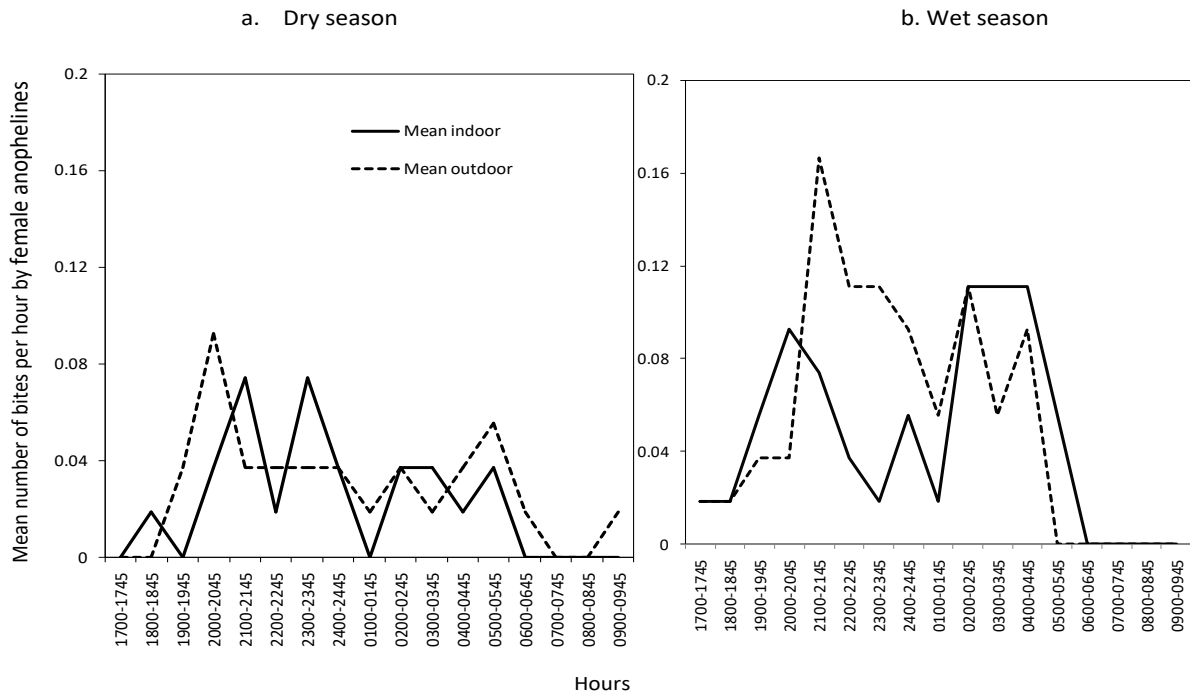
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704 Fig. 1: Typical house in the present study region (a) and HLC method (b)

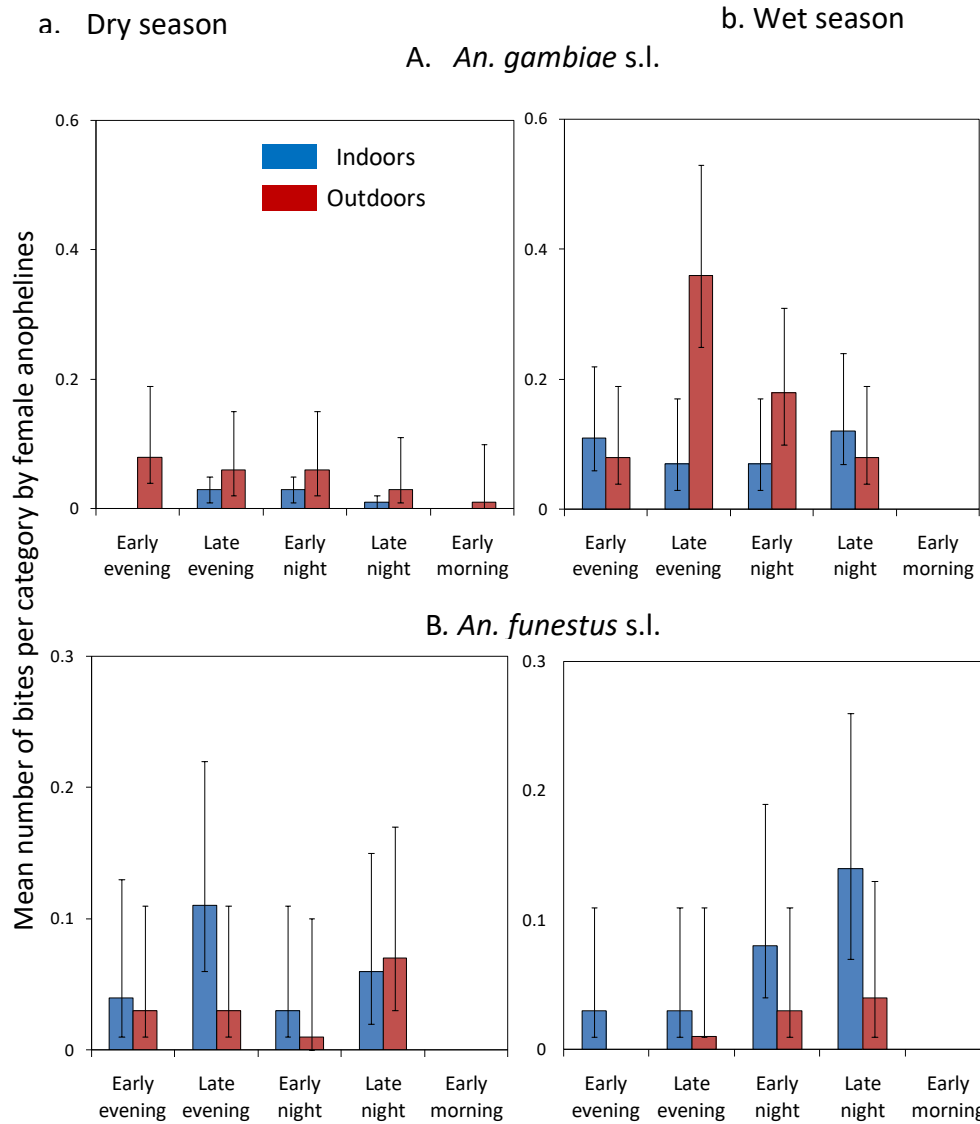
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708 during the dry and wet seasons.

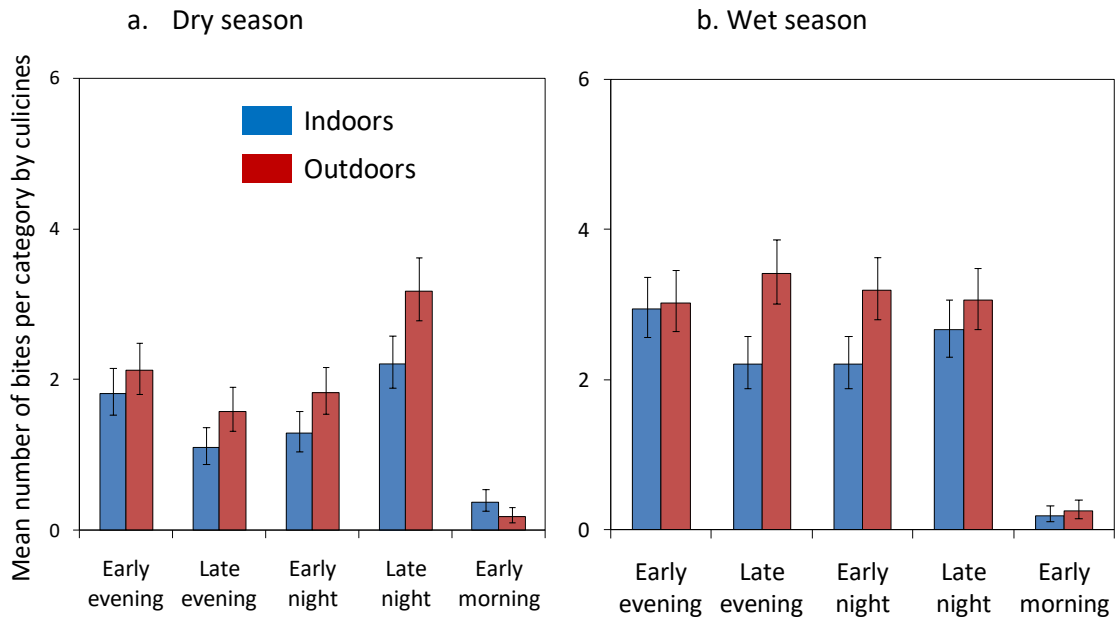


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710 Fig. 3: Mean number of bites (95% CI) per category by female *An. gambiae* s.l. (A) and

711 *An. funestus* s.l. (B) both indoors and outdoors during the dry and wet seasons.

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714 Fig 4: Mean number of bites (95% CI) per category by female culicines both indoors

715 and outdoors during the dry and wet seasons.

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