Biting time of day in malaria mosquitoes is modulated by nutritional status

Catherine E. Oke[1], Samuel S. C. Rund[3,4], Maxwell G. Machani[5], Abdul Rahim Mohammed Sabtiu[6], Yaw Akuamoah-Boateng[6], Yaw A. Afrane[6], Sarah E. Reece[1,2]

[1]Institute of Ecology and Evolution and [2]Institute of Immunology and Infection Research, School of Biological Sciences, University of Edinburgh, Edinburgh, UK; [3]Center for Research Computing and [4]Department of Biological Sciences, University of Notre Dame, Notre Dame, USA; [5]Entomology Department, Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya; [6]Department of Medical Microbiology, University of Ghana Medical School, College of Health Sciences, University of Ghana, Accra, Ghana.

Corresponding author:

Catherine E. Oke, Email: catherine.oke@ed.ac.uk

Abstract

Background: Transmission of vector-borne pathogens follows daily rhythms, occurring at the time of day that vectors forage for blood. Control measures such as insecticide-treated bed nets (ITNs) have been particularly successful for reducing malaria transmission, because they exploit the nocturnal biting behaviour of the *Anopheles* spp. that vector malaria. However, shifts in biting behaviour to earlier or later hours when people are unprotected can undermine the efficacy of ITNs. Despite the implications for malaria transmission, the mechanisms underlying these shifts remain poorly understood. Because food availability mediates activity and foraging rhythms, and ITNs block access to human blood as a food source, we hypothesized that nutritional deprivation could cause mosquitoes to shift their biting behaviour to earlier or later in the diel cycle.

Methods: We provided female *Anopheles gambiae s.l.* mosquitoes with a blood meal on day 3 post-emergence, and access to one of three feeding treatments that varied in nutritional resources: (i) 0.5% sucrose, (ii) 10% sucrose, or (iii) 10% sucrose plus an additional blood meal on day 6. We released mosquitoes into a semi-field system on day 10 with human-mimic traps to investigate how food availability impacted the time of day that mosquitoes host seek.

Results: Nutritional resources determine both the likelihood and time of day that host seeking occurs. Specifically, low-resourced mosquitoes were 2-3 fold more likely to host seek overall, and 5-10 fold more likely to host seek at an earlier time of day than well-resourced mosquitoes (fed 10% sucrose with and without an additional blood meal), which predominantly sought a host in the second half of the night time.

Conclusions: We reveal that mosquito nutritional condition drives plasticity in biting time of day, suggesting it is an underappreciated contributor to residual malaria transmission. Furthermore, our results suggest that targeting mosquito nutrition (e.g. sugar-baited traps) could influence mosquito behaviour in ways that affect the success of ITNs. More broadly, understanding the drivers of biting time of day variation is crucial for the future success of vector control tools and controlling malaria transmission.

Key words:

Anopheles, transmission, vector behaviour, daily rhythms, food availability

Background

The transmission of vector-borne diseases (VBDs), such as malaria and dengue, relies on the foraging rhythms of insect vectors, which take up pathogens from an infected vertebrate host during a blood meal and transmit them to a new host during a subsequent blood-feeding event (1,2). For example, *Plasmodium* parasites, the causative agent of malaria, are vectored between humans by *Anopheles* spp. mosquitoes, which are generally nocturnally active and preferentially bite between the hours of 11pm and 4am (*i.e.* the 'classical' time window) (3–5). Insecticide-treated bed nets (ITNs) exploit these rhythmic foraging behaviours, and are consequently one of the most effective methods for curbing the spread of malaria (6,7), averting 68% of deaths since 2000 (8). Despite this success, residual transmission occurs, in part due to the evolution of physiological insecticide resistance (e.g. biochemical and morphological modifications) and behavioural resistance in vector populations (9). In particular, millions of clinical malaria cases are predicted to be occurring annually due to mosquitoes altering the time of day they bite (10).

There are increasing reports that *Anopheles* spp. are shifting their biting time of day to earlier in the evening ('early' biting) or later in the morning ('late' biting) when humans are unprotected by ITNs (4,5,11–15), undermining the success of ITNs, especially in high-coverage regions. However, the extent to which mosquitos can shift biting time of day and the range of potential drivers are poorly understood. *Anopheles* spp. biting rhythms are weakly heritable, so shifts in biting time of day are expected to be predominantly driven by phenotypic plasticity (16). Phenotypic plasticity evolves to enable organisms to alter (via epigenetic mechanisms, for example) aspects of phenotype, including behaviours, in manners that maximise fitness in response to environmental variation (17). Furthermore, variation in physiological condition and resource limitation can cause animals to alter their foraging rhythms (18) in adaptive (i.e. fitness enhancing) ways (19). For example, low food availability promotes a shift to diurnal foraging patterns in small nocturnal rodents, which minimises energy loss by remaining in burrows during particularly cold nights (20,21). Similarly, mosquitoes may garner greater benefits from shifting biting time of day when they are in poor nutritional condition, because the fitness benefits associated with acquiring a blood meal at suboptimal times of day

outweigh the increased risks of desiccation and predation. Such context-dependent variation in the costs and benefits of plastic shifts in biting time may explain why biting times have seemingly not significantly changed in some *Anopheles* spp. populations despite high ITN use (22,23), and why the evolution of behavioural resistance varies across populations in manners that correlate with the abundance of sources of nutrition for mosquitoes (24).

Mosquitoes rely on blood and sugar meals for reproduction and survival, with female mosquitoes utilising proteins in blood for egg production, and using sugar sources to accumulate energy reserves for powering energetically demanding processes, such as flight (25). Their feeding ecology is under circadian clock control (26), with females seeking blood predominantly at night and approximately every 3 days to fuel gonotrophic cycles of egg development and oviposition (27), and feeding on sugar sources in between these cycles to promote survival until future biting opportunities (28,29). Mosquitoes are likely to vary in their nutritional status because the type and abundance of sugar sources available to mosquitoes varies across habitats and seasons (25,30), and vector control tools such as ITNs can limit access to protein rich blood meals (31). In response to variation in food sources, mosquitoes exhibit plasticity in multiple feeding behaviours. If a preferred host is not found, mosquitoes are more likely to feed on sugar (32) or choose an alternative host (23), and will feed on low-sugar plant tissues when sugar-rich plants are unavailable (33). However, despite the importance of mosquito foraging rhythms for malaria transmission and that physiological condition alters foraging rhythms in other taxa, the effect of nutritional resource availability on mosquito biting time of day is unknown.

Here, we perturb the nutritional resources provided to adult mosquitoes to test whether nutritional constraints cause a shift in biting time of day. Using a semi-field system, we simulated scenarios in which mosquitoes have taken an initial blood meal (simulating the point at which malaria parasites would be acquired) and then resided in environments that provide varying access to further blood meals and sugar concentrations. Our three scenarios represented mosquitos that: (i) were unsuccessful at acquiring a subsequent blood meal and only have access to low levels of sugar (0.5% sucrose), (ii) were unsuccessful at acquiring a subsequent blood meal but have access to high levels of sugar (10% sucrose), and (iii) successfully acquired a subsequent blood meal and have access to high levels of sugar (10% sucrose plus an additional blood meal). We hypothesised that nutritionally deprived mosquitoes, provided with limited sugar and blood, would be more likely to host seek and

103 shift their biting behaviour to earlier or later in the diel cycle, compared to better-resourced
104 mosquitoes. We also supplemented our data with a preliminary field collection to investigate if biting
105 time of day of wild-caught adult mosquitoes correlates with nutritional condition. We discuss the
106 potential outcomes of shifts in biting time of day in response to resource availability for understanding
107 residual transmission patterns, and how this may impact parasite development and transmission
108 between hosts.

109

110 **Methods**

111 We performed a semi-field experiment to assess if mosquito nutritional status impacts host seeking at
112 different times of day. We reared wild-caught larvae, and maintained blood fed female *Anopheles*
113 *gambiae s.l.* mosquitoes from the F_2 generation on diets that varied in nutritional resources, before
114 releasing them into an enclosed semi-field system with traps mimicking human odour. The three traps
115 were programmed to separately capture mosquitoes biting at early (evening), classical (during the
116 night) and late (morning) times of day. In addition, we collected wild adult *Anopheles* mosquitoes over
117 the course of one night, to test for correlations between mosquito nutritional status and the time of day
118 they attempted to bite.

119

120 *Study sites*

121 For the semi-field experiment, we collected *Anopheles gambiae s.l.* larvae from three urban sites
122 across the Greater Accra region of Ghana (Tuba, 5° 30' 47" N 0° 23' 16" W; Teshie, 5° 35′ 0″ N, 0° 6′
123 0″ W; East Legon, 5° 38' 16.39" N, 0° 9' 40.33" W) during the dry season in Dec 2023-Jan 2024
124 (Additional file 1, Fig. S1). Adult *An. gambiae s.l.* collections were conducted in Teshie during the dry
125 season in February 2024. The most abundant malaria vector species in these three sites are *An.*
126 *gambiae s.s*. and *An. coluzzii*, which both have a high Human Biting Index and predominantly bite
127 between 10pm and 4am (34,35). We transported larvae to the insectary at the Department of Medical
128 Microbiology, University of Ghana Medical School, Accra, and raised them to adults. We conducted
129 mosquito behavioural experiments in January-February 2024 in an enclosed semi-field system (the
130 “MalariaSphere”, (38,39)) at the University of Ghana campus in Legon, Accra. The average (± SEM)

131 minimum and maximum daily temperatures during the experimental releases were 25.8±0.6°C and
132 34.5±0.4°C respectively (40). The enclosure was 5.8 x 4.2 x 2.8m and covered in an insect-proof
133 screen to prevent mosquito escape and/or entry from the external environment.

134

135 *Semi-field host seeking experiment*

136 To ensure sufficient sample sizes, we conducted semi-field experiments using the F_2 progeny of wild-
137 caught *An. gambiae s.l.* larvae (Fig 1A). We pooled collected larvae and then transferred them into
138 large tubs of water. We kept larvae at similar densities throughout the rearing procedure and fed them
139 on TetraMin Baby fish food. The emerged adults formed the F_0 generation, which we maintained in
140 cages under standard rearing conditions (26±2°C, 80% relative humidity with a 12L:12D cycle), with
141 *ad libitum* access to 10% sucrose solution in water. We provided a blood meal via direct-feeding (36),
142 and placed soaked cotton wool in petri dishes into the cages for egg laying. We reared the resulting
143 F_1 generation larvae and adults under the same conditions, and their eggs were used to produce the
144 experimental F_2 generation. On day 1 post-emergence of F_2 adults, we randomly allocated female
145 mosquitoes to three experimental cages (n=100-150 per cage) and allocated each cage to one of
146 three feeding treatments (Fig.1A): (i) 0.5% sucrose in water (0.5% suc), (ii) 10% sucrose in water
147 (10% suc) or (iii) 10% sucrose in water plus an additional blood meal on day 6 post-emergence (10%
148 suc + bm). We maintained all treatment groups on their respective sucrose solutions *ad libitum* from
149 day 1 post-emergence until the host seeking experiment. In addition, to mimic mosquito life history in
150 nature, we provided all groups with an initial blood meal on day 3 post-emergence. We gave
151 mosquitoes the opportunity to lay eggs after all blood meals. On day 9-10 post-emergence, we
152 transferred mosquitoes from each cage to paper cups (n=70-100 per cup), where they were housed
153 for six hours with access to water only prior to assaying biting time of day. We reared six batches of
154 mosquitoes to conduct a total of six semi-field system releases.

155 We placed three BG-Sentinel traps (Biogents, Germany) in the semi-field enclosure, with an MB5
156 human-mimic lure developed to attract anopheline mosquitoes (Biogents, Germany) and a CO_2 flow
157 of approximately 21g/hour, to mimic human scent and breath. We programmed each trap to turn on at
158 either 6pm-10pm to capture 'early' biters, 11pm-3am to capture 'classical' biters, or 4am-8am to
159 capture 'late' biters (Fig. 1B-D). We chose these timings because mosquitoes in this region

predominantly bite between 11pm and 4am, but biting can occur from approximately 6pm and persist into the morning beyond 6am (34). Thus, these time windows also correspond to when people are typically indoors and protected by ITNs (classical), or are outdoors and unprotected (early and late) (37). CO_2 flow was controlled with BG-CO_2 timers (Biogents, Germany), ensuring CO_2 was only emitted when the respective trap was switched on.

Within the MalariaSphere, released mosquitoes could access resting sites (four clay pots around the enclosure). We did not provide water or food sources because this would have confounded the experimental treatment groups. We colour-marked mosquitoes from each feeding treatment using aerosolised fluorescent powder (FTX series, Swada London), applied following Machani et al (38). We rotated the colour of each treatment group across sequential releases to ensure any differential impacts of colour on mosquito behaviour did not confound treatment groups. We carried out six releases over a period of three weeks, with mosquitoes being released into the enclosure between 5pm-5:30pm, just prior to dusk at 6pm (n=235-300 mosquitoes per release). A period of at least 48 hours between each release and checking all resting sites with a Prokopack aspirator ensured that any remaining untrapped mosquitoes in the MalariaSphere had been removed or died before the next release. We collected mosquitoes from the traps, at 8am the day after the release, then cold-anaesthetised and counted the number caught in each trap.

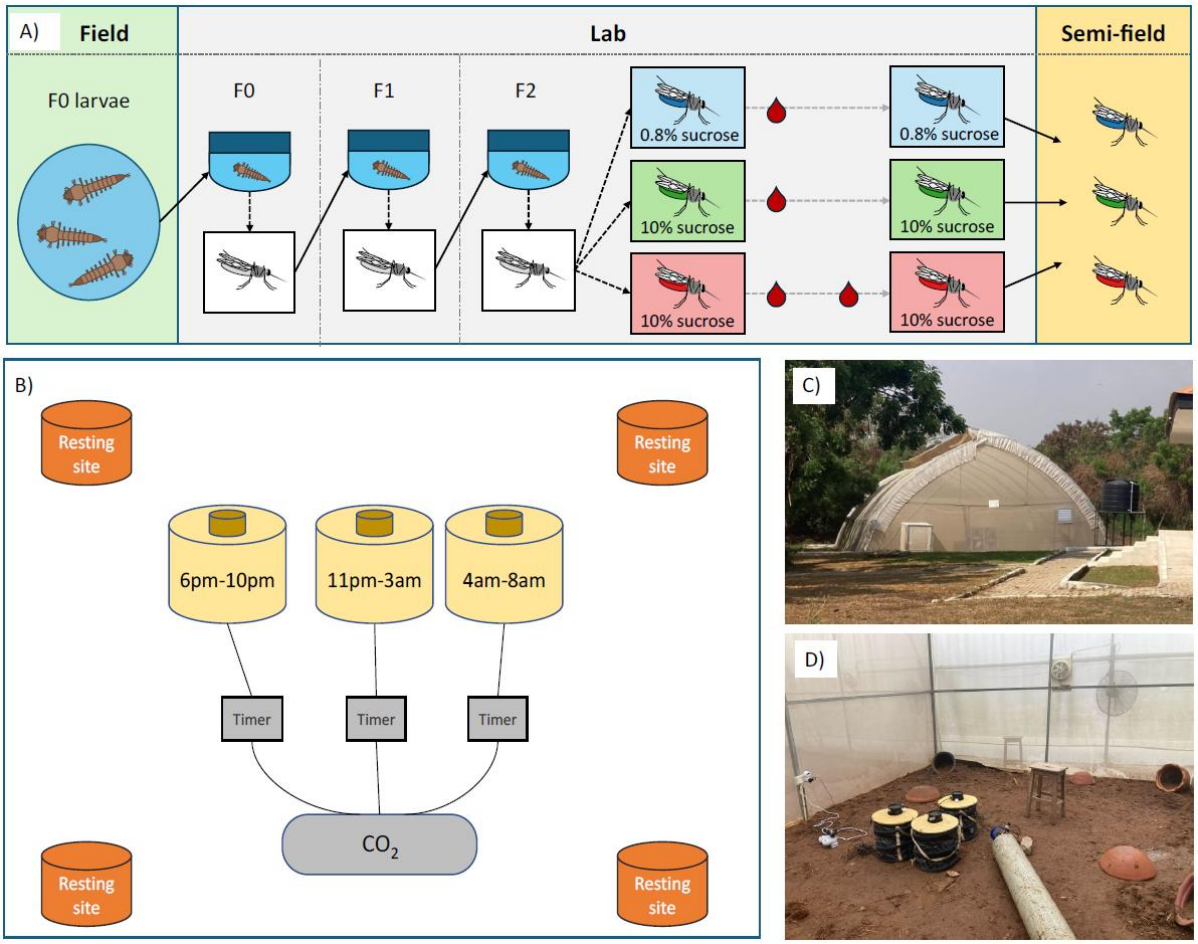


Figure 1. The experimental design for the semi-field behavioural experiment. Schematic diagrams of the entire experiment, from wild larvae collection to release of mosquitoes reared under the three feeding treatments into the semi-field enclosure (A), and the semi-field assay set-up (B), featuring four resting sites for mosquitoes, and traps baited with human odour which were programmed to turn on at 6pm-10pm, 11pm-3am and 4am-8pm respectively. These windows correspond to approximately ZT12-ZT16, ZT17-ZT21, and ZT22-ZT2 respectively, where Zeitgeber time (ZT) defines the hours since lights on and ZT0/24 is dawn. Each trap was linked to the CO_2 source, using separate BG-CO_2 timers to ensure the CO_2 was only flowing when the corresponding trap that was switched on. Photographs show the semi-field facility (C) and the behavioural assay set-up (D).

Wild mosquito collections

In addition to the experiment, we also conducted a preliminary investigation into correlations between biting time of day and nutritional content in wild mosquitoes, by collecting adult mosquitoes (n=95)

193 overnight between 6pm and 7am using the human landing catch (HLC) technique. This technique
194 involves trained human collectors exposing their lower legs and collecting mosquitoes that land on
195 them before they bite, and is the gold standard for investigating human exposure to disease vectors
196 (39). Every hour, we snap-froze collected mosquitoes on dry ice and identified specimens to genus
197 level according to morphological characteristics (40), noting the number of *Anopheles* spp.
198 mosquitoes collected per hourly bin. We placed individual anopheline mosquitoes in 1.5ml
199 microcentrifuge tubes and stored them on dry ice, transferring them to a -20°C freezer after the
200 overnight collection period had ended.

201

202 *Nutrition assays*

203 Immediately prior to each semi-field release in the experiment, we collected a subset of mosquitoes
204 from each feeding treatment group (n=14-19 per group, total n=48) to confirm our resource
205 perturbations impacted nutritional status. We also quantified mosquito nutritional status in individual
206 wild-caught mosquitoes (n=66). Mosquitoes were frozen at -20°C for lipid, glycogen and total sugar
207 analyses using modified Van Handel protocols (41–43). Firstly, we removed a wing from each
208 mosquito to quantify body size. Wing length is a commonly used proxy for adult body size because
209 dry weight and wing length are highly correlated (44), and is included in statistical analyses to ensure
210 any size variation does not confound differences in nutritional content. We lysed individual
211 mosquitoes in 100µl 2% sodium sulphate and added 750µl of 1:2 chloroform:methanol. We
212 centrifuged samples at 12000 rpm for 3 minutes; the supernatant was used for lipid and total sugars
213 analysis, and the precipitate was used for glycogen analysis. We conducted lipid, glycogen and total
214 sugars assays following Oke et al (45). For wild-caught mosquitoes, we used 250µl supernatant for
215 total sugars analysis.

216

217 *Statistical analysis*

218 We used R v. 4.1.3 to perform data analysis. We confirmed that adult body size did not differ across
219 the treatments using a linear model with wing length, mosquito release batch and their interaction as
220 main effects (Additional file 1, Fig. S2). To analyse nutritional content (lipid, glycogen, total sugars) of

221 individual mosquitoes, we used linear mixed models with feeding treatment as a main effect and
222 mosquito release batch as a random effect, accounting for any within treatment variation in body size.
223 We used binomial generalised linear mixed models (glmm) with mosquito release as a random effect
224 to account for any between-release variation to investigate how feeding treatment impacted the
225 overall proportion of mosquitoes caught across the night, with feeding treatment as a main effect, and
226 how feeding treatment impacted the proportion of mosquitoes caught across the different biting time
227 of day windows, with feeding treatment, trap time and their interaction as main effects. We estimated
228 proportion of caught mosquitoes and relative odds ratios (OR) ± SE from models using the *emmeans*
229 (46) package. We square root transformed nutrition data to meet assumptions of normality and
230 homogeneity of variance. We minimised all models using likelihood ratio tests and AICc for non-
231 nested models, and confirmed model assumptions using the *easystats* (47) package. To account for
232 differences between mosquito release batches, we present estimated marginal means ± SEM
233 (*emmeans* package), predicted from models. Whenever the minimised model contained a significant
234 effect of feeding treatment, we conducted post hoc pairwise comparisons using the Tukey method
235 with the *emmeans* package.

236 To investigate how nutritional content between field-caught and lab-reared mosquitoes varied, we
237 used linear models to compare between the field-caught, 0.5% suc, 10% suc and 10% suc + bm
238 groups. We performed a chi-square test to investigate if the number of field-caught mosquitoes
239 differed throughout the night by grouping hourly bins into 'early' biters (6pm-11pm), 'classical' biters
240 (11pm-4am) and 'late' biters (4am-7am). Using linear models, we analysed the nutritional content
241 (lipid, glycogen, total sugars) of individual wild-caught mosquitoes with biting time of day as an
242 unordered factor, and assessed the correlation between wing length and nutritional content. Nutrition
243 data were square root transformed to meet assumptions of normality and homogeneity of variance.
244 We conducted post hoc pairwise comparisons using the Tukey method with the *emmeans* package to
245 compare nutritional content between field-caught and lab-reared mosquitoes.

246

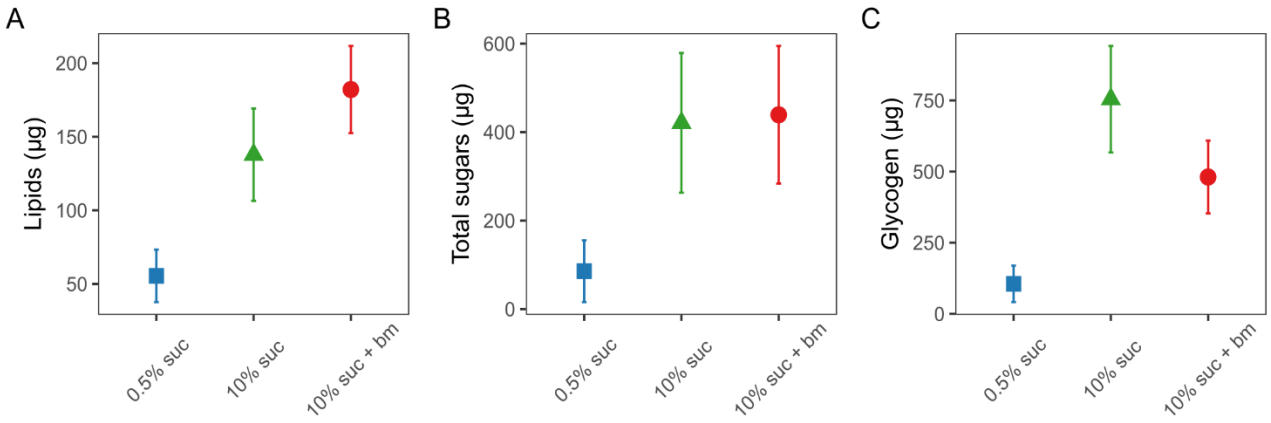
247 **Results**

248 *Resource availability perturbs mosquito nutritional status*

249 First, we confirmed that mosquitoes fed on the lowest concentration of sucrose (0.5%) had lower
250 nutritional reserves than mosquitoes fed 10% sucrose with or without an additional blood meal.
251 Mosquitoes fed on 0.5% sucrose had significantly lower lipid levels (55.5±17.8μg), approximately 2.5-
252 fold lower than those fed with 10% sucrose (138±31.4μg) and 3.3-fold lower than those fed 10%
253 sucrose plus an additional blood meal (182±29.6μg) (χ^2_2=17.6, $p < 0.001$; Fig. 2A, Table 1). A similar
254 pattern was observed for total sugars: mosquitoes fed with 0.5% sucrose had the lowest total sugar
255 levels (85.6±69.9μg), approximately 5-fold lower than those given 10% sucrose (420±158μg) and
256 10% sucrose plus an additional blood meal (439±156μg) (χ^2_2=30.8, $p < 0.001$; Fig. 2B, Table 1).
257 Finally, mosquitoes fed with 0.5% sucrose had the lowest glycogen levels (105±64.2μg),
258 approximately 7-fold lower than those given 10% sucrose (754±187μg), with mosquitoes given 10%
259 sucrose plus an additional blood meal exhibiting intermediate levels (481±128μg) (χ^2_2=21.7, $p <$
260 0.001; Fig. 2C, Table 1).

261

262



263

264 **Figure 2.** Concentrations (μg) per mosquito of lipids (A), total free sugars (B) and glycogen (C) in
265 individual mosquitoes under differing feeding treatments on day 10 post-emergence. Nutritional
266 perturbations began on day 1 post-emergence and all treatment groups received a blood meal on day
267 3 post-emergence (with an additional blood meal given to the 10% suc + bm group on day 6). Data
268 presented are estimated marginal means ± SEM.

269

270

271 **Table 1. Post hoc pairwise comparisons for nutritional content of mosquitoes provided with**

272 **different feeding treatments.** Significant p-values (<0.05) are highlighted in bold.

		Test statistic	*p-value*
Lipids	0.5% suc – 10% suc	*t* = 2.75	**0.02**
	0.5% suc – 10% suc + bm	*t* = -4.54	**<0.001**
	10% suc – 10% suc + bm	*t* = -1.16	0.48
Total sugars	0.5% suc – 10% suc	*t* = 5.13	**<0.001**
	0.5% suc – 10% suc + bm	*t* = -6.13	**<0.001**
	10% suc – 10% suc + bm	*t* = -0.21	0.98
Glycogen	0.5% suc – 10% suc	*t* = 4.66	**<0.001**
	0.5% suc – 10% suc + bm	*t* = -3.69	**0.002**
	10% suc – 10% suc + bm	*t* = 1.55	0.28

273

274

275 *Resource availability affects host seeking tendency and biting time of day*

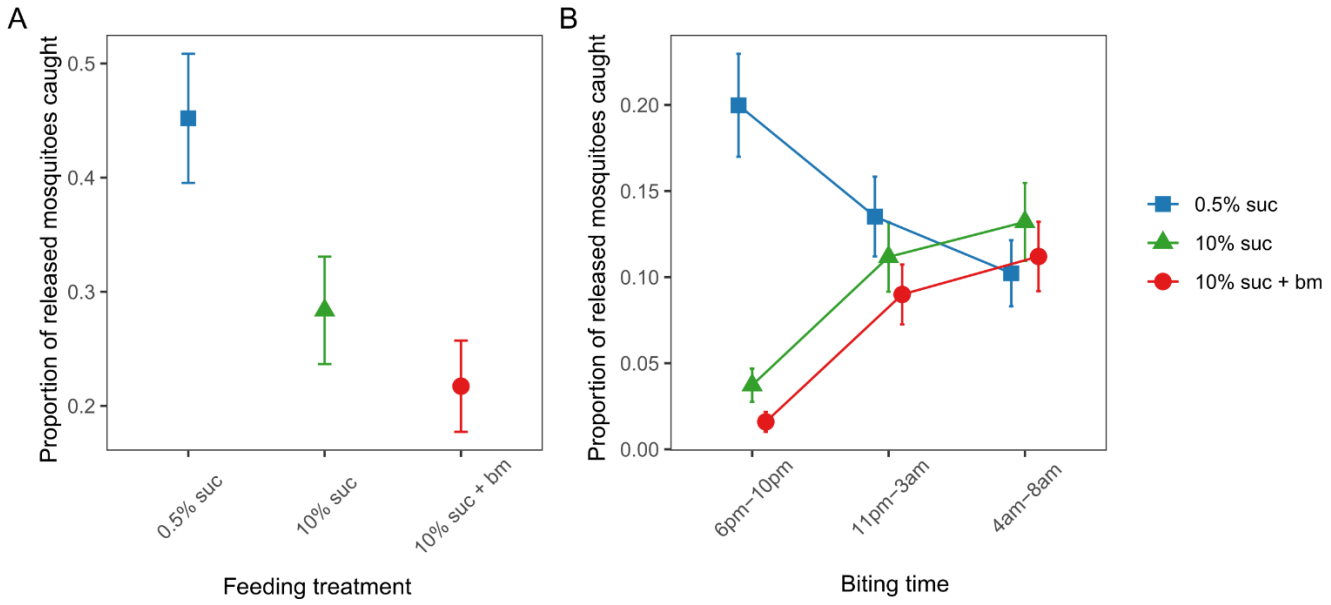
276 Across all six releases and trapping times of day, the total mosquitoes trapped ranged from 21% to
277 49%, with a mean of 32.4% of mosquitoes caught in the traps. The likelihood of host seeking at any
278 time of day correlated negatively with the level of nutritional resources provided (χ^2_2=65.8, p < 0.001;
279 Fig. 3A). Specifically, mosquitoes fed 0.5% sucrose were 2-fold more likely to be trapped than those
280 fed 10% sucrose (odds ratio (OR): 2.08±0.28) and 3-fold more likely than those fed 10% sucrose plus
281 an additional blood meal (OR: 2.97±0.42). Mosquitoes fed only 10% sucrose were 1.4-fold more likely
282 to be trapped than those fed 10% sucrose plus an additional blood meal (OR: 1.43±0.21).

283

284 We also found that nutritional resources differentially affected the time of day of host seeking
285 (interaction between treatment and trapping time: χ^2_4=95.4, p < 0.001; Fig 3B). The majority of early
286 biting mosquitoes were from the 0.5% sucrose group, whereas mosquitoes with more resources were
287 more likely to be caught at classic and late times of day. Specifically, mosquitoes fed 0.5% sucrose
288 were approximately 5- and 10-fold more likely to be caught in the early biting window (20±2.9%) than
289 those fed with 10% sucrose (3.7±0.9%) and 10% sucrose plus an additional blood meal (1.6±0.5%),

290 respectively. At the classical and late trapping times of day, the proportion of mosquitoes fed 0.5%
291 sucrose caught was similar to mosquitoes fed 10% sucrose with or without an additional blood meal.
292 Mosquitoes fed 10% sucrose with or without an additional blood meal followed similar temporal
293 patterns across all trapping times of day, with approximately 4-fold more of these mosquitoes being
294 trapped during the classical (10% sucrose: 11±2.0%, 10% sucrose + bm: 9.0±1.7%) and late biting
295 windows (10% sucrose: 13±2.3%, 10% sucrose + bm: 11±2.0%) than during the early biting window
296 (10% sucrose: 3.7±0.9%, 10% sucrose + bm: 1.6±0.5%).

297



298

299 **Figure 3.** The proportion of released mosquitoes from each feeding treatment that were trapped
300 across the entire night (A) and that were caught in the evening (early, 6pm-10pm), classical night time
301 biting window (11pm-3am) and morning (late, 4am-8am) biting (B). Data presented are estimated
302 marginal means ± SEM.

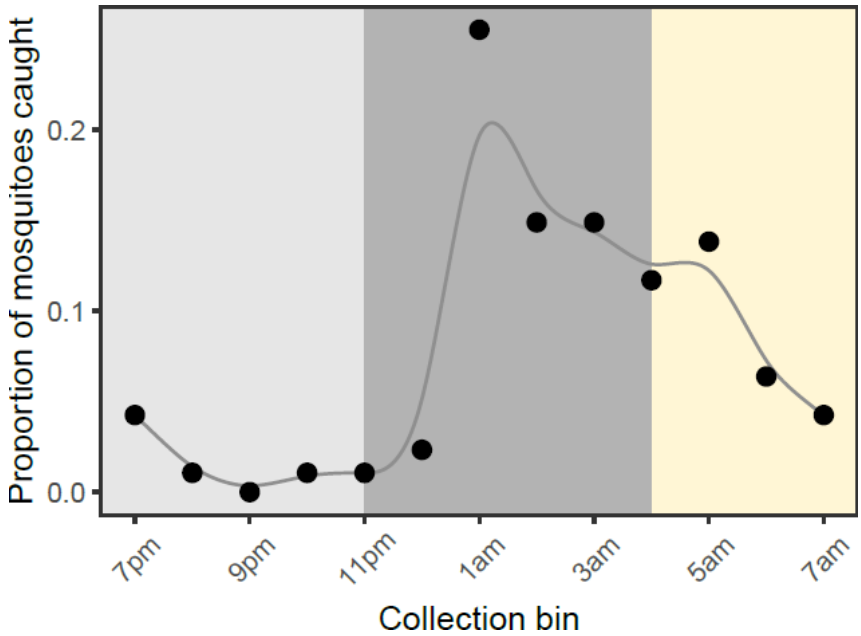
303

304 *Biting time distribution and nutritional status of wild host-seeking mosquitoes*

305 We conducted a preliminary collection of wild host seeking *An. gambiae s.l.* mosquitoes to assess
306 whether mosquito nutritional status correlates with biting time of day. We caught a total of 95 wild host
307 seeking mosquitoes during our overnight collection, with a peak in biting between 12am-1am. We

308 grouped hourly bins into ‘early’ biters (6pm-11pm), ‘classical’ biters (11pm-4am) and ‘late’ biters (4am-
309 7am) and found that there were differences in the number of mosquitoes caught across these
310 windows. Specifically, 68% of mosquitoes were caught during the classical biting window, compared
311 to 7% and 24% caught during the evening and morning windows respectively, confirming that this
312 population predominantly bite during the ‘classical’ time of day (χ^2_2= 45.0, $p < 0.001$; Fig 4).

313



314

315 **Figure 4.** The proportion of wild adult *An. gambiae s.l.* mosquitoes caught by HLC collections across
316 hourly bins between 6pm and 7am. Early, classical, and late bins are indicated by shaded regions
317 from left to right. To highlight how the pattern changes over the course of the night, data points are
318 connected by an X-spline.

319

320 Although wild mosquitoes varied in their nutritional content, we found no differences between lipid
321 ($F_{9,56}$ = 0.75, p = 0.66), sugars ($F_{9,56}$ = 1.06, p = 0.40) or glycogen ($F_{9,56}$ = 0.40, p= 0.93) contents
322 across hourly bins (Additional file 1, Fig S3). However, we found that wild host-seeking mosquitoes
323 had similar nutritional reserves to our experimental mosquitoes fed a 0.5% sucrose diet (estimated
324 marginal means for field-caught mosquito lipids: 39.7±5.5µg, sugars: 65.2±13µg, and glycogen:
325 51.4±11µg), but had significantly lower reserves than mosquitoes fed 10% sucrose and 10% sucrose

326 plus an additional blood meal (lipid: $F_{3,110}$=24.8, p<0.001; sugars: $F_{3,110}$=25.9, p<0.001; glycogen:
327 $F_{3,110}$=58.1, p<0.001; Table 2).

328

329 **Table 2. Post hoc pairwise comparisons of the nutritional content of field-caught mosquitoes**
330 **versus. lab-reared mosquitoes on different feeding treatments.** Significant p-values (<0.05) are
331 highlighted in bold.

		Test statistic	***p-value***
Lipids	field – 0.5% suc	*t* = -1.26	0.59
	field – 10% suc	*t* = -4.83	**<0.001**
	field – 10% suc + bm	*t* = -7.93	**<0.001**
Total sugars	field – 0.5% suc	*t* = -1.14	0.67
	field – 10% suc	*t* = -4.96	**<0.001**
	field – 10% suc + bm	*t* = -8.07	**<0.001**
Glycogen	field – 0.5% suc	*t* = -1.97	0.21
	field – 10% suc	*t* = -11.3	**<0.001**
	field – 10% suc + bm	*t* = -8.87	**<0.001**

332

333

334 **Discussion**

335 By altering sugar provision and access to blood meals, and using traps that mimic human breath and
336 scent, we find that resource availability modulates the time of day that *Anopheles gambiae s.l.*
337 mosquitoes in Ghana search for a blood meal. Mosquitoes on a low sucrose diet had significantly
338 lower levels of lipids, glycogen and sugar, and were 5-10 fold more likely to host seek in the evening
339 (early) than mosquitoes on a high sucrose diet with or without a blood meal (Fig. 2, 3B). In contrast,
340 better-resourced mosquitoes were most likely to host seek during the classical biting time window and
341 into the morning (Fig. 2, 3B). We also find that as resources increased, the overall tendency to host
342 seek decreased; mosquitoes on a low sucrose diet were 2-fold more likely to be caught compared to
343 mosquitoes given a higher concentration of sucrose, and 3-fold more likely than those with access to
344 10% sucrose and an additional blood meal (Fig. 3A). Finally, to probe the ecological relevance of our

345 experimental results, we also undertook a trapping session for wild host-seeking *An. gambiae s.l.*
346 mosquitoes. While the nutritional content of wild mosquitoes varied, we found that levels were similar
347 to the profiles of our low-resourced experimental mosquitoes fed 0.5% sucrose (Fig. 4). Given that
348 mosquito flight during host seeking is energetically consuming (48), this suggests that our
349 experimental feeding treatments resulted in mosquitoes with nutritional contents within an ecologically
350 realistic range.

351 Foraging at unusual times of day can be detrimental because it exposes organisms to rhythmic
352 environmental risks, such as the active phase of predators and suboptimal temperature/humidity, and
353 misalignment between metabolic and other circadian rhythms may impair digestion and metabolism
354 (1). However, under nutritional stress, finding food becomes more critical for survival, outweighing the
355 environmental risks and physiological costs (18,21). For example, a poorly-resourced mosquito may
356 begin host seeking earlier in the night because their lack of energy reserves reduce the chance of
357 surviving until later at night (or the morning, depending on whether ITNs limit access to hosts at
358 night). Our results are consistent with this hypothesis because poorly-resourced mosquitoes with low
359 nutritional reserves are more likely to host seek, predominantly in the early evening when
360 temperatures are higher and humidity is lower, and they risk being killed because human hosts are
361 alert. Although we did not quantify survival, we did not encounter any live mosquitoes fed on a 0.5%
362 sucrose diet whilst clearing the semi-field systems the day after each release, but regularly
363 encountered live mosquitoes fed on higher sucrose solutions and/or an additional blood meal. This
364 indicates that mosquitoes fed 0.5% sucrose were low-resourced and did not have sufficient reserves
365 to survive until morning. Overall, our results suggest that early biting could be an adaptive plastic
366 temporal shift to maximise fitness in sugar-poor regions and where hosts are not easily accessible
367 due to high ITN use.

368 Better-resourced mosquitoes on high sucrose diets (with or without an additional blood meal) had
369 higher nutritional reserves and waited until the classical time window to host seek, in alignment with
370 their circadian physiology and when environmental risks are likely to be lower, which also supports
371 our hypothesis. However, we also found that better-resourced mosquitoes were just as likely as
372 poorly resourced mosquitoes to be trapped later in the morning. Such late biting by well-fed
373 mosquitoes was unexpected but may be due to the lack of water/food sources provided in the semi-
374 field enclosures, forcing untrapped mosquitoes to host seek to mitigate dehydration caused by the

375 rise in temperature and the reduction in humidity in the morning (49,50). Alternatively, well-resourced
376 mosquitoes which had failed to locate a host (*i.e.* had not entered a trap) during the night may have
377 become resource-depleted because flight is energetically demanding (48), leading to an increased
378 propensity to continue host seeking because they do not have sufficient energy to survive until the
379 subsequent night. Regardless of the explanation(s), our results reveal that adult nutritional condition
380 drives variation in biting time of day.

381 Our semi-field system allowed mosquitoes to experience ecologically realistic temperature and
382 humidity fluctuations and provided sufficient flight space, but excluded other ecological factors,
383 including predation and access to alternative food sources. Thus, to fully investigate how resource
384 availability impacts biting time, future studies should consider adding plants of varying sugar contents
385 and alternative blood hosts into semi-field systems. However, these omissions were necessary in our
386 experiment to isolate the effect of adult nutritional status on biting behaviour. To probe the ecological
387 relevance of our experimental results, we also undertook a preliminarily trapping session for wild host-
388 seeking *Anopheles gambiae* s.l. mosquitoes. We found that they had similar nutritional profiles to our
389 low-resourced mosquitoes fed on 0.5% sucrose, suggesting that our experimental perturbations
390 represented natural conditions. The low sample size for wild caught mosquitoes precludes comparing
391 nutritional reserves between host seeking times of day but does suggest that adult nutritional content
392 does not correlate with body size (a product of larval condition) (Additional file 1, Fig. S4). Thus,
393 catching lowly resourced mosquitoes at the time of host seeking is consistent with our experimental
394 results revealing that adult nutrition influences biting rhythms. Further field studies are necessary to
395 build on our findings, including sampling host-seeking mosquitoes across multiple sites, both indoors
396 and outdoors, throughout a 24-hour period, examining their nutritional status, and investigating when
397 mosquitoes become active.

398 Like previous studies across Africa (3,35,51), we found a large proportion of wild mosquitoes
399 (approximately 20%) host seeking during the morning when humans are unprotected by ITNs. This
400 pattern of 'late' biting has become more pronounced since the widespread use of ITNs. Because
401 biting time shifts have a genetic component (16), and universal coverage of ITNs is approximately
402 75% in Ghana (52), late biting could be an evasion strategy evolving in the mosquito population
403 where the larval collections for our experiment were carried out, rather than an entirely plastic
404 behavioural change. Further work is needed to ascertain the extent to which biting time of day is

genetically determined and/or due to behavioural plasticity, because plasticity itself evolves and can facilitate or constrain evolution that genetically segregates mosquito populations into early, classical or late biters. Modelling suggests that early biting is more likely to evolve in sugar-poor environments than sugar-rich environments, for example, due to differences in the abundance and diversity of plants (24). However, in areas of fluctuating resource availability or ITN coverage, plastic genotypes that can switch between early, classical, or late biting depending on their environmental conditions should have higher fitness (17).

Both our semi-field experiment and preliminary field data reveal significant variation in biting time of day, and intuition suggests that early biting by poorly-resourced mosquitoes could sustain malaria transmission. However, the overall impact of shifts in mosquito biting time of day for malaria transmission is difficult to predict, because of complex and potentially opposing effects on both mosquito and parasite fitness. While early biting causes greater contact between vectors and hosts in regions with high ITN coverage, malaria parasite development is less productive in low-resourced mosquitoes, and mosquito survival is reduced (45). Thus, the poor nutritional state of early biters could curtail onward transmission through lower parasite numbers (53) and because vectors may not survive long enough for malaria parasites to complete their development (54,55). In addition, parasite establishment in mosquitoes is impacted by daily temperature rhythms (56) and parasite infectivity and mosquito susceptibility vary between day and night (1,57,58). Disruption to foraging rhythms also reduces fitness in other insects (59), and in mosquitoes could lead to mismatch between metabolic processes and blood digestion (60). The net outcome for malaria transmission of these myriad and potentially synergistic and antagonistic effects of biting time of day is challenging to predict. However, recognising that transmission is time of day dependent is necessary for accurately predicting epidemiology, the risk of clinical malaria cases, and the trajectory of parasite evolution.

Conclusions

Our results suggest that plasticity in biting time of day, driven by variation in adult mosquito nutritional condition, may be an underappreciated contributor to residual malaria transmission. In particular, nutritional stress in mosquito populations may lead to shifts in biting behaviour, reducing ITN efficacy. This highlights that mosquito nutrition and its influence on the timing of biting behaviour should be

434 considered when implementing and assessing vector control strategies, especially those which target
435 mosquito foraging behaviour, such as ITNs and sugar-baited traps. More broadly, investigating the
436 drivers of variation in biting time of day and the overall impact on pathogen transmission is critical for
437 assessing and improving the efficacy of current and future vector control tools. This is especially
438 important for malaria, where up to 30% of biting is reported to occur when humans are unprotected by
439 ITNs and is predicted to lead to millions of additional clinical cases per year (3,10). However, we also
440 expect our findings to apply to other vector-borne diseases that rely on vector foraging rhythms for
441 between-host transmission.

442

443 **Declarations**

444 *Ethics approval and consent to participate*

445 The study protocols for mosquito colony blood feeds and human landing catches (HLCs) were
446 ethically reviewed and approved by the Ghana Health Service Ethics Review Committee (GHS-ERC:
447 021/07/23). Volunteers involved in colony blood feeds were tested for malaria prior to direct-feeding,
448 and the same person fed the mosquitoes throughout the experiment. Permission to conduct HLCs in
449 study sites was obtained from the District Assembly Representative, and written consent was
450 obtained from all collectors. Collectors had access to malaria prophylaxis and did not report any
451 adverse events.

452

453 *Consent for publication*

454 Not applicable.

455

456 *Availability of data and materials*

457 The datasets supporting the conclusions of this article are available in the Edinburgh DataShare
458 repository and can be accessed with [https://doi.org/LINK AVAILABLE UPON ACCEPTANCE].

459

460

461 *Competing interests*

462 The authors declare that they have no competing interests.

463

Funding

This work was funded by a Varley-Gradwell Travelling Fellowship in Insect Ecology (2023-2024) to C.E.O, and by a Wellcome Trust Investigator Award (202769/Z/16/Z) and Wellcome Trust Discovery Award (227904/Z/23/Z) to S.E.R. Research equipment and travel funds for M.G.M. and S.S.C.R. were funded by an Initiation Grant from the University of Notre Dame, Notre Dame Research office. Y.A. A. is funded by the National Institute for Health (D43 TW 011513). The funders had no role or influence on the design of this study, data collection, analyses, and interpretation of the data collected, as well as in writing this manuscript.

Authors' contributions

Conceptualisation: CEO, SSCR, YAA, SER. Methodology: CEO, SSCR, MGM, YAA, SER. Investigation: CEO, SSCR, MGM, ARMS, YAB. Analysis: CEO. Writing-original draft: CEO, SER. Writing-review and editing: CEO, SSCR, MGM, YAA, SER. All authors read and approved the final manuscript.

Acknowledgements

We thank Anisa Abdulai for experimental and technical assistance, and the serology lab team at the Noguchi Memorial Institute for Medical Research for kindly letting us use their facilities.

References

1. Rund SSC, O'Donnell AJ, Gentile JE, Reece SE. Daily rhythms in mosquitoes and their consequences for malaria transmission. Insects. 2016;7(2):1–20.

2. Westwood ML, O'Donnell AJ, de Bekker C, Lively CM, Zuk M, Reece SE. The evolutionary ecology of circadian rhythms in infection. Nature Ecology and Evolution. 2019;3(4):552–60.

3. Sangbakembi-ngounou C, Costantini C, Longo-pendy NM, Ngoagouni C, Akone-ella O, Rahola N. Diurnal biting of malaria mosquitoes in the Central African Republic indicates residual transmission may be " out of control ." Proceedings of the National Academy of Sciences. 2022;119(21):e2104282119.

4. Thomsen EK, Koimbu G, Pulford J, Jamea-Maiasa S, Ura Y, Keven JB, et al. Mosquito behavior change after distribution of bednets results in decreased protection against malaria exposure. Journal of Infectious Diseases. 2017;215(5):790–7.

5. Moiroux N, Gomez MB, Pennetier C, Elanga E, Djènontin A, Chandre F, et al. Changes in anopheles funestus biting behavior following universal coverage of long-lasting insecticidal nets in benin. Journal of Infectious Diseases. 2012;206(10):1622–9.

6. Oke CE, Ingham VA, Walling CA, Reece SE. Vector control: agents of selection on malaria parasites? Trends in Parasitology. 2022;38(10):890–903.

7. World Health Organisation. World Malaria Report 2023. Geneva; 2023.

8. Bhatt S, Weiss DJ, Cameron E, Bisanzio D, Mappin B, Dalrymple U, et al. The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015. Nature. 2015;526(7572):207–11.

9. Huijben S, Paaijmans KP. Putting evolution in elimination: Winning our ongoing battle with evolving malaria mosquitoes and parasites. Evolutionary Applications. 2018;11(4):415–30.

10. Sherrard-Smith E, Skarp JE, Beale AD, Fornadel C, Norris LC, Moore SJ, et al. Mosquito feeding behavior and how it influences residual malaria transmission across Africa. Proceedings of the National Academy of Sciences of the United States of America. 2019;116(30):15086–96.

11. Russell TL, Beebe NW, Cooper RD, Lobo NF, Burkot TR. Successful malaria elimination strategies require interventions that target changing vector behaviours. Malaria Journal. 2013;12(1):1–5.

12. Sougoufara S, Diédhiou SM, Doucouré S, Diagne N, Sembène PM, Harry M, et al. Biting by Anopheles funestus in broad daylight after use of long-lasting insecticidal nets: A new challenge to malaria elimination. Malaria Journal. 2014;13(1):1–7.

13. Yohannes M, Boelee E. Early biting rhythm in the afro-tropical vector of malaria, Anopheles arabiensis, and challenges for its control in Ethiopia. Medical and Veterinary Entomology. 2012;26(1):103–5.

14. Cooke MK, Kahindi SC, Oriango RM, Owaga C, Ayoma E, Mabuka D, et al. “A bite before bed”: Exposure to malaria vectors outside the times of net use in the highlands of western Kenya. Malaria Journal. 2015;14(1):1–15.

15. Carrasco D, Lefèvre T, Moiroux N, Pennetier C, Chandre F, Cohuet A. Behavioural adaptations of mosquito vectors to insecticide control. Current Opinion in Insect Science. 2019;34:48–54.

16. Govella NJ, Johnson PCD, Killeen GF, Ferguson HM. Heritability of biting time behaviours in the major African malaria vector Anopheles arabiensis. Malaria Journal. 2023;22(1):1–9.

17. Pigliucci M. Evolution of phenotypic plasticity: Where are we going now? Trends in Ecology and Evolution. 2005;20(9):481–6.

18. Van Der Veen DR, Riede SJ, Heideman PD, Hau M, Van Der Vinne V, Hut RA. Flexible clock systems: adjusting the temporal programme. Phil Trans R Soc B. 2017 Nov 19;372(1734):20160254.

19. Van Der Vinne V, Riede SJ, Gorter JA, Eijer WG, Sellix MT, Menaker M, et al. Cold and hunger induce diurnality in a nocturnal mammal. Proc Natl Acad Sci USA. 2014 Oct 21;111(42):15256–60.

20. Van Der Vinne V, Gorter JA, Riede SJ, Hut RA. Diurnality as an energy-saving strategy: energetic consequences of temporal niche switching in small mammals. Journal of Experimental Biology. 2015 Aug 1;218(16):2585–93.

21. Van Der Vinne V, Tachinardi P, Riede SJ, Akkerman J, Scheepe J, Daan S, et al. Maximising survival by shifting the daily timing of activity. Ghalambor C, editor. Ecology Letters. 2019 Dec;22(12):2097–102.

22. Sougoufara S, Harry M, Doucouré S, Sembène PM, Sokhna C. Shift in species composition in the Anopheles gambiae complex after implementation of long-lasting insecticidal nets in Dielmo, Senegal. Medical and veterinary entomology. 2016;30(3):365–8.

23. Kreppel KS, Viana M, Main BJ, Johnson PCD, Govella NJ, Lee Y, et al. Emergence of behavioural avoidance strategies of malaria vectors in areas of high LLIN coverage in Tanzania. Scientific Reports. 2020;10(1):1–11.

24. Stone C, Chitnis N, Gross K. Environmental influences on mosquito foraging and integrated vector management can delay the evolution of behavioral resistance. Evolutionary Applications. 2016;9(3):502–17.

25. Barredo E, Degennaro M. Not Just from Blood: Mosquito Nutrient Acquisition from Nectar Sources. Trends in Parasitology. 2020;36(5):473–84.

26. Rund SSC, Bonar NA, Champion MM, Ghazi JP, Houk CM, Leming MT, et al. Daily rhythms in antennal protein and olfactory sensitivity in the malaria mosquito Anopheles gambiae. Sci Rep. 2013 Aug 29;3(1):2494.

27. Mitchell SN, Catteruccia F. Anopheline reproductive biology: Impacts on vectorial capacity and potential avenues for malaria control. Cold Spring Harbor Perspectives in Medicine. 2017;7(12):14.

28. Foster WA. Behavioural ecology of plant-mosquito relations. In: Ignell R, Lazzari CR, Lorenzo MG, Hill SR, editors. Sensory ecology of disease vectors [Internet]. The Netherlands: Wageningen Academic Publishers; 2022. p. 171–234. Available from: https://doi.org/10.3920/978-90-8686-932-9

29. Ebrahimi B, Jackson BT, Guseman JL, Przybylowicz CM, Stone CM, Foster WA. Alteration of plant species assemblages can decrease the transmission potential of malaria mosquitoes. Journal of Applied Ecology. 2018;55(2):841–51.

30. Gu W, Müller G, Schlein Y, Novak RJ, Beier JC. Natural plant sugar sources of Anopheles mosquitoes strongly impact malaria transmission potential. PLoS ONE. 2011;6(1).

31. Killeen GF, Smith TA, Ferguson HM, Mshinda H, Abdulla S, Lengeler C, et al. Preventing childhood malaria in Africa by protecting adults from mosquitoes with insecticide-treated nets. PLoS Medicine. 2007;4(7):1246–58.

32. Gary RE, Foster WA. Diel timing and frequency of sugar feeding in the mosquito Anopheles gambiae, depending on sex, gonotrophic state and resource availability. Medical and Veterinary Entomology. 2006;20(3):308–16.

33. Müller G, Schlein Y. Plant tissues: the frugal diet of mosquitoes in adverse conditions. Medical Vet Entomology. 2005 Dec;19(4):413–22.

34. Sabtiu ARM, Sr IK, Mfum COA, Akuamoah-Boateng Y, Doe RT, Boadu EN, et al. Malaria transmission risk in the city of Accra, Ghana: vector behavior and distribution [Internet]. 2025 [cited 2025 July 2]. Available from: https://www.researchsquare.com/article/rs-6008722/v1

35. Akuoko OK, Dhikrullahi SB, Hinne IA, Mohammed AR, Mfum Owusu-Asenso C, Coleman S, et al. Biting behaviour and the spatio-temporal dynamics of malaria vectors in different ecological zones in Ghana. Parasites & Vectors [Internet]. 2024;17(16). Available from: https://doi.org/10.21203/rs.3.rs-1975878/v1

36. Harrington LC, Foy BD, Bangs MJ. Considerations for Human Blood-Feeding and Arthropod Exposure in Vector Biology Research: An Essential Tool for Investigations and Disease Control. Vector-Borne and Zoonotic Diseases. 2020 Nov 1;20(11):807–16.

583 37. Nzioki I, Machani MG, Onyango SA, Kabui KK, Githeko AK, Ochomo E, et al. Current
584 observations on shifts in malaria vector biting behavior and changing vulnerability to malaria
585 transmission in contrasting ecosystems in Western Kenya. Parasites & Vectors. 2023;1–19.

586 38. Machani MG, Ochomo E, Amimo F, Mukabana WR, Githeko AK, Yan G, et al. Behavioral
587 responses of pyrethroid resistant and susceptible Anopheles gambiae mosquitoes to insecticide
588 treated bed net. PLoS ONE. 2022;17(4):e0266420.

589 39. Gimnig JE, Walker ED, Otieno P, Kosgei JK, Olang GB, Ombok MO, et al. Incidence of Malaria
590 among Mosquito Collectors Conducting Human Landing Catches in Western Kenya. The
591 American Journal of Tropical Medicine and Hygiene. 2013 Feb 6;88(2):301–8.

592 40. Gillies MT, Coetzee M. A Supplement to the Anophelinae of Africa South of the Sahara.
593 Publications of the South African Institute for Medical Research. 1987;55:1–143.

594 41. Van Handel E, Day JF. Assay of lipids, glycogen and sugars in individual mosquitoes:
595 correlations with wing length in field-collected Aedes vexans. Journal of the American Mosquito
596 Control Association. 1988;4(4):549–50.

597 42. Van Handel E. Rapid determination of glycogen and sugars in mosquitoes. Journal of the
598 American Mosquito Control Association. 1985;1:299–301.

599 43. Van Handel E. Rapid determination of total lipids in mosquitoes. Journal of the American
600 Mosquito Control Association. 1985;(1):302–4.

601 44. Lehmann T, Dalton R, Kim EH, Dahl E, Diabate A, Dabire R, et al. Genetic contribution to
602 variation in larval development time, adult size, and longevity of starved adults of Anopheles
603 gambiae. Infection, Genetics and Evolution. 2006 Sept;6(5):410–6.

604 45. Oke CE, O'Donnell AJ, Schneider P, Reece SE. Plasticity in malaria parasite development:
605 mosquito resources influence vector-to-host transmission potential. Front Malar. 2024 Nov
606 1;2:1481816.

607 46. Lenth RV. emmeans: Estimated Marginal Means, aka Least-Squares Means [Internet]. 2023.
608 Available from: https://cran.r-project.org/package=emmeans

609 47. Makowski D, Ben-Shachar M, Lüdecke D. The {easystats} collection of R packages [Internet].
610 2020. Available from: https://easystats.github.io/easystats/

611 48. Kaufmann C, Briegel H. Flight performance of the malaria vectors Anopheles gambiae and
612 Anopheles atroparvus. Journal of Vector Ecology. 2004;29(1):140–53.

613 49. Hagan RW, Didion EM, Rosselot AE, Holmes CJ, Siler SC, Rosendale AJ, et al. Dehydration
614 prompts increased activity and blood feeding by mosquitoes. Scientific Reports. 2018;8(1):1–12.

615 50. Lin S, Senapati B, Tsao CH. Neural basis of hunger-driven behaviour in Drosophila. Open
616 Biology. 2019;9(3).

617 51. Odero JI, Abong'o B, Moshi V, Ekodir S, Harvey SA, Ochomo E, et al. Early morning anopheline
618 mosquito biting, a potential driver of malaria transmission in Busia County, western Kenya.
619 Malaria Journal. 2024;23(1):1–13.

620 52. Afagbedzi SK, Alhassan Y, Kenu E, Malm K, Bandoh DAB, Peprah NY, et al. Universal coverage
621 and utilization of free long-lasting insecticidal nets for malaria prevention in Ghana: a cross-
622 sectional study. Front Public Health. 2023 May 25;11:1140604.

623 53. Aleshnick M, Ganusov VV, Nasir G, Yenokyan G, Sinnis P. Experimental determination of the
624 force of malaria infection reveals a non-linear relationship to mosquito sporozoite loads. PLoS
625 Pathogens. 2020;16(5):e1008181.

54. Ohm JR, Baldini F, Barreaux P, Lefevre T, Lynch PA, Suh E, et al. Rethinking the extrinsic incubation period of malaria parasites. Parasites and Vectors. 2018;11:178.

55. Shaw WR, Holmdahl I, Itoe M, Werling K, Marquette M, Paton D, et al. Multiple blood feeding in mosquitoes shortens the Plasmodium falciparum incubation period and increases malaria transmission potential. PLoS Pathogens. 2020;16(12):e1009131.

56. Suh E, Grossman MK, Waite JL, Dennington NL, Sherrard-Smith E, Churcher TS, et al. The influence of feeding behaviour and temperature on the capacity of mosquitoes to transmit malaria. Nature Ecology and Evolution. 2020;4(7):940–51.

57. Pigeault R, Caudron Q, Nicot A, Rivero A, Gandon S. Timing malaria transmission with mosquito fluctuations. Evolution Letters. 2018;2(4):378–89.

58. Schneider P, Rund SSC, Smith NL, Prior KF, O'Donnell AJ, Reece SE. Adaptive periodicity in the infectivity of malaria gametocytes to mosquitoes. Proceedings of the Royal Society B: Biological Sciences. 2018;285(1888).

59. Gill S, Le HD, Melkani GC, Panda S. Time-restricted feeding attenuates age-related cardiac decline in Drosophila. Science. 2015;347(6227):1265–9.

60. Rund SSC, Hou TY, Ward SM, Collins FH, Duffield GE. Genome-wide profiling of diel and circadian gene expression in the malaria vector Anopheles gambiae. Proceedings of the National Academy of Sciences of the United States of America. 2011;108(32).

Additional files

Additional file 1 (.pdf)

Supplementary Figures.

Figure S1 is a map of larval and adult collection sites, Figure S2 is the wing length of lab-reared mosquitoes under different feeding treatments, Figure S3 provides information about the nutritional contents of wild adult mosquitoes collected at different times of day, and Figure S4 is the correlation between wing length and nutritional content in wild adult mosquitoes.