Novel sampling methods for monitoring *Anopheles arabiensis* from Eritrea

5 J.D. Charlwood[1,2], A.K. Andegiorgish [1,3], Y. Asfaha[1], L. Tekle Weldu[1], F. Petros[1], L.
6 Legese[1], R. Asfaha[1], S. Mihreteab[4], C. LeClair[5] and A. Kampango[6,7]

8   1- Epidemiology and Public Health, College of Health Sciences, Asmara, Eritrea
9   2- Global Health and Tropical Medicine, Instituto de Higiene e Medicina Tropical, Lisbon,
10   Portugal
11   3- Department of Epidemiology and Biostatistics, Xi'an Jiaotong University, Shaanxi,
12   China
13   4- Malaria Control Program, Asmara, Eritrea
14   5- Medecins sans Frontiers, Belgium.
15   6- Instituto Nacional de Saude, Maputo, Mozambique
16   7- Department of Zoology and Entomology, University of Pretoria, Pretoria, South Africa

18 e-mail: jdcharlwood@gmail.com
19   akidane2016@gmail.com
20   yonatanestifanos737@gmail.com
21   Robiyoasfaha16@gmail.com
22   lidiaghezae@gmail.com
23   smuqubay@gmail.com
24   corey_leclair@hotmail.com
25   akampango@gmail.com
26   liya47tekle@gmail.com

28   Keywords
29   *Anopheles arabiensis*. Outdoor sampling. Tent-trap. Resting Behaviour. Control

Abstract

33 Background: Studies comparing novel collection methods for host seeking and resting

34 mosquitoes *An. arabiensis* were undertaken in a village in Eritrea. Techniques included an odour

35 baited trap, a novel tent-trap, human landing collection and three methods of resting collection.

36 A technique for the collection of mosquitoes exiting vegetation is described. Pre-gravid rates

37 were determined by dissection of host seeking insects and post-prandial egg development among

38 insects collected resting.

40   Results: Overall 5382 host-seeking, 2296 resting and 357 *An. arabiensis* exiting vegetation were

41   collected. The Furvela tent-trap was the most efficient, risk-free method for the collection of

42   outdoor host-seeking insects, whilst the Suna trap was the least effective method. Mechanical

43   aspirators (the CDC backpack or the Prokopack aspirator) were superior to manual aspiration in

44   a dark shed but there was no advantage over manual aspiration in a well-lit one. An estimated

45   two-thirds of newly-emerged mosquitoes went through a pre-gravid phase, feeding twice before

46   producing eggs. Mosquitoes completed gonotrophic development in a dark shed but left a lighter

47   shed soon after feeding. One blood-fed female marked in the village was recaptured two days

48   after release exiting vegetation close to the oviposition site and another shortly after oviposition

49   attempting to feed on a human host three days after release. Exit rates of males from vegetation

50   peaked three minutes after the initial male had left. Unfed and gravid females exited

51   approximately six minutes after the first males.

52

53   Conclusions: Furvela tent-traps are suitable for the collection of outdoor biting *An. arabiensis* in

54   Eritrea whilst the Prokopack sampler is the method of choice for the collection of resting insects.

55   Constructing well-lit, rather than dark, animal sheds, may encourage otherwise endophilic

56   mosquitoes to leave and so reduce their survival and hence their vectorial capacity.

57
58   Introduction
59
60   Efforts to eliminate malaria have increased in recent years and a number of countries are

61   approaching a situation in which local cases of the disease no longer occur [WHO, 2017a]. To

62   enter the elimination-phase the malaria burden should be reduced to an incidence rate of less

63   than one per 1000 persons at risk [WHO, 2018]. In Eritrea the malaria burden has declined from

64   110 cases/1000 in 1998 to 6 cases/1000 in 2017 [WHO,2018], thus, approaching elimination. In

65   order to reach the target of elimination, however, residual malaria transmission must be

66   addressed. This includes monitoring of potential vectors and their control by application of

67   appropriate interventions in existing and newly-active foci [Killeen, 2104].

68   Given the substantial geographic heterogeneities in malaria burden, in Eritrea and elsewhere,

69   assessment of the bionomics of local anophelines is likely to require a range of bespoke

70   interventions for different populations. *Anopheles arabiensis* is the most common vector in east

71   Africa, to date it is the only recorded vector in Eritrea [Shililu et al., 2004 Wiebe et al., 2017],

72   and is noted for displaying ecological and behavioural plasticity such as readily biting animals or

73   humans, either indoors or outdoors [Sinka et al., 2010]. As such, control is challenging and its

74   importance as a vector is likely to increase in the future.

75

76   In 2017 larvae of *An. arabiensis* were found, on one occasion, on the outskirts of Asmara, the

77   capital, 2,200m above sea level in the Eritrean Highlands (JD Charlwood, unpublished data). At

78   lower altitudes on the Escarpment mosquito populations are associated with streams, which exit

79   from small-scale dams used to create reservoirs that supply nearby villages with water during the

80   long dry season. Dams and streams are separated by several kilometres and, with typically

81   limited dispersal, mosquitoes are likely to occur in a metapopulation-patchwork of semi-isolated

82   sub-populations, whose ecology is defined as much by the environment as by intrinsic

83   characteristics [Verdonschot & Besse-Lototoskaya, 2014]. A knowledge of mosquito bionomics

84   in such populations might help in the design of novel control techniques, especially those based

85   on environmental management rather than insecticides.

86   Assessing outdoor mosquito activity is especially important because outdoor transmission may

87   maintain residual malaria once the major conventional techniques, indoor residual spraying with

88   insecticide (IRS) and use of LLINs, have been deployed [Killeen et al., 2013].

89   Traditionally the 'gold standard' for sampling outdoor biting mosquitoes has been the human

90   landing catch (HLC) in which collectors acting as bait catch mosquitoes attempting to feed on

91   their exposed lower legs [Silver, 2008]. Whilst this technique is likely to be the sample that best

92   reflects biting exposure outdoors there are a number of problems inherent to it. Human landing

93   catches require a considerable amount of supervision, are expensive to run, and, most

94   significantly, they often expose the collector to pathogen transmission [Achee et al., 2015].

95   These considerations have recently led the World Health Organization (WHO) to prioritize the

96   search for substitutes to HLC [WHO, 2017b] and whilst a number of alternatives have been

97   suggested, 'tent-traps', which catch blood-seeking mosquitoes, prior to biting appear particularly

98   promising [Charlwood et al., 2017]. Collectors are likely to differ in their efficiency, so that

99   numbers caught may be independent of the actual number biting. Humans produce a large range

100   of volatile chemicals [Penn et al., 2007] and vary in their attractiveness to mosquitoes. Individual

101   humans not only attract different numbers of mosquitoes but also different species [Knols et al.,

102   1995] so that it becomes difficult to extrapolate from the numbers collected to exposure to

103   disease. These difficulties are common to all techniques that involve humans as baits so

104   alternatives that do not require human involvement may be the most suitable sample. One such

105   sampling technique is the recently developed Suna trap which was both an effective sampling

106   device and control tool in Kenya [Homan et al., 2016].

107

108   Outdoor biting insects may, nevertheless, rest inside houses or sheds either before or after

109   feeding, providing a target for control if their location can be identified [Killeen et al., 2016].

110   Suitable sampling methods for resting mosquitoes are, therefore, another priority. This may be

111   by collection of mosquitoes from houses, sheds or vegetation, e.g. using mechanical or manual

112   aspirators when the insects are resting during the day or when they leave their resting sites at

113   dusk.

114

115   A study designed to compare different collection techniques was, therefore, undertaken in

116   September-October 2016 in a village on the escarpment of the Eritrean Highlands. Two types of

117   tent-trap were compared with an odour baited trap (the SUNA trap [Homan et al., 2016]) and

118   HLC for the collection of mosquitoes' host-seeking outdoors whilst the CDC backpack aspirator

119   [Clark et al., 1994] and the Prokopack aspirator [Vazquez-Prokopec, 2009] were compared to

120   manual aspiration for the collection of those resting indoors. In addition, a novel method for the

121   sampling of insects leaving vegetation at dusk was developed.

122

123   Methods

124   Sample sites

125   The study took place between the 7th and 23rd of October 2016 in the environs of the village of

126   Adi Boskal (15° 41' 41.67" N 38° 38' 54.59" E) at an altitude of 1536m above sea level in Zoba

127   Anseba, Eritrea (Fig 1). The village comprises 25 stone-walled houses and is located on a steep

128   hillside 70m above a field that abutted a stream; the outlet from the local micro-dam. At the time

129   of the survey no rain was recorded and the stream, in which large numbers of *Anopheles* larvae

130   were observed, was very slow flowing with filamentous algae. In addition to the houses there

131   were two animal sheds in the village. At night five goats and a calf were kept in the first shed

132   (shed 1: 3.2m length x 1.8m width x 1.8m height) and 10 goats and 5 sheep were kept in the

133   other (shed 2: 3.8m x 3.1m x 1.6-1.8m). Close to shed 1, four cows and a donkey were kept in an

134   open-sided, thatched roof corral at night.

135   At other times of the year mosquito numbers are generally very low and in the 12 months prior to

136   the study there had been no cases of malaria from the village reported at the local hospital in the

137   nearby town of Elaberid.

138   Study design
139
140   A comparison between three methods of collecting post prandial resting mosquitoes were

141   conducted in the two animal sheds shown as red triangles in Figure 1 and five methods of

142   collecting host-seeking mosquitoes across a transect, shown as yellow circles in Fig 1, were

143   undertaken. To evaluate the performance of the different types of collection method a

144   randomised block experimental design was applied. As such, collection methods were randomly

145   assigned to sites (blocks) situated approximately 45 metres apart to avoid possible interference

146   between collection methods. Then, collectors were also randomly assigned to these locations to

147   perform either HLC catches or act as bait in tent-traps. Collection methods were rotated between

148   sites to account for any possible environmental heterogeneity on trap performance. Accordingly,

149   collectors were rotated between collection methods to reduce the influence of differential

150   attractiveness between collectors on mosquito catch. At each site collection were performed for 5

151   replicate-days.

152   A sample of mosquitoes exiting vegetation by the edge of the river valley below the village and

153   a sample from swarms observed over the river bed were also collected at sunset.

154

155 Mosquito collection
156
157 *Resting mosquitoes*

158

159 Mosquitoes resting in the animal sheds were collected either with 1) a CDC-backpack aspirator

160 [Clark et al., 1994], 2) a home-made Prokopack aspirator [Vazquez-Prokopec, 2009] or 3) by

161 manual aspiration. The supplementary file 2 'Sampling routine' indicates the dates when the

162 different collection techniques were used in each shed. Removal sampling [Southwood, 1978]

163 was undertaken in order to determine if the different collection methods produced similar

164 population estimates.

165
166 Light levels inside the two sheds were measured using a Hand-held LX1010B light-meter

167 (Yingxinguang, Guandong, China) that registered down to 1 Lux.

168 *Host seeking mosquitoes*
169
170 The efficiency of the Suna trap was compared to both tent-traps and HLC in the field below the

171 village, shown in Fig 1. The supplementary file 'Sampling routine' indicates the location on the

172 transect and date when the different collection techniques were used. At the same time a sentinel

173 tent-trap (with two doors and two traps attached) was run on a nightly basis in one corner of the

174 field (shown as the white square in Fig 1).

175
176 **Landing collections**
177
178 Human landing collections (HLC) were performed by two teams of three people, in the field

179 below the village, with each individual working a three or four-hour shift (19:00-23:00; 23:00-

180 03:00 and 03:00-06:00). Collectors, using a torch and an aspirator, caught mosquitoes as they

181 attempted to feed on their exposed lower legs and feet. Collected mosquitoes were separated into

182 four-hourly groups. When not working the collectors acted as bait in tent-traps. Thus, the

183 collectors from the earliest shift replaced the sleepers in the tents who worked the second shift

184 and they themselves replaced the sleepers in the other tents who undertook the last shift of

185 landing collection. The period and location during which the individual collectors worked was

186 alternated on different nights to reduce the influence of differential attractiveness on mosquito

187 catch.

188

189 **Furvela tent-trap Type I**

190

191 Furvela tent-traps [Charlwood et al., 2017] were also used for the collection of outdoor biting

192 insects. Odour and exhaled gases from a host leave the tent through an approximately 8cm

193 opening, equivalent to the diameter of a CDC light-trap, in the door of the tent. A CDC trap

194 (without the light, lid or grid) is placed outside the tent, horizontally, 2 to 3cm from this opening.

195 On approach to the opening the insects are sucked into the trap and held in a conical collection

196 bag.

197

198 In the second part of the study the BG-Lure (a synthetic lure, which, consisted of a mixture of

199 ammonia, L-lactic acid, and caproic acid, in undeclared proprietary concentrations (BioGents

200 HmGb, Regensburg, Germany) designed to mimic human odour to attract mosquitoes [Homan et

201 al., 2016], was attached to the outside opening of the tent just below the CDC trap (Fig 2). This

202 facilitated the dispersion of volatiles from the lure. Collectors slept for two sequential nights in

203 the tents during this phase of the experiment. On one night, the lure was in use and on the other

204 there was no lure included. Tents were taken down and re-erected every day.

205

206 **Furvela tent-trap Type II**

207

208   A further comparison included a novel design for the attachment of the CDC trap to the tent. In

209   this case an A4 sized plywood board with an 8cm diameter hole (the size of the internal diameter

210   of the CDC trap) was placed inside the tent at the opening. Two L-shaped clips were used to

211   attach the board to the trap with wing nuts. The clips were positioned at right angles to the tent

212   zip. In this way the size of the opening and the distance of the trap from the tent were more

213   easily standardized than in the basic tent-trap (Fig 3). A video describing the setting up of the

214   trap is available at https://youtu.be/3UCOhfPGgiw.

215

216   **Suna Trap**

217

218   The Suna trap (Biogents, Germany) (Fig 4) is a conical trap (52 cm high x 39 cm diameter) that

219   has been used to control malaria vectors on Rusinga Island, western Kenya where it reduced

220   malaria transmitted by *An. funestus* but was less effective against *An. gambiae* s.l. [Homan et

221   al., 2016]. A 12v battery drives a fan that sucks mosquitoes up through a tube, with a 10cm

222   diameter opening, into a collection bag. Netting can be placed between the tube and the fan so

223   that collected mosquitoes are not damaged when caught. A non-return gate that is activated when

224   the fan is switched off, or when the tube is removed, means that under these circumstances, the

225   tube acts as a collection cage. The remainder of the base of the trap is perforated with numerous

226   small holes through which the odour from a BG-Lure that is placed inside the trap is blown.

227   There is also the possibility of adding carbon-dioxide to the trap via an external source connected

228   with a tube to an outlet in the trap. In the trial in Rusinga Island 2-butanone was included as a

229   substitute for carbon dioxide [Homan et al., 2016]. The lack of an effect against *An. gambiae*

230   s.l. may have been due to the absence of carbon dioxide in the attractive mix. In the present

231   experiment two traps were used. In one trap carbon dioxide was generated using a sugar and

232   yeast mixture in 2L of water in a 5L plastic bottle [Smallegange et al., 2010] and in the other

233   carbon dioxide was not used.

234
235   The traps were suspended 70cm above the ground using metal tripods, with the funnel opening

236   set 30cm above the ground (Fig 4).

237
238   *Exit collections from vegetation*
239

240   Males and recently-emerged virgin females leave their diurnal resting site at dusk to mate, and

241   gravid females do so to oviposit. Endophilic mosquitoes can be collected at this time by placing a

242   netting barrier over the open door of houses [Charlwood, 2011]; a technique that was adapted here

243   for the collection of mosquitoes leaving their outdoor resting sites. In this case a double-sized (1.8

244   x 2.2m), rectangular mosquito net was mounted horizontally in front of an area in the vegetation

245   subjectively considered to be darker than the other vegetation bordering the stream, and the edges

246   sealed with sheets. A collector sat inside the net and caught mosquitoes as they emerged (Fig.5)

247   Collections were separated into three-minute intervals and light levels were recorded every minute.

248   At the same time swarming insects were observed by looking towards the lightest part of the sky

249   over the dry river bed in areas close to the exit site.

250   Mosquito processing
251
252   Insects from all collections were sexed, categorized to species or species group using the keys of

253   Gillies and DeMeillon [1968] and Gillies and Coetzee [1987] and females sorted according to their

254   abdominal condition into unfed, part-fed, engorged, semi-gravid and gravid according to Fig 6.

255   The abdomens of a sample of blood-fed mosquitoes from the resting collections were squashed on

256   filter paper and are available on request. A subsample of *An. gambiae* complex mosquitoes

257   collected were identified to species by PCR using the techniques of Scott et al [1993].

258   Resting collections
259

260   In order to determine the duration of resting inside the sheds after feeding, the proportion of

261   gravid: blood-fed females on days following three or more sequential days of prior collections

262   was compared to days when the sheds had been left undisturbed for a day previously. *Anopheles*

263   are generally gonotrophically concordant and each blood meal gives rise to complete egg batch.

264   Should blood-fed insects have remained in the shed then they would have become semi-gravid or

265   gravid by the second day, in which case the ratio of semi-gravid and gravid insects to the rest of

266   the population would increase; but if they normally left shortly after feeding then the proportion

267   of semi-gravid and gravid insects would not increase despite this respite in sampling.

268   *Anopheles arabiensis* may also take a pre-gravid blood meal during the first oviposition cycle

269   that is used for adult nutrition rather than egg development. By maintaining blood-fed females

270   alive for 24hrs it is possible to separate those females that will develop eggs and will become

271   gravid and those that will not develop eggs, despite being blood fed on collection. These females

272   are pre-gravid and are almost entirely newly emerged [Gillies, 1955]. The number of such pre-

273   gravid females is, therefore, one indicator of adult recruitment to the population, and their

274   relative frequency an indicator of mosquito survival rates [Charlwood et al., 2003]. On six days

275   engorged mosquitoes collected resting were kept in the insectary for 24hrs and the proportion

276   pre-gravid (i.e. the proportion that did not develop eggs) determined. Results were compared to

277   estimates obtained by dissection of host-seeking mosquitoes. In this case virgin females, with

278   undeveloped ovaries, that attempt to feed, will not develop eggs and so would go through a pre-

279   gravid phase.

280

281 In order to determine post-prandial behaviour a series of three capture-recapture experiments

282 using engorged mosquitoes from resting collections were performed. Engorged insects, collected

283 resting from shed 1, were counted, dusted with fluorescent powder and released back into the

284 shed. Mosquitoes from all subsequent collections (resting, host-seeking and exiting vegetation)

285 were scanned with UV-light for colour which revealed previous marking.

286

287 Host seeking collections

288

289 Mosquitoes from the tent-trap collections were dissected for parity, sac-stage and mated status

290 according to the schema outlined in Charlwood et al. [2003].

291 **Statistical analysis**

292

293 Mosquito density by trapping method, period and shed was expressed in terms of Williams

294 geometric mean (M_w). Williams means have been shown to be a robust measure of central

295 tendency compared to arithmetic means, as they are less sensitive to both abundance and

296 periodicity of insect occurrence [Williams, 1937, Haddow, 1954]. The absolute density of

297 mosquitoes in each shed was estimated using the maximum weighted likelihood method of

298 Zippin [1956, 1958] as modified by Carle and Strud [1978]. In this case the population size, N, is

299 estimated as the smallest integer greater than the total catch, T, that satisfies the following

300 inequality: $\left(\frac{N+1}{N-T+1}\right)\left(\frac{kN-M+T+0.5K}{kN-M+1+0.5k}\right)^k \leq 1$, where k is the total number of removal periods. The

301 parameter M is estimated by the equation $M = \Sigma_{i=1}^{k}(k-i)c_i$, where c_i is the number of

302 individuals sampled.

303 Generalized Linear Multilevel Models (GLMMs) with negative binomial error distribution and

304 log-link functions were applied to model the difference in sampling catches produced by the

305   three resting collection methods, that is, Manual Aspirator, Backpack and Procopack. Collection

306   method (a three levels factor: Manual Aspirator, Backpack and Procopack), shed (a binary

307   factor: Shed 1 and Shed 2) and collection technique (a five-level factor: $1^{st} – 5^{th}$ rounds) were

308   considered as fixed factors. To accommodate potential hierarchical (correlation) and explicit

309   nesting structure of repeated measurements across collectors and during the study period, as

310   evidenced by exploratory analysis, both collector and time (in days) effects were modelled as

311   crossed random factors. This allowed the performance of each collection method to vary among

312   collectors and days within sheds. Model fit was assessed by visual inspection of the graph of

313   standardized model residuals against fitted observations [Hartig, 2020]. The amount of variation

314   of mosquito counts explained by predictors was determined by both marginal and conditional

315   coefficients of determination R^2, proposed in Nakagawa & Schielzeth [2013]. Additionally, Akaike

316   Information Criterion test (AICc) was applied to guide the selection of the best model fit. The

317   most parsimonious model (the one with the lowest AICc) was preferred among the others. Final

318   estimation of the mean and 95% Credible Interval bands (95% CrI) of fixed effect coefficients

319   were obtained via simulations from posterior distribution of best model fit parameters. A total of

320   2000 random simulations were performed using the function sim of the arm packages [Gelman &

321   Yu-Sung, 2018]. The performance of the CDC Backpack Aspirator and Prokopack in relation to

322   Manual Aspiration was estimated in terms of Incidence Rate Ratio (IRR). The Tukey *post-hoc*

323   multiple comparisons test was applied to determine the significance of the difference between

324   main effect treatment levels and interacting effects levels. GLMM and post-hoc tests were

325   performed using the packages lme4 v.1.1-23 [40] and emmeans v.1.4.8 [Lenth, 2020], respectively.

326   Similarly, GLMMs was also applied to investigate the significance of the difference between

327   mosquito catches obtained by Tent-traps and Suna trap. All the data processing tasks and

328   statistical analysis were performed using the R software version 4.0.2 [R Core team, 2020].

329

330   Ethical approval for the study was provided by the Research Ethical Clearance Committee of the

331   Asmara College of Health Sciences on the 18/09/2017.

332   **Results**
333
334   Temperatures during the night dropped to a minimum of 11.8ºC outdoors but were 4ºC warmer

335   inside the tents (minimum of 15.1ºC inside the tents). All 37 *An. gambiae* s.l. from resting

336   collections identified to species by PCR were *An. arabiensis*. Since this is the only member of

337   the species complex to have ever been found in Eritrea [Shililu et al., 2004] we assume that this

338   was the only species collected. A total of 5382 host seeking, 2296 resting and 357 *An. arabiensis*

339   exiting vegetation were collected during the experiment. In addition, 1 male and 1 female *An.*

340   *demellioni*, 6   *An. garnhami* and 1 *An. turkhudi* were collected resting towards the end of the

341   study.

342
343   Greatest numbers of host-seeking females were collected in the sentinel tent-trap run for 23

344   consecutive nights during the experiment (geometric mean of 162 per night 95% C.I. 117.13,

345   224.87). The majority of host-seeking parous mosquitoes had large follicular sacs, indicating a

346   rapid return to host-seeking after oviposition [Charlwood et al., 2018]. Total numbers collected

347   declined during the experiment whilst at the same time the parous rate among the 281

348   mosquitoes dissected [Charlwood et al., 2018] increased from 21% to 56% (Fig 7). Thus, there

349   was a decline in output of newly emerged insects and the population was an ageing one.

350   Collections from 49 tent-traps, eight all night landing collections and 28 resting collections were

351   positively correlated (Pearson's r= 0.64 n=5, n.s, between tent and landing collections; r=0.76 n

352   = 5, P =0.05 between tent and resting collections; r=0.97, n = 12, P = >0.01 between landing and

353   resting collections). The data from all collections is provided in the supplementary file 3.

354

355   Resting collections

356   ***Mosquito counts by collection method***

357   Shed 1 was darker than shed 2 (20 vs 40 Lux measured at 09:00). Temperature extremes were

358   also greater in the more open Shed 2 (maximum and minimum temperatures recorded close to

359   the roof of the sheds over 24hrs being 46.7 °C and 18.2 °C in Shed 2 compared to 39.6 °C and

360   17.2 °C in Shed 1). A total of 1561 and 735 female and 93 and 19 male *An. arabiensis* were

361   collected from Shed 1 and Shed 2, respectively from 134 rounds of sampling. The estimated

362   abundance of mosquitoes in the two sheds according to the different methods of collection with

363   their 95% confidence intervals are shown in Table 1.

364   Relatively high mosquito counts were obtained from Shed 1 using either the Backpack or

365   Procopack compared to manual aspiration (Table 1). Manual aspiration produced a total of 198

366   mosquitoes (Shed 1) and 208 mosquitoes (Shed 2), Backpack (723 vs. 157) and Procopack (640

367   vs. 370). Results of estimation of total abundance of indoor resting mosquito population by

368   removal test (Table 1) suggest that manual aspiration underestimated density in both sheds. On

369   the other hand, the Backpack may have underestimated the population abundance at Shed 2

370   compared to manual aspirator and, especially, Procopack. The overall estimate of total

371   abundance (± 95%CI) by combining catches from all the three methods were 1580 (1567 – 1585)

372   and 750 (738 – 754) from shed 1 and shed 2, respectively. The probability of capture was 0.603

373   (0.581 – 0.624) and 0.566 (0.534 – 0.598).

374

375 ***Sampling regime and collection performance***

376

377 There was a sharp reduction in the number of mosquitoes collected after the first sampling round,

378 irrespective of collection method or shed but the three methods produced similar numbers caught

379 after the second round of collection (Fig 8). The distribution of mosquito counts by the three

380 methods were heavily (right) skewed (supplementary file 4). Further exploratory analysis

381 suggested that the counts could plausibly be described by a negative binomial distribution

382 function. Additionally, since collections were repeatedly performed by the same individuals at

383 the same sheds (shed 1 and shed 2) over the study period some amount of dependency

384 (correlation structure) between observations was introduced (supplementary file 4).

385 The best model fit of sampling method performance (AICc = 852.2) was one that included

386 collection method and shed as main effects factors and an interaction effect between collection

387 methods and sampling regime. Collectors and day of collection were considered as random

388 factors. Both fixed and random factors explained 86.6% of the variability in mosquito counts

389 (Figure 9). Results of mosquito Incidence Rate Risks (IRR ± 95CrI) indicate that a mosquito

390 was, respectively, 1.45 (0.89 – 2.32) and 1.83 (1.17 – 2.29) more likely to be sampled by CDC

391 Backpack and Prokopack compared to manual aspiration. However, the difference was

392 statistically significant only between manual aspiration vs Prokopack. The results also showed

393 that the likelihood of collecting any mosquito varied significantly between sheds; that is, the

394 chance of collecting at Shed 2 was 41.6% lower compared to Shed 1. There were also significant

395 interaction effects between collection methods and sampling regimes.

396

397

398 There was a significant difference between the relative proportions of *An. arabiensis* females in

399 different abdominal stages collected from shed 1 and shed 2 on the days when no collections

400 were conducted the previous day (X^2 with Yates correction 23. 5, $p < 0.00002$). (Table 2) . On

401 the 10 days when resting collections had been conducted the previous day the relative

402 proportions of *An. arabiensis* by abdominal stage from either shed were similar X^2 with Yates

403 correction = 2.3963, p =0.12) but the proportion of semi-gravid and gravid *An. arabiensis*

404 females collected from Shed 1 were significantly higher than Shed 2, the lighter shed, following

405 days in which no previous collections had been undertaken (X^2 with Yates correction is 7.3493, p

406 = 0.0067). The number of females collected from Shed 2 was not significantly different from

407 numbers collected following days where previous collections had been undertaken (DRR = 0.84,

408 95% CI: 0.56–1.26, P=0.41).

409 ***Pre-gravid rates***

410 Seventy-two (35%) of the 204 engorged mosquitoes collected resting and kept in the insectary

411 for 24hrs failed to develop eggs, and so were pre-gravid. This proportion was similar to the 86

412 (31%) of the 281 dissected insects that were virgins (X^2= 1.6, P = 0.21). Fifty-one (37%) of the

413 recently emerged insects dissected had a mating plug. These insects may develop eggs after a full

414 blood meal and implies that at least two thirds of newly emerged insects went through a pre-

415 gravid stage.

416 Exit collections
417
418 Males started to exit the vegetation when light levels fell to approximately 500 Lux each day

419 (mean = 506 Lux; standard deviation = 80 Lux; range 373-610 Lux). Peak exit rates of males

420 occurred three minutes after the initial mosquito was collected. Both unfed and gravid females

421 were collected leaving the vegetation a few minutes after the males (Figure 10). One of the 13

422 gravid females collected had been and marked 2 days earlier and released, engorged, back into

423 Shed 1.

424

425 Performance of host-seeking sampling methods

426

427 The number collected in the HLC decreased with time during the night as did the temperature.

428 The geometric mean number per night in the HLC was 35.02 (s.d. 12.9). After eight nights of

429 collection a total of 40 *An. arabiensis* (mean of 5 per night 95% C.I. 1.18- 8.82) were collected

430 in the Suna trap with carbon dioxide and only 10 were collected when the trap was used without

431 carbon dioxide (mean 1.25 per night, 95% C.I. 0-3.6) (Table 3). The Suna trap also failed to

432 collect any mosquitoes when it (with added carbon dioxide) was moved alongside a likely flight

433 path of the mosquitoes by the vegetation 15m from the sentinel tent-trap, or close to the base of

434 the hill leading to the village. Furvela traps type I and type II collected, respectively, 6.23 (4.90 –

435 7.97) and 4.63 (3.42 – 7.96) significantly more mosquitoes than did the HLC. Both types of

436 Furvela tent traps collected relatively similar numbers of mosquito (Incidence Ratio = 1.36;

437 95%CI= 0.917-2.013) (Figure 11).

438

439 A previously marked blood-fed mosquito, was recaptured (with notably large follicular sacs),

440 host-seeking in the sentinel tent-trap, two days after release in Shed 1.

441 The addition of the lure to the tent-trap, either in the sentinel trap or the experimental tents, did

442 not result in an increase in the number of mosquitoes collected ($t^7 = 0.28$, P = 0.79 for sentinel

443 tent trap; $t^7= 0.48$, P = 0.64 for experimental tents).

444

445 Discussion

446

447 Monitoring outdoor biting and resting malaria vectors has assumed a greater importance as a

448 result of worldwide efforts to eliminate the disease [WHO, 2018]. Previous work has highlighted

449 the potential importance of outdoor exposure to *An. arabiensis* in Eritrea [Shililu et al., 2004].

450   Two alternatives were tested to HLC for the monitoring of outdoor biting mosquitoes in Adi

451   Boskal, the Suna trap and the Furvela tent-trap. In comparisons between an earlier version of the

452   Furvela trap and HLC, where *An. gambiae* was the principle vector collected, the methods were

453   similar at low densities but at higher densities HLC was considered to be more efficient [Govella

454   et al., 2009]. Nevertheless, several thousand mosquitoes have been caught in a night with a

455   single trap [Charlwood et al., 2011, 2012, 2013].

456   The Suna trap caught very small numbers of *An. arabiensis* compared to the other collection

457   methods. Cribillier et al., [2020] have recently shown that adding heat to a trap increases its

458   efficiency. Thus, one reason why the Suna trap failed to collect mosquitoes may be because the

459   trap did not have a sufficient 'heat signature' that might otherwise aid mosquitoes in location

460   when in close proximity to potential hosts. It is also possible that the lure used failed to elicit a

461   response from the mosquito. The fact that numbers were not affected in the sentinel trap when a

462   lure was attached to the opening and the relative inefficiency of the trap against *An. gambiae* s.l.

463   in Kenya [Homan et al., 2016] and Tanzania [Cribillier et al., 2020] indicates that this might be

464   the case. On the other hand, our results indicate that Furvela tent-traps are a suitable, indeed a

465   more efficient, alternative to HLC for the collection of outdoor biting *An. arabiensis* in this area.

466   At the temperatures experienced, collectors' legs became cold and may also have not given off

467   heat cues that assist host location. It is therefore possible that the difference between tent-trap

468   collections and HLC may be less apparent at warmer temperatures. Nevertheless, there remain

469   considerable advantages in using tent-traps, not the least of which are getting a good night's

470   sleep, not being exposed to pathogens and having a uniform collection efficiency between tents.

471   The method used to attach the CDC-trap to the tent, however, did not significantly affect the

472   number of *An. arabiensis* collected.

473   In the present experiments the sentinel trap caught more than twice as many mosquitoes as the

474   other tents. There are a number of possible reasons for this: the odour of the host in the tent

475   (JDC) whilst being unappealing to humans may have been particularly attractive to mosquitoes;

476   the tent was larger than the other tents and the enhanced visual profile may have attracted more

477   mosquitoes [Hawkes & Gibson, 2016]. Because mosquitoes tend to fly along the edge of

478   vegetation [Charlwood and Wilkes, 1981] those that rested in vegetation close to the river, or

479   that oviposited prior to host seeking may have been funnelled through the opening where the

480   sentinel trap was located. Thus, the trap may have sampled from a more concentrated population

481   than the other traps and so caught more mosquitoes as a result. Setting up tent-traps in such

482   locations may enhance collections, which is especially useful at low mosquito population

483   densities.

484

485   The collection of resting females also forms an important component of vector monitoring. The

486   results of sequential sampling using either the CDC-backpack aspirator, a Prokopack aspirator,

487   or manual aspiration were equivocal. For removal sampling to function adequately a number of

488   assumptions must be met: the catching procedure must not affect the probability of an animal

489   being caught; the population must remain stable during the catching period, sampling effort must

490   be the same in each round of collection and, most importantly, the chance of being caught must

491   be equal for all animals. A relatively large proportion of the population must also be caught to

492   obtain reasonably precise estimates. Numbers collected on each trapping interval must also

493   decline for estimates to be meaningful [Southwood, 1978]. Whilst we were unable to estimate

494   total numbers in the sheds on all but a few occasions our results indicate that between a quarter

495   and two thirds of the total sample was obtained during the initial round of collection depending

496   on the shed and method used (Table 1). Thus, a single round of collection from an indoor resting

497   site is a suitable sampling procedure. It being better to sample five sheds, once than one shed five

498   times.

499

500   The number of mosquitoes collected by manual aspiration, especially in Shed 1 was lower than

501   the other methods. This is probably because not only was the shed dark but there were many hard

502   to see hiding places due to the shed walls being made of stones. It was not easy to illuminate

503   these hiding places with a torch. These considerations did not affect collection by mechanical

504   aspirator. In Tanzania, Maia *et al* [2011] found that CDC backpack and Prokopack aspirators

505   were equivalent in efficiency for collecting mosquitoes in general, but that the Prokopack was

506   easier to use and there was increased consistency across the numbers of mosquitoes collected by

507   four different collectors operating the Prokopack compared with the CDC-BP. Also, in Tanzania

508   the Prokopack was considered to be a better method for collection of resting insects than manual

509   aspiration [Charlwood et al., 2018b]. It is now the method of choice for such samples.

510

511   The blood-fed *An. arabiensis* released in the animal shed in the middle of the village and

512   recaptured gravid two days later exiting vegetation close to the oviposition site at dusk indicates

513   that females probably arrive there half-gravid. Seeking a resting site close to the oviposition site

514   when half gravid makes sense if the oviposition site is some distance from the (indoor) feeding

515   site. It is undertaken when the insect still may have the ability to use energy from the blood meal

516   for flight (although by being outside nectar sources may be available) and means that the insects

517   can start hunting early in the night and shortly after oviposition. They will not have used energy

518   unnecessarily flying from the village to the oviposition site before starting to hunt, which they

519   did shortly after oviposition, as shown by the high proportion of dissected females with large

520   follicular sacs [Charlwood et al., 2018a], including one marked and released in the same shed

521   two days previously. The marked mosquito collected from the tent-trap had presumably fed on a

522   non-human host prior to release. These results emphasise the rapid gonotrophic cycle in this

523   species and also suggests a lack of 'host fidelity' in the mosquito.

524

525   Male *arabiensis* in Adi Boskal appeared to spend much of their lives in vegetation close to the

526   emergence site. It was from here that they were collected at dusk. Emergence from vegetation

527   was, as expected, related to illumination and, as expected, males left before the females. Most of

528   the females collected exiting vegetation were unfed. Such females are largely newly emerged

529   virgins who would presumably mate in swarms, which were seen at this time in the river bed,

530   before ascending the hill to the village for their first blood meal. How, or where, pre-gravid

531   virgin females, who feed in the village, mate remains unknown. Less than 5% of the insects

532   collected from the animal sheds were males and alternative sites for males to rest were not

533   obvious. Unfortunately searching for and monitoring of swarms in the village was not

534   undertaken. Similarly, the question of how the mosquito (and the other anophelines collected)

535   maintain themselves at times of low population density is not known and whether they do form

536   part of a meta-population (which implies occasional extinction) remains moot.

537
538   The likelihood that blood-fed insects will complete gonotrophic development inside human-

539   made structures appeared to depend on illumination, the darker of the two sheds in Adi Boskal

540   contained a higher proportion of gravid insects on the days when the sheds had not been sampled

541   the previous day compared to the lighter shed. The absence of any difference between days when

542   sampling had been curtailed on the previous day in the lighter shed indicates that the insects were

543   leaving before they became gravid. This implies that the insects had left the lighter shed to rest

544   outside. Resting outside shortly after feeding may have a significant mortality risk for

545   mosquitoes (and may be one reason why species that rest inside houses are better vectors than

546   those that rest outdoors) [Gillies, 1954b]. Constructing well-lit, rather than dark, animal sheds,

547   may encourage otherwise endophilic mosquitoes to leave and so reduce their survival and hence

548   reduce malaria transmission.

549

550   Given that the population was not stable during the study, but rather suffered a decline in output,

551   estimation of survival by parous rate determination was not possible. The data was also

552   insufficient to determine survival rates by time-series analysis. Nevertheless, just over a third of

553   all the insects from the resting collections were pre-gravid whilst almost half the mosquitoes

554   collected from the tent-trap had stage I ovaries, suggesting that they were also pre-gravid

555   females. Thus, newly emerged mosquitoes comprised a large proportion of the collections,

556   indicating a relatively low survival rate in the mosquito. The higher proportion of mosquitoes

557   that were considered to be pre-gravid by dissection compared to those from resting collections

558   may reflect mortality amongst such females before they could obtain a blood meal (as appears to

559   happen with young *An. coluzzii* from São Tomé [Charlwood et al., 2003]. All older females

560   were gonotrophically concordant and took two/three days per oviposition cycle.

561

562   Owing to the low night-time temperatures in Adi Boskal, people enter their houses early in the

563   evening. Making sure that houses are mosquito-proof and that villagers have access to LLINs

564   should help protect them from malaria. Whether anything other than current control techniques

565   (LLINs over all beds in the village) needs to be used in Adi Boskal is moot. There had been no

566   cases of malaria reported from Adi Boskal in the previous year and so the village would seem to

567   be a case of *anophelism sans malaria*. Although it is not certain that the mosquito provides any

568   benefit to local ecosystems it may do so. Treating cattle or spraying the shed with insecticide

569   would also, perhaps, enhance the development of resistance in the mosquito and waste precious

570   resources.

571   Conclusions

572

573   At the time of the study, although it would readily bite humans if they were available, the

574   *Anopheles arabiensis* population from Adi Boskal was apparently a largely zoophagic one. Most

575   of the newly emerged female mosquitoes went through a pre-gravid phase after which they

576   became gonotrophically concordant with a rapid gonotrophic cycle. A proportion of the semi-

577   gravid and gravid insects would leave their resting site to rest in vegetation close to the stream

578   below the village prior to oviposition. Male mosquitoes rested in vegetation and swarmed close

579   to the stream below the village. Furvela tent-traps set up in areas where there may be a flyway.

580   are a suitable alternative to human landing catches for the collection of outdoor biting *An.*

581   *arabiensis* whilst the Prokopack mechanical aspirator is the method of choice for the collection

582   of resting insects. Constructing well-lit, rather than dark, animal sheds, may encourage otherwise

583   endophilic mosquitoes to leave and so reduce their survival and hence their vectorial capacity.

584   **Availability of data and materials**

585

586   The data file associated with the work described here is available as a supplementary file 3 (Adi

587   Boskal collections.xls). The analysis file is also available as a supplementary file 4 (Analysis)

588

589   Abbreviations

590   CDC Centres for Disease Control

591   HLC Human Landing Collection

592   IRS Indoor Residual Spray

593   LLIN Long Lasting Insecticide-treated Net

594   WHO World Health Organization

595   References

596
597
598   **Achee NL, Youngblood L, Bangs MJ, Lavery JV, James S. 2015.** Considerations for the use of
599   human participants in vector biology research: a tool for investigators and regulators. *Vector Borne*
600   *Zoonotic Disease* **15**: 89–102.

601   **Carle FL, Strub MR. 1978.** A new method for estimating population size from removal data.
602   *Biometrics* **34:** 621–630.

603   **Charlwood JD. 2011.** Studies on the biology of male *Anopheles gambiae* Giles and *Anopheles*
604   *funestus* Giles from southern Mozambique. *Journal of Vector Ecology* **36**: 382-394.

605   **Charlwood JD, Macia GA, Manhaca M, Sousa B**, **Cuamba N, Bragança M. 2013.** Population
606   dynamics and spatial structure of human-biting mosquitoes, inside and outside of houses, in the
607   Chockwe irrigation scheme, southern Mozambique. *Geospatial Health*. **7**: 309-320.

608   **Charlwood JD, Pinto J, Sousa CA, Ferreira C, Petrarca V, do Rosario VE. 2003**, 'A mate or a
609   meal' – Pre-gravid behaviour of female *Anopheles gambiae* from the islands of São Tomé and
610   Príncipe, West Africa. *Malaria Journal* **2:** Article 9

611   **Charlwood JD, Rowland M, Protopopoff N, Le Clair C. 2017.** The Furvela tent-trap Mk 1.1 for
612   the collection of outdoor biting mosquitoes. *PeerJ*. **5**: Article 3848
613
614   **Charlwood JD, Protopopoff N, Rowland M, LeClair C. 2018b.** Studies on the resting and feeding
615   behaviour of *Anopheles gambiae* and *An. arabiensis* from Muleba District, North-eastern Tanzania.
616   *Medical and Veterinary Entomology.*
617
618   **Charlwood JD, Tomás EVE, Andegiorgish A, Mihreteab S, Le Clair C. 2018a.** `We like it wet': a
619   comparison between dissection techniques for the assessment of parity in *Anopheles arabiensis* and
620   determination of sac stage in mosquitoes alive or dead on collection. *PeerJ* **5**: Article 155

621   **Charlwood JD, Tomás EVE, Egyir-Yawson A, Kampango A, Pitts RJ. 2012.** Feeding frequency
622   and survival of *Anopheles gambiae* from a rice growing area of Ghana. *Medical and Veterinary*
623   *Entomology*. **26**: 263-70.

624   **Charlwood JD, Tomás EVE, Salgueiro P, Egyir-Yawson A, Pitts RJ, Pinto J. 2011.** Studies on
625   the behaviour of peridomestic and endophagic M form *Anopheles gambiae* from a rice growing area
626   of Ghana. *Bulletin of Entomological Research* **101**: 533–539.
627
628   **Charlwood JD, Wilkes TJ. 1981.** Observations on the biting activity of *Anopheles triannulatus*
629   *bachmanni* from the Mato Grosso, Brazil. *Acta Amazonica* **11**: 411-413.
630

631

632   **Clark GG, Seda H, Gubler DJ. 1994**. Use of the "CDC backpack aspirator" for surveillance of
633   *Aedes aegypti* in San Juan, Puerto Rico. *Journal of the American Mosquito Control Association* **10**:
634   119-124.

635

636   **Cribellier A, Spitzen J, Fairbairn H, van de Geer C, van Leeuwen JL, Muijres FT. 2020.** Lure,
637   retain, and catch malaria mosquitoes. How heat and humidity improve odour-baited trap performance.
638   *Malar J* **19,** 357 https://doi.org/10.1186/s12936-020-03403-5

639

640   **Gelman A, Yu-Sung S. 2018.** arm: Data Analysis Using Regression and Multilevel/Hierarchical

641   Models. *R package version 110-111*.

642   **Gillies MT, Coetzee MMT. 1987.** *A supplement to the anophelinae of Africa south of the Sahara*
643   *(Afrotropical Region)*. Johannesburg: South African Institute for Medical Research. 143pp.

644

645   **Gillies MT, De Meillon B. 1968.** *The Anophelinae of Africa south of the Sahara (Ethiopian*
646   *zoogeographical region)*. Johannesburg: South African Institute for Medical Research. 343pp

647

648   **Gillies MT. 1954.** Studies in house leaving and outside resting of *Anopheles gambiae* Giles and

649   *Anopheles funestus* Giles in East Africa. 2. The exodus from houses and the house resting population.

650   *Bulletin of Entomological Research* **45**: 375-387.

651

652   **Gillies MT. 1955.** The recognition of age-groups within populations of *Anopheles gambiae* by the

653   pre-gravid rate and the sporozoite rate. *Annals of Tropical Medicine & Parasitology* **48:** 58-74.

654

655   **Gillies MT, Hamon J, Davidson G, de Meillon B, Mattingly PF. 1961.** *A practical guide*

656   *for malaria entomologist in the African region of WHO*. WHO, Geneva. 276pp

657

658   **Govella NJ, Chaki PP, Geissbuehler Y, Kannady K. Okumu F.O., Charlwood J.D., Anderson R.**
659   **A., Killeen G. F. 2009**. A new tent trap for sampling exophagic and endophagic members of the
660   *Anopheles gambiae* complex. *Malaria Journal* **8**: Article 8.

661   **Haddow AJ. 1954.** Studies of the biting-habits of African mosquitos. An appraisal of methods
662   employed, with special reference to the twenty-four-hour catch. *Bulletin of Entomological Research*
663   **45**: 199–242.

664

665   **Hartig F. 2020.** DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression
666   Models. *R package version 0320*

667

668   **Hawkes F, Gibson G**. **2016.** Seeing is believing: the nocturnal malarial mosquito *Anopheles coluzzii*
669   responds to visual host-cues when odour indicates a host is nearby. *Parasites & Vectors*. **9**:320.

670   **Homan T, Hiscox A, Mweresa CK, Masiga D, Mukamba WR, OriaP, Maire N, di Pasquale A,**
671   **Silkey M, Alaii J, Bousema T, Leeuwis C, Smith TA, Takken W. 2016**. The effect of mass
672   mosquito trapping on malaria transmission and disease burden (SolarMal): a stepped-wedge cluster-
673   randomised trial. *Lancet* **6736**: 30445-30447.

674
675 **Killeen GF, Govella NJ, Lwetoijera DW, Okumu FO. 2016.** Most outdoor malaria transmission by
676 behaviourally resistant Anopheles arabiensis is mediated by mosquitoes that have previously been
677 inside houses. *Malaria Journal* **15**: Article 225
678
679 **Killeen GF, Seyoum A, Sikaala C, , Zomboko AS, Gimnig JE, Govella NJ, White MT. 2013.**
680 Eliminating malaria vectors. *Parasites & Vectors.* **6**: Article 172
681
682 **Killeen GF. 2014.** Characterizing, controlling and eliminating residual malaria transmission. *Malaria*
683 *Journal* **13**: Article330
684
685 **Knols BGJ, Takken W, Charlwood JD, De Jong R. 1995.** Species-specific attraction of Anopheles
686 mosquitoes (Diptera, Culicidae) to different humans in South-East Tanzania. *Proceedings of*
687 *Experimental and Applied Entomolgy (N.E.V.) Amsterdam*. **6**: 201-206.

688 **Lenth R. 2020.** emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version
689 1.4.8; https://CRAN.R-project.org/package=emmeans

690 **Maia MF, Robinson A, John A, Mgando J. Simfukwe E, Moore SJ. 2011.** Comparison of the
691 CDC backpack aspirator and the Prokopack aspirator for sampling indoor- and outdoor-resting
692 mosquitoes in southern Tanzania. *Parasites & Vectors* **4**: Article 124

693 **Nakagawa S, Schielzeth H. 2013.** A general and simple method for obtaining R2 from generalized
694 linear mixed-effects models. *Methods in Ecology and Evolution* **4**: 133-142.

695 **Penn DJ, Oberzaucher E, Grammer K, Fischer G, Soini HA, Wiesler D, Novotny MV, Dixon**
696 **SJ, Xu Y, Brereton RG. 2007.** Individual and gender fingerprints in human body odour. *Journal of*
697 *the Royal Society Interface*. **4**: 331-340.

698 **R Core Team. 2020**. R: A language and environment for statistical computing. Vienna, Austria: R
699 Foundation for Statistical Computing

700 **Scott JA, Brogdon WG, Collins FH. 1993.** Identification of single specimens of the *Anopheles*
701 *gambiae* complex by the polymerase chain reaction. *American Journal of Tropical Medicine &*
702 *Hygiene* **49:** 520–529.
703
704 **Shililu J, Ghebremeskel T, Seulu F, Mengistu S, Fekadu H, Zerom M, Asmelash GE, Sintasath**
705 **D, Mbogo C, Githure J, Brantly E, Beier JC, Novak RJ. 2004**. Seasonal abundance, vector
706 behavior, and malaria parasite transmission in Eritrea. *Journal of the American Mosquito Control*
707 *Association* **20**:155–64.
708
709 **Silver JB**. 2008. *Mosquito ecology, field sampling methods.* Dordrecht: Springer. 1477pp.
710
711 **Sinka ME, Bangs MJ, Manguin S, Coetzee M, Mbogo CM, Hemingway J, Patil AP, Temperley**
712 **WH, Gething PW, Kabaria CW, Okara RM, van Boeckel T, Godfray HCJ, Harbach RE, Hay**
713 **SI. 2010.** The dominant Anopheles vectors of human malaria in Africa, Europe and the Middle East:
714 occurrence data, distribution maps and bionomic précis. *Parasites & Vectors*. **3**: Article 117
715

716 **Smallegange RC, Schmied WH, van Roey KJ, Verhulst NO. Spitzen J, Mukabana WR, Takken**
717 **W. 2010**. Sugar-fermenting yeast as an organic source of carbon dioxide to attract the malaria
718 mosquito *Anopheles gambiae*. *Malaria Journal* **9**: Article 292
719
720 **Southwood TRE. 1978.** *Ecological methods: with particular reference to the study of insect*
721 *populations.* Chapman & Hall. 524pp
722
723 **Vazquez-Prokopec G. 2009.** A New, cost-effective, battery-powered aspirator for adult mosquito
724 collections. *Journal of Medical Entomology* **46**: 1256-1259.
725
726 **Verdonschot PFM, Besse-Lototoskaya AA. 2014.** Flight distance of mosquitoes (Culicidae): A
727 metadata analysis to support the management of barrier zones around rewetted and newly constructed
728 wetlands. *Limnologica*. **45**: 69-79.
729
730 **Wiebe A, Longbottom J, Gleave K, Sinka ME, Massey NC, Cameron E, Bhatt S, Gething PW,**
731 **Hemingway J, Smith DL, Coleman M, Moyes CL. 2017.** Geographical distributions of African
732 malaria vector sibling species and evidence for insecticide resistance. *Malaria Journal* **16**: Article 85
733
734 **Williams CB. 1937.** The use of logarithms in the interpretation of certain entomological problems.
735 *Annals of Applied Biology* **24**: 404–414.
736
737 **World Health Organization. 2017a.** Global vector control response -2030. 2017. Geneva: World
738 Health Organization.
739
740 **World Health Organisation. 2017b.** Ethical issues associated with vector-borne diseases. Geneva:
741 World Health Organisation.
742
743 **World Health Organization: World malaria report. 2018**. Geneva: World Health Organization.
744
745 **Zippin C. 1956.** An evaluation of the removal method of estimating animal populations. *Biometrics*
746 **12:** 163-89.
747
748 **Zippin C. 1958.** The removal method of population estimation. *Journal of Wildlife Management* **22:**
749 82-90.
750

751 Acknowledgements
752

753 We would like to thank the students of the College of Health Sciences, especially Kokob Tesfay,
754 for their assistance during the experiments. We would also like to thank the editor and reviewers
755 for their perceptive comments which helped us to considerably improve the manuscript.

756

Figure 1

Map of the study area © 2019 Google Image Landsat/Copernicus

The village of Adi Boskal, Anseba Zoba, Eritrea, showing the location of the outdoor trapping sites (yellow circles with numbers representing catch number), the sentinel tent-trap (white square) and the two sheds used for the collection of resting insects (red triangles). Also shown is the location where the exit collections took place © 2019 Google Image Landsat/Copernicus

Figure 2

Furvela tent-trap with lure attached

Position of the lure attached below the opening of the Furvela tent-trap. photo © JD Charlwood

Figure 3

Furvela tent-trap type II

Close up of the attachment of the CDC trap body to the tent type II trap. A - plywood board with circular opening equivalent to the size of the CDC trap, B - L-shaped supports for the CDC trap, C - CDC trap body with light and grid removed, D - Tent with zip open to allow odor and carbon dioxide easy egress. Photo © JD Charlwood

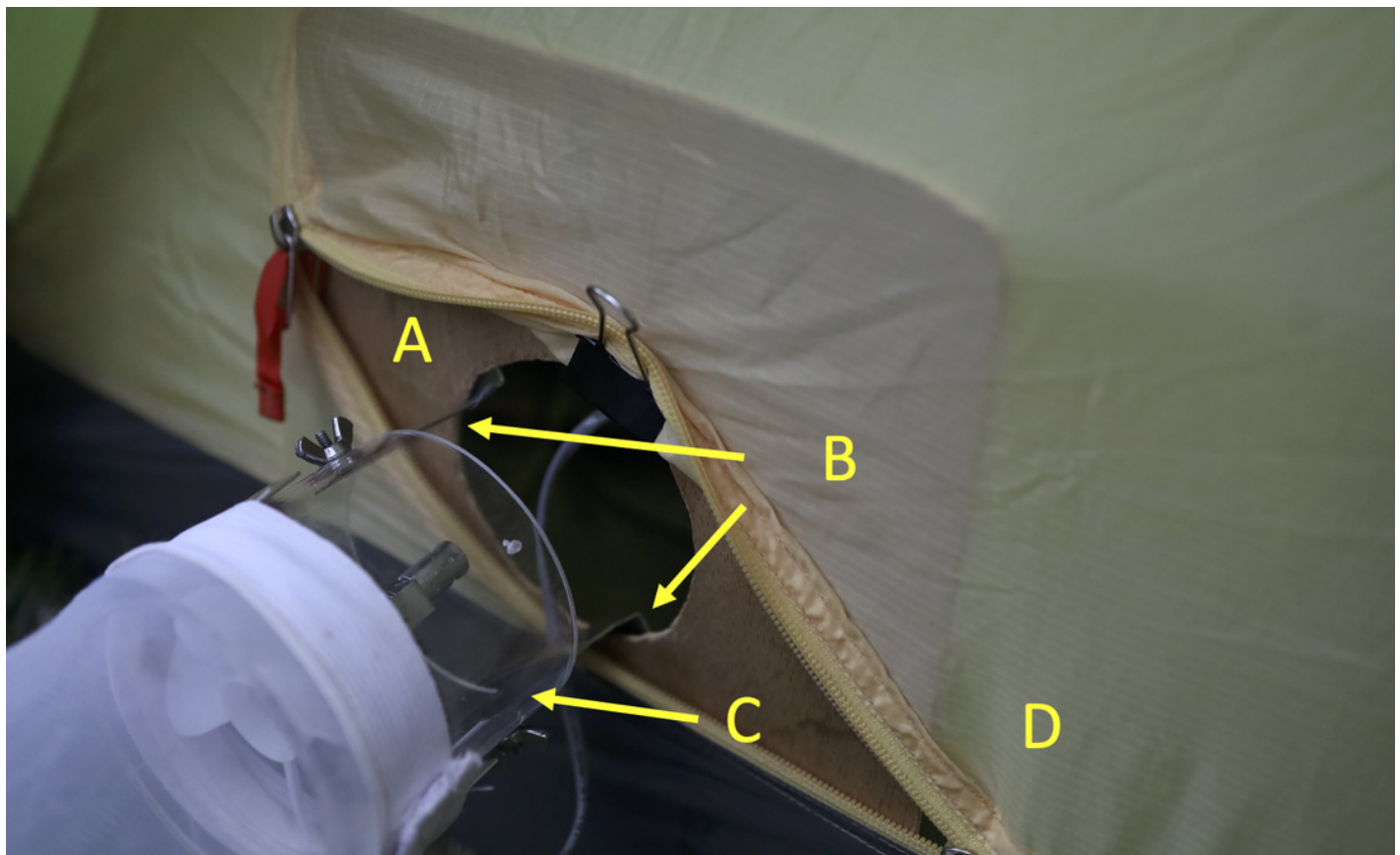



Figure 4

Suna trap with carbon dioxide

Suna trap in situ in an area close to Adi Boskal. A - Tripod supporting the trap, B - Trap cover, C - Trap inlet, D - 12 volt lead/acid battery powering the trap, E - 5 litre bottle containing the sugar/yeast mix used to generate carbon dioxide, F - Overflow bottle. An artificial lure is also placed inside the trap.Note the tent-trap in the distance. Photo © JD Charlwood

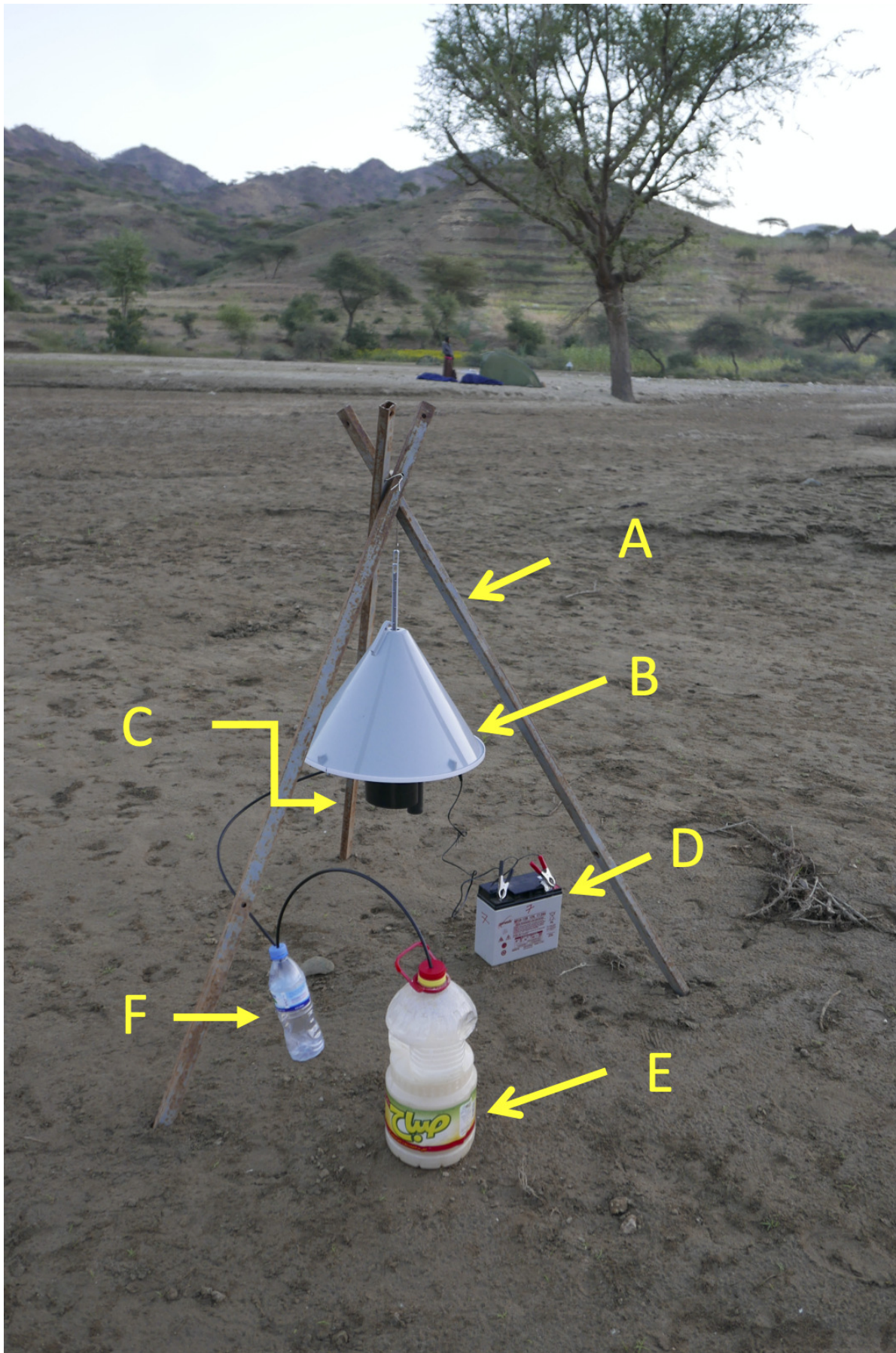



Figure 5

Set up of mosquito net over vegetation for use in the collection of mosquitoes leaving diurnal resting at dusk

(A) Double sized mosquito net placed horizontally over opening in vegetation, (B) Material used to cover any excess openings, (C) Pole to support net in place.

Figure 6

Daigram of mosquito abdomens at different stages of gonotrophic development

A) unfed, no blood or developing eggs visible; B) Engorged, abdomen full of fresh blood; C) Semi-gravid, anterior half of abdomen with dark blood and posterior half showing developing eggs; D) Gravid, abdomen full of eggs (Redrawn from Gillies et al., 1961)

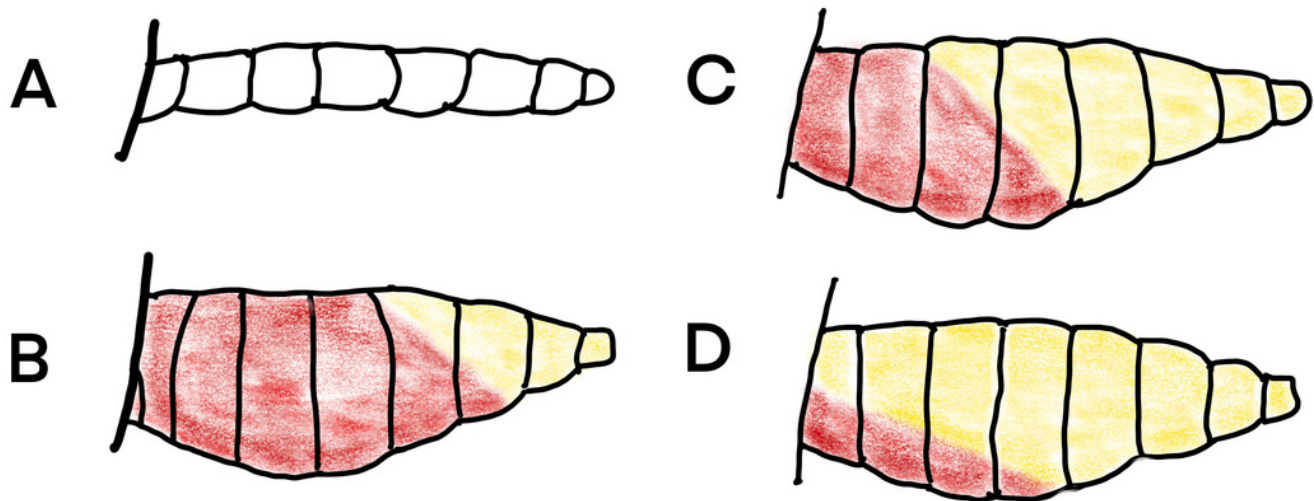



Figure 7

Tent traps and parous rates

Mean number of *Anopheles arabiensis* collected in experimental tent traps (on a log scale) and proportion of the collection that was parous by date of collection, Adi Boskal, Anseba zoba, Eritrea.

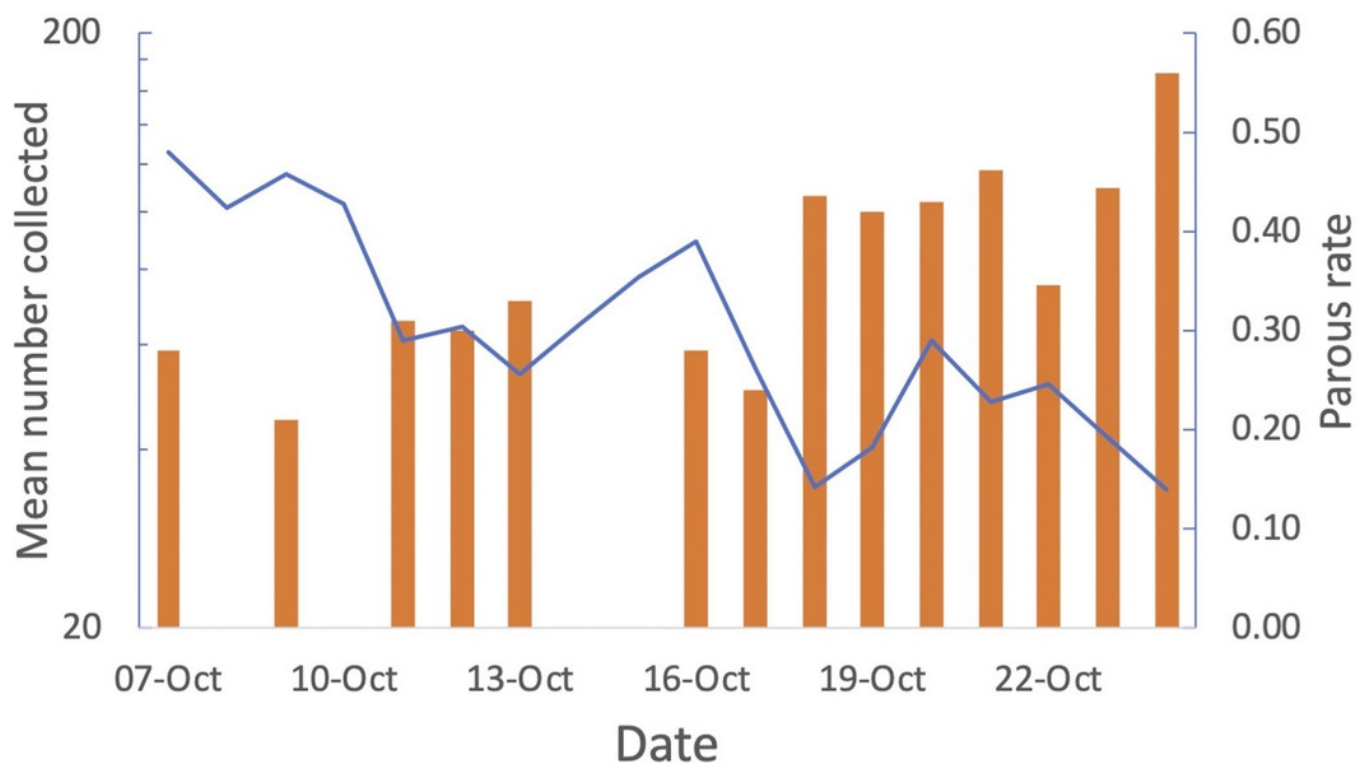



Figure 8

Resting collections of *Anopheles arabiensis*

Relationship between collection methods, shed and sampling round on numbers of *Anopheles arabiensis* caught resting, Adi Boskal, Eritrea. (A) Shed 2, Round 1 (B) Shed 2, Round 2. (C) Shed 2 Round 3. (D) Shed 2 Round 4. (E) Shed 2 Round 5. (F) Shed 1 Round 1. (G) Shed 1 Round 2. (H) Shed 1 Round 3. (I) Shed 1 Round 4. (J) Shed 1 Round 5. In each box the method used from left to right was Manual aspirator, Backpack aspirator and Procopack aspirator.

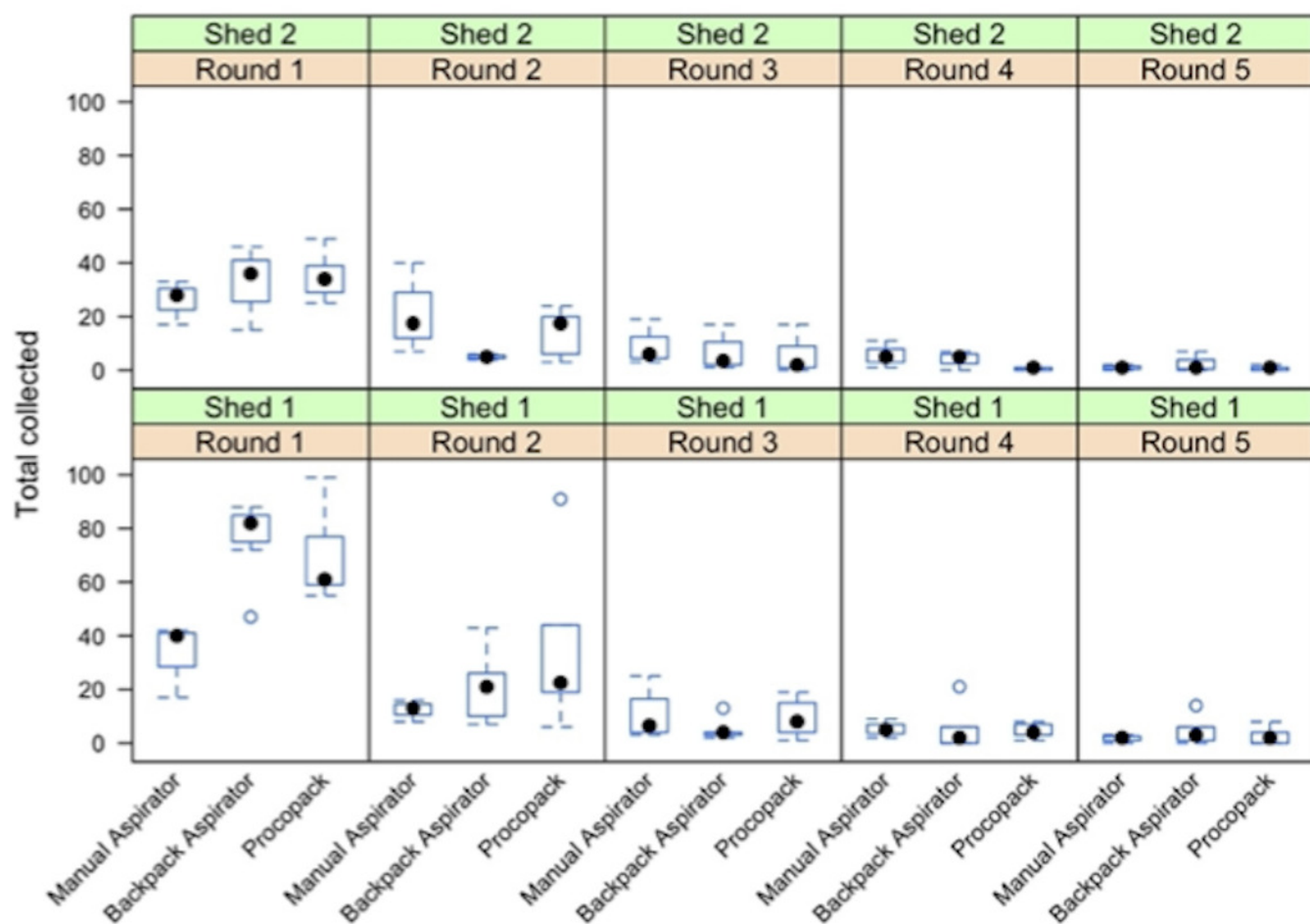



Figure 9

Comparison between sampling techniques for indoor resting mosquitoes

Performance of Backpack and Prokopack sampling indoor resting *Anopheles arabiensis*
compared to manual aspiration (reference), Adi Boskal, Eritrea

Backpack Aspirator

Procopack

Shed 2

Manual Aspirator:Round 2

Backpack Aspirator:Round 2

Procopack:Round 2

Manual Aspirator:Round 3

Backpack Aspirator:Round 3

Procopack:Round 3

Manual Aspirator:Round 4

Backpack Aspirator:Round 4

Procopack:Round 4

Manual Aspirator:Round 5

Backpack Aspirator:Round 5

Procopack:Round 5

Random Effects

$\sigma^2 = 0.25$

$ICC = 0.42$

Marginal $R^2 = 0.770$

Conditional $R^2 = 0.866$

0.01   0.05 0.1   0.5   1   5   10

Incidence Rate Ratios

Figure 10

Collection of mosquitoes exiting vegetation at sunset

Numbers of male, unfed female and gravid female *Anopheles arabiensis* exiting vegetation according to light-level, Adi Boskal, Eritrea.

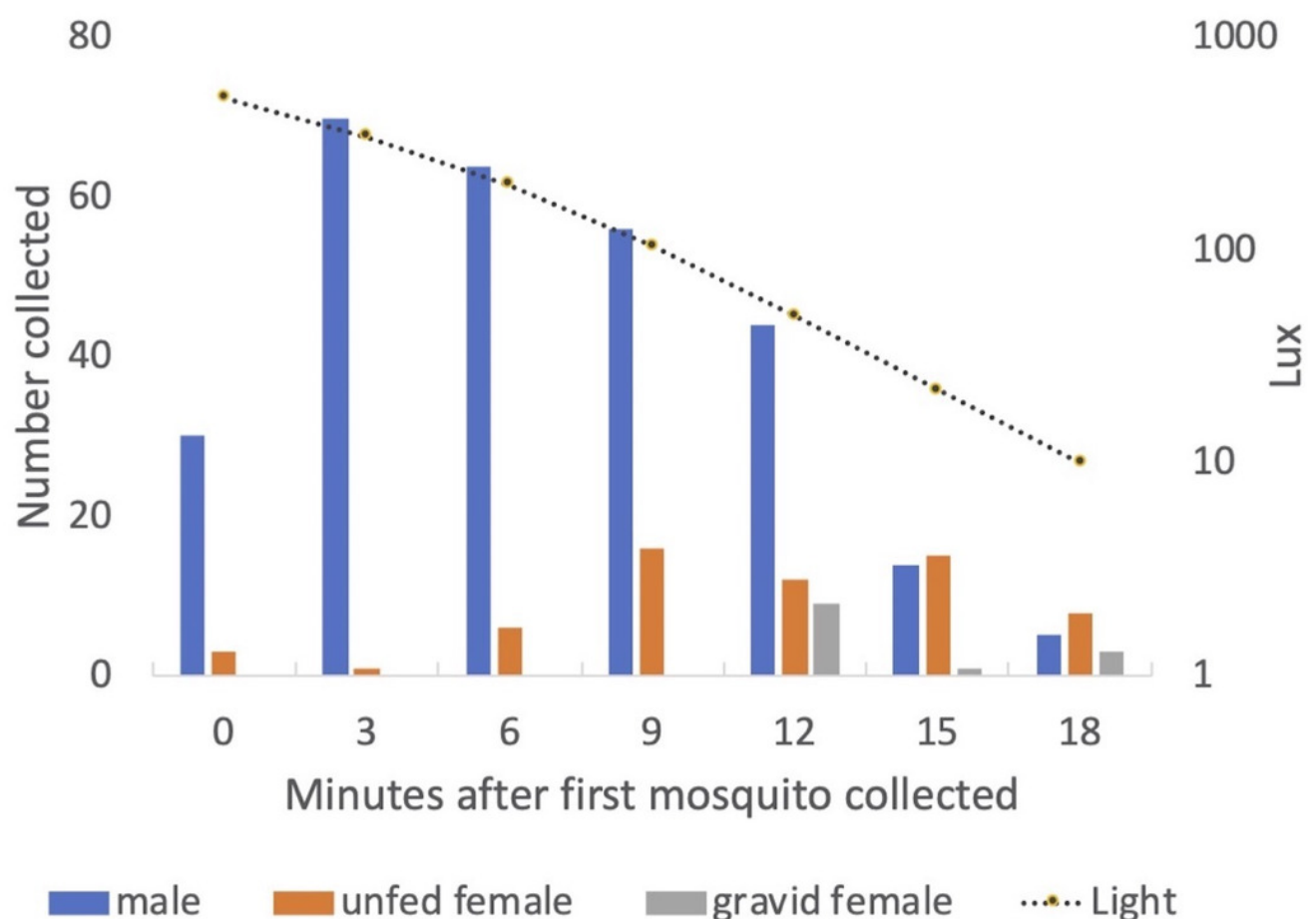
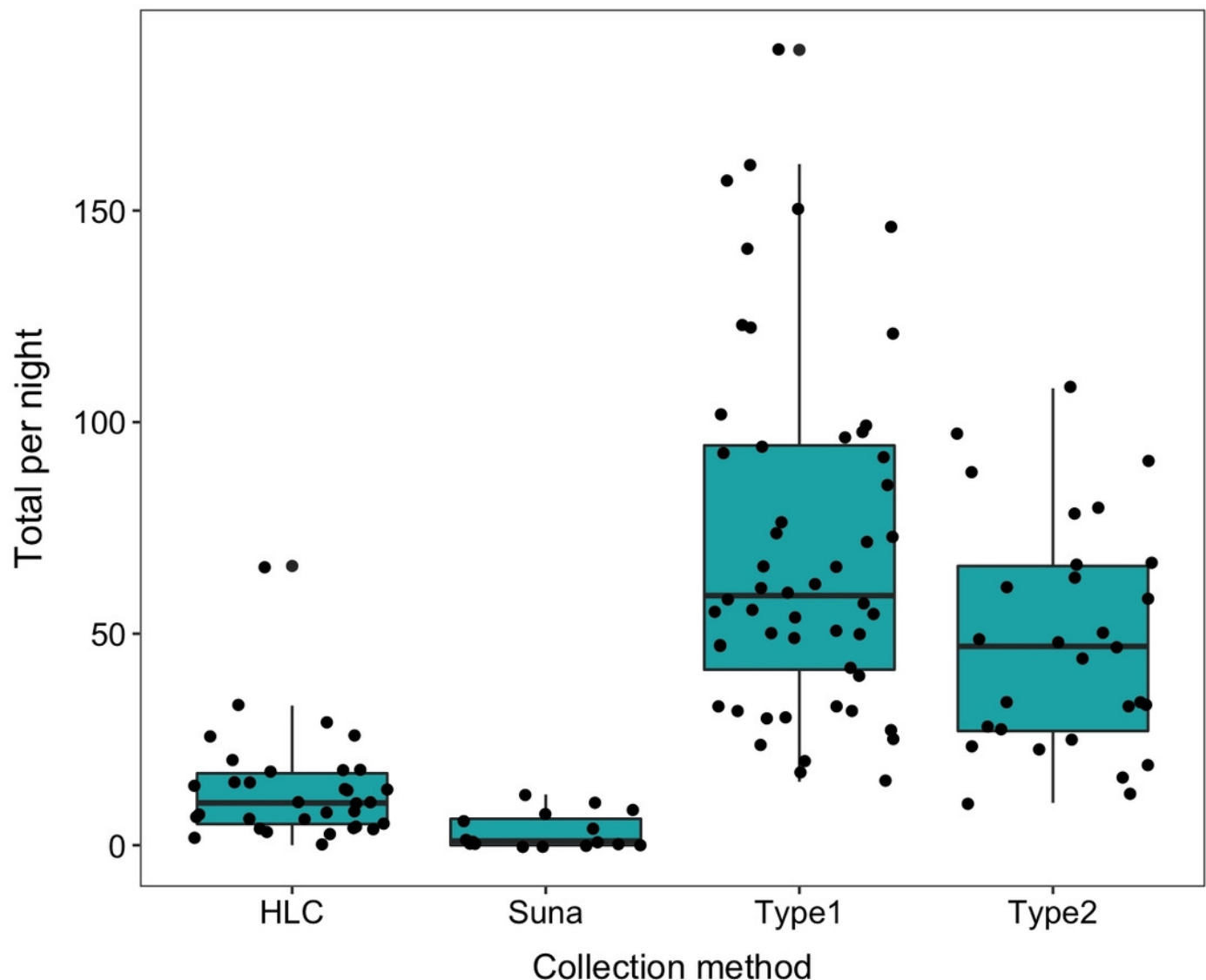



Figure 11

Comparison between collection methods of host seeking mosquitoes.

Density of host-seeking *Anopheles arabiensis* obtained by different sampling methods in Adi Boskal, Eritrea

Comparison of abundance of *An. arabiensis* found resting indoors at site 1 and site 2 estimated by removal sampling. N : Estimated total abundance and p : probability of mosquito collection

Method	Parameter	Shed 1				Shed 2			
			SE	95% CI	Proportion in round 1		SE	95% CI	Proportion in round 1
Manual Aspirator	Total	100			0.34	165			0.25
	Estimate	207	4.42	198.33 - 215.67		218	4.77	208.65 - 227.35	
	p	0.46	0.03	0.39 - 0.53		0.45	0.03	0.39 - 0.52	
Backpack	Total	382			0.67	56			0.34
	Estimate	725	1.78	721.52 - 728.48		161	2.85	155.41 - 166.59	
	p	0.67	0.02	0.64 - 0.70		0.51	0.04	0.43 - 0.58	
Procopack	Total	318			0.54	302			0.59
	Estimate	648	3.43	641.27 - 654.73		371	1.27	368.51 - 373.49	
	p	0.58	0.02	0.55 - 0.61		0.67	0.02	0.63 - 0.71	

Mean number of *An. arabiensis* collected from two animal sheds when collections had or had not been made the previous day, Adi Boskal, Anseba zoba, Eritrea. (UF – unfed, PF – part-fed, BF – blood-fed, SG – semi-gravid, GR – gravid).

	UF %, [95% C.I.]	PF %, [95% C.I.]	BF %, [95% C.I.]	SG %, [95% C.I.]	GR %, [95% C.I.]
No Previous Collection					
Total Shed 1	4	26	189	80	145
Total Shed 2	10	35	126	44	34
Shed 1	0.9,[0.02, 1.8]	5.9,[3.7, 8.0]	42.6,[38.0, 47.2]	18.0,[14.4, 21.6]	32.7,[28.3,37.0]
Shed 2	4.0,[1.6, 6.5]	14.1,[9.7, 18.4]	50.6,[44.4, 56.8]	17.7,[12.9, 22.4]	13.7,[9.4, 17.9]
Previous Collection					
Total Shed 1	19	36	167	79	55
Total Shed 2	15	42	131	41	45
Shed 1	5.3,[3.0, 7.7]	10.1,[7.0, 13.2]	46.9,[41.7, 52.1]	22.2,[17.9, 26.5]	15.5, [11.7, 19.2]
Shed 2	5.5,[2.8, 8.2]	15.3,[11.1, 19.6]	47.8,[41.9, 53.7]	15.0,[10.7,19.2]	16.4,[12.0, 17.9]

Number of collections, total collected geometric mean number per collection and standard deviation of *An. arabiensis* collected host seeking, Adi Boskal, Anseba zoba, Eritrea.

Collection	HLC	Tent	Sentinel	Suna with CO_2	Suna without CO_2
n	8	19	30	8	8
Total collected	296	1248	2107	40	10
Geometric Mean	35.0	72.4	162.2	3.1	0.6
S.D.	12.9	19.3	45.7	4.6	2.7