A comparative assessment of adult mosquito trapping methods to estimate spatial patterns of abundance and community composition in southern Africa

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Abstract

Background. Assessing adult mosquito populations is an important component of disease surveillance programs and ecosystem health assessments. Inference from adult trapping datasets involves comparing populations across space and time, but comparisons based on different trapping methods may be biased if traps have different efficiencies or sample different subsets of the mosquito community.

Methods. We compared four widely-used trapping methods for adult mosquito data collection in Kruger National Park (KNP), South Africa: Centers for Disease Control miniature light trap (CDC), Biogents Sentinel trap (BG), Biogents gravid *Aedes* trap (GAT), and a CO_2-baited net trap. We quantified how trap choice and sampling effort influence inferences on the regional distribution of mosquito abundance, richness, and community composition.

Results. The CDC and net traps together collected 96% (47% and 49% individually) of the 955 female mosquitoes sampled and 100% (85% and 78% individually) of the 40 species or species complexes identified. The CDC and net trap also identified similar regional patterns of community composition. However, inference on the regional patterns of abundance differed between these traps because mosquito abundance in the net trap was influenced by variation in weather conditions. The BG and GAT traps collected significantly fewer mosquitoes, limiting regional comparisons of abundance and community composition.

Conclusion: This study represents the first systematic assessment of trapping methods in in natural savanna ecosystems in southern Africa. We recommend the CDC trap or the combined use of the net and CDC trap for future monitoring and surveillance programs.

Keywords

Arboviruses, community composition; Kruger National Park; Mosquito; South Africa; Trap Bias; Vector

Background

Adult mosquito sampling is a key component of mosquito surveillance [1–4], but trapping success may vary across studies due to differences in trapping methods. Different traps vary in their ability to catch certain species [5–8]. For example, the dominant species attracted with light-baited traps may differ from those attracted to traps baited with carbon dioxide or live hosts [9]. Additionally, sampling conditions such as the number of nights over which sampling occurs and weather conditions may also influence trapping success [10], with some species and traps potentially more affected than others. This variation in trapping outcome may not limit inference on the presence or absence of common species (e.g. the information used in global risk maps [11,12]). However, it does limit inference based on comparing patterns of diversity or abundance across space or time [7]. Given that these comparisons are required to evaluate the ecological or anthropogenic drivers of mosquito populations and disease risk, choice of trapping methods presents challenges and opportunities for optimizing sampling efforts.

Previous studies have evaluated trapping methods in Europe, North America, and South America [5–8,13] while studies in southern Africa remain relatively limited. The mosquito fauna (Diptera: Cluicidae) of southern Africa consist of over 216 species, many of

65 which are endemic to the region [14]. Additional studies evaluating trapping methods are
66 needed to evaluate if traps designed for other locations and species perform equally well in
67 the rich communities in southern Africa. For example, a recent comparison of four traps in
68 Germany found that the Biogents Sentinel trap (BG trap) collected the highest number of
69 individuals in urban and snowmelt forest environments where *Culex* species predominate.
70 In contrast, the Centers for Disease Control miniature light trap (CDC trap) collected the
71 most mosquitoes in floodplain environments where *Aedes vexans* predominate, suggesting
72 that the preferred trapping device may vary by habitat and community composition [7].
73 Qualitative evaluations in southern Africa suggest different traps likely collect different
74 subsets of the mosquito community [15]. However, the two studies systematically
75 evaluating trapping methods in southern Africa focus only on house-based trapping in
76 residential areas [16,17].
77 An evaluation of trapping methods is also needed to influence the design of future
78 mosquito surveillance efforts. Such efforts could provide baseline information for public
79 health interventions by identifying hotspots with a high abundance of vector species
80 [18,19]. There were more than 26,000 estimated cases of malaria in southern Africa in
81 2017 (Botswana, Namibia, South Africa, Lesoto, Swaziland) [20]; key malaria vectors
82 include members of the *Anopheles gambiae* complex *(An. arabiensis, An. gambiae, An.*
83 *merus)* [21–23] and the *An. funestus* complex [24]. Mosquito-borne livestock infections are
84 also a concern, as outbreaks of West Nile virus, Rift Valley fever, Sindbis, and Wesselsbron
85 occur [25,26]. Key vectors for these viral infections include, *Aedes caballus, Ae.*
86 *circumluteolus, Ae. dentatus, Ae. juppi, Ae. mcintoshi, Ae. ochraceus, Culex pipiens, Cx.*
87 *quinquefasciatus, Cx. univittatus,* and *Cx. theileri* (based on suspected or known prime

88 vectors in Africa, reviewed elsewhere [26]). Previous work has characterized the
89 distribution [27], ecological drivers [28], and consequences for malaria risk [29] of key
90 Anopheline species. Understanding the distribution and drivers of non-Anophelinae
91 mosquito species in southern Africa (but see [15,30]) could facilitate informed
92 management of a broader range of mosquito-borne disease or the development of vector
93 control programs that target multiple infections.

94 In this study, we assessed four commonly used trapping methods for adult mosquito
95 data collection in natural savanna ecosystems. We compared estimates of abundance and
96 community composition from four traps: Centers for Disease Control miniature light trap
97 (CDC), Biogents Sentinel trap (BG), Biogents gravid *Aedes* trap (GAT), and a CO_2-baited net
98 trap. We also evaluated the importance of trapping method for inferring the regional
99 distribution of mosquito abundance, mosquito community composition, and the
100 distribution of key disease vectors.

101

102 Methods

103 *Study location*

104 We sampled twenty sites within five regions of Kruger National Park (KNP), South
105 Africa (Fig 1). Sampling sites within KNP were chosen to cover the geographic extent of the
106 park and to capture the variability in rainfall, geology and vegetation types within KNP
107 (between 22°31' and 25°31 S and 30°45 and 32°00 E) [31]. This region experiences
108 summer rainfall between November and April, and we selected our sampling to occur from
109 March to April 2017, when mosquito populations are generally high. We sampled 5 out of
110 the 22 management regions within the park: Malelane, Skukuza, Satara, Shingwedzi, and

Punda Maria. Within each region, we sampled at four sites, which were selected based on multiple criteria (Fig 1). The primary selection criterion was to sample from water bodies that represented diverse types of wetlands (temporary ponds, permanent ponds, rivers). Additional criteria stipulated that the water bodies were at least 2 km away from one another to avoid sampling mosquitoes from adjacent water bodies and within 25 km from one another for sampling logistics. These distances are justified based on mean mosquito dispersal rates, which range from 35m to 1.4 km depending on the species and habitat [32–35]. Although dispersal farther than 2 km is possible, it is uncommon [36] and we do not expect it to influence our results because only one pair of sites in Shingwedzi was located within 1km of each other due limited surface water availability in the area.

Trapping and identification

We trapped at each site within a region for four consecutive nights and moved sequentially between regions each week in random order (Skukuza: 20-March to 23-March-2017; Malelane 27-March to 30-March-2017; Satara 3-April to 6-April-2017; Shingwedzi 17-April to 20-April; Punda Maria 24-April to 27-April-2017). The four traps we used were the Centers for Disease Control miniature light trap with an incandescent light (Bioquip Inc, USA), a Biogents Sentinel trap (Biogents AG, Germany), the Biogents gravid *Aedes* trap (Biogents AG, Germany), and the net trap. Although the net trap is not commercially available, it is easily and inexpensively made from netting and poles (see [15,30]). For consistency in sampling and to minimize any bias due to the traps' location, we set up the traps at a similar distance away from the water body (15-25m) and consistently rotated their positions around the water body at each site. The four positions were defined based on their cardinal direction around the water body. Position 1 was set

134 directly east of the water body and positions 2-4 were set approximately 30-50m away
135 from each other moving northward around the water body. For the first two nights of
136 trapping, the BG trap was set in position 1, the GAT trap in position 2, the net trap in
137 position three, and the CDC trap in position 4. For the second two nights of trapping, the
138 traps were rotated one position, such that the CDC trap was moved to position 1.

139 Our trap-use protocol was based on preliminary trapping work and expertise from
140 mosquito surveillance in southern Africa [15,30]. Specifically, we equipped the CDC trap,
141 BG trap, and net trap with a closed container containing 200-400g of dry ice. To ensure the
142 dry ice would last overnight, we reduced sublimation by wrapping the dry ice in multiple
143 layers of newspaper and ensuring the container was closed except for 2 small holes. We
144 placed the dry ice containers inside the BG traps, at the center of the net trap, and hanging
145 with the CDC trap according to the manufacturer's instructions. In addition to the spatial
146 locations defined above, we hung the CDC light trap on a branch so it was approximately
147 1m from the ground, and we placed the BG and GAT traps on the ground. We ensured the
148 traps were placed close to the vegetation, but not directly under it, which has been shown
149 to improve trapping success [10]. If available, we placed the net trap in slight shade and
150 pulled the netting down to leave an approximately 10cm opening from the ground. Our
151 trapping schedule required traps to be set up in the field before dusk (4-6pm) and emptied
152 again at dawn (6-7am). The timing of this sample collection was used because the net trap
153 requires clearing at dawn, as mosquitoes may leave the trap after sunrise. Due to rainfall,
154 we excluded one trapping night at all sites in Satara and Punda Maria from all analyses.

155 During the study, five sampling events (nights at one site) with the BG trap, two
156 sampling events with the CDC trap, and one sampling event with the net trap were lost due

157 to animal interference that damaged the trap. An additional 11 nights of BG trap sampling
158 were lost due to other logistical reasons, primarily permanent damage by hyenas, which we
159 hypothesize is due to the Octenol bait. Our statistical analyses account for trap loss by
160 excluding data or comparisons from damaged traps.

161 Directly after emptying the traps, we stored the mosquitoes on dry ice in the field
162 and at -20°C until identification. For identification of Anophelinae mosquitoes, we used
163 identification literature from Gilles & Coutzee [37]. All Anophelinae were identified to
164 species except for members of *An. funestus* complex and the *An. gambiae* complex (referred
165 to here as *An. gambiae s.l*)., which require molecular methods for identification [38]. For
166 identification of Culicinae, we used the key by Peter Jupp [14]. Species were identified
167 independently in duplicate by coauthors. Because species identification in the *Aedes vexans*
168 complex (*Ae. vexans, Ae. hirsutus, Ae. fowleri, Ae. durbanensis, Ae. natronius*), and the *Aedes*
169 *dentatus* complex (*Ae. dentatus, Ae. subdentatus Ae. pachyurus, Ae. bevisi, Ae. cumminsii*)
170 were inconsistent at the species level, we aggregated them to the species complex level.

171 *Abiotic measurements*

172 Temperature and rainfall within KNP follow a north-south gradient, with the highest
173 values in the south-west [39]. Temperature, relative humidity, and wind speed may
174 influence trapping success; they were monitored using a Kestrel 3000 handheld weather
175 meter (NK Inc., Boothwyn, PA, USA). We calculated the median temperature, relative
176 humidity, and wind speed across sites within a region for each morning at the time of
177 collection (Table S1). All environmental variables were standardized for analysis, by
178 centering and dividing by one standard deviation.

179 *Assessing how the number of traps and trapping nights influences mosquito richness*

We assessed how observed species richness (the total number of unique species) saturates with sampling effort. We aggregated the data successively over 1, 2, 3, or 4 nights and calculated the cumulative proportion of species identified with an increasing proportion of nights. We also evaluated each trap's ability to estimate richness by comparing richness estimated in pairs of trap types at each site aggregated across all sampling nights using a Wilcoxon signed-rank test and a Bonferroni correction for multiple comparisons. This analysis accounts for trap losses by excluding comparisons from damaged traps.

Assessing whether trap type influences estimates of mosquito abundance and inference on the regional patterns of abundance

To test for differences in abundance, we compared the number of mosquitoes collected per night between pairs of traps. First, we quantified the relationship between trap type and abundance with a Wilcoxon signed-rank test and a Bonferroni correction for comparisons among the 4 traps. Then, we quantified the influence of trap type for inferences on the regional patterns of abundance using linear regressions with Poisson errors and a log-link function. The regression analyses assessed the relationship between a trap's nightly mosquito counts with region of the park (*region*) and weather conditions (wind speed, temperature, relative humidity (*RH*)). These assumptions result in the following full model,

$$\log(\mu_i) = \propto_{j[i]} + \beta_{1,k} region_k + \beta_2 windspeed_i + \beta_3 temperature_i + \beta_4 RH_i + \varepsilon_i \qquad (1)$$

where μ_i is the expected number of mosquitoes captured at evening *i* in the trap, ε_i is the Poisson distributed random error, and $\propto_{j[i]}$ is the site-specific intercept.

202 We fit the regression model separately to data from the CDC trap, the net trap, the
203 BG trap, and data from aggregating abundance across all traps at a site. Because no
204 individuals were collected in the GAT trap on most nights, we did not fit the model to data
205 from the GAT trap alone. For each dataset, we conducted model selection using backward
206 selection based on AICc and select the model with the lowest AICc value. From the selected
207 model, we evaluate $\beta_{1,k}$ to determine how the average counts of mosquitoes in region k
208 differed from counts in Malelane, and we evaluate $\beta_2, \beta_3, \beta_4$ to determine how the average
209 counts of mosquitoes vary with wind speed, temperature, and relative humidity,
210 respectively. If the relative values of $\beta_{1,k}$ are consistent among traps (e.g. $\beta_{1,1} > \beta_{1,2} > \beta_{1,3}$)
211 for models fit to data from different traps, then we conclude that trap choice does not
212 influence spatial comparisons among regions. We fit all regression models in R [40] using
213 the lme4 package [41].

214 *Assessing whether trap type influences estimates of mosquito community composition and*
215 *inference on the regional patterns of mosquito communities*

216 To assess if different traps provide different estimates of community composition,
217 we first evaluated if certain species were particularly attracted to one trap over the other.
218 We assessed species-specific trap bias by calculating the difference in the number of
219 individuals for each species sampled between each pair of traps collected at a site over all
220 nights of trapping. Because traps were paired at each site, we tested for differences
221 between the traps using a Wilcoxon signed-rank test and a Bonferroni correction for
222 multiple comparisons. We only compared the species-specific trap bias of common species,
223 defined as being observed in the dataset more than three times. Because 23 common

224 species were compared (k = 23), significant differences between traps occur when p-values
225 are less than p = 0.05/23. We assessed trap bias for rare species visually.
226 To test for differences in community composition among traps, we used a
227 nonparametric analysis of similarities analysis (ANOSIM), visualized potential differences
228 with nonmetric multidimensional scaling (NMDS), and quantified the influence of trap type
229 for inferences on the regional patterns of community composition with hierarchical
230 clustering. For all analyses, we calculated Bray-Curtis dissimilarity matrices based on the
231 trap-specific (BG, CDC, net) abundances of all taxa within a site aggregated across sampling
232 nights. The ANOSIM analysis is a non-parametric test for differences in mosquito
233 communities among traps that compares the ranks of Bray-Curtis dissimilarity measures
234 from samples collected from the same vs. different traps [42]. To visualize this, we created
235 an ordination of traps and sites in mosquito community space for each region of the park.
236 Before all ordinations, we applied a Wisconsin transformation followed by a square root
237 transformation to the species matrices, which standardizes by species maxima and reduces
238 the influence of highly abundant taxa, respectively [43]. The ordinations converged on a
239 stable two-dimensional solution, based on stress values. We conducted all community
240 analyses in R using the vegan package [44].
241 *Describing regional patterns of disease vectors*

242 We additionally describe how known prime vectors for West Nile virus (*Cx. pipiens,*
243 *Cx. quinquefasciatus, Cx. theileri, Cx. univittatus*), Rift Valley fever (*Ae. dentatus, Ae.*
244 *mcintoshi, Ae. ochraceus*), Sindbis (*Cx. univittatus*), and Wesselsbron (none found) are
245 distributed across regions [21,26]. Additional known prime vectors for these infections
246 were not found in the study (Ae. caballus, *Ae. circumluteolus, Ae. juppi*). Chikungunya and

247 dengue fever outbreaks are less common in South Africa [14], but we describe the
248 distribution of their vector, *Ae. aegypti* [21] because additional known prime vectors in
249 Africa were not found (*Ae. africanus, Ae. albopictus, Ae. cordellieri, Ae. furcifer*, *Ae.*
250 *luteocephalus*, *Ae. neoafricanus*, *Ae. taylori*). We focus on the known prime vectors for a
251 conservative description, but additional mosquito species are considered suspected vectors
252 (reviewed in [26]). We use the numbers of *Cx. pipiens* complex to approximate the numbers
253 of *Cx. pipiens* and *Cx. quinquefasciatus; Cx. univittatus* complex for *Cx. univittatus; Ae.*
254 *dentatus* complex for *Ae. dentatus.* Although this assumption may hide epidemiologically
255 important variation, it is a valid approximation for these infections because multiple
256 members of the complex are known or suspected vectors. However, we do not plot the
257 distribution of malaria vectors because the most abundant species of *An. gambiae s.l.* in
258 KNP, *An. quadriannulatus*, is not a malaria vector [15].
259

260 **Results**

261 We collected 955 female mosquitoes, 946 (99.1%) of which were identified to the
262 level of species or species complex (Table S2). The most common species included
263 members of the *Culex univittatus* complex, *Aedes vexans* complex, and *Culex pipiens*
264 complex. We also collected over 50 *Anopheles gambiae* s.l., *Anopheles pretoriensis*, and
265 *Culex theileri* females.

266 *Mosquito communities can be characterized with the net and CDC traps after multiple*
267 *trapping nights*

268 Based on all traps together, mosquito community richness was sensitive to the
269 number of sampling nights (Fig 2; Fig S1). Taken across all sites in Malelane, 89% (24/27)

270 of the total number of unique species identified after four trapping nights were collected by
271 night 2 and 96% (26/27) were collected by night 3. In Shingwedzi, 79% (19/24) of the
272 species were collected by night 2 and 96% (23/24) by night 3. In Punda Maria, 53% (8/15)
273 of the species were collected by night 2 and 87% (13/15) by night 3. We note that these
274 percentages overestimate the percent of richness captured because species accumulation
275 curves suggest more than four nights of sampling are required to estimate total species
276 richness (Fig 2). The CDC and net trap together sampled all of the mosquito species
277 captured (range across regions, 93-100%), while the BG and GAT trap captured far fewer
278 species (Fig 2). Estimates of richness did not significantly differ between samples from the
279 net or CDC trap (Wilcoxon signed-rank test, p-value = 0.940), but estimates based on the
280 BG trap were lower than both the net and CDC trap (p-values <0.001).

281 *Daily mosquito abundance estimates were comparable between the net and CDC traps but not*
282 *the BG and GAT trap*

283 Most female individuals were collected in the CDC or the net trap, while the BG and
284 GAT trap captured far fewer individuals (Fig 3a). Together, the CDC and net trap sampled
285 96% of the individuals collected (range across regions, 94-99%). Estimates of mosquito
286 abundance did not significantly differ based on samples from the net or CDC trap (Wilcoxon
287 signed-rank test, p-value = 0.095), but estimates based on the BG trap were lower than
288 both the net and CDC trap (p-values <0.001; Fig 3b). Regression analyses showed that trap
289 choice influences comparisons between regions. All traps identified similar trends, with the
290 highest numbers captured in Malelane and the lowest numbers captured in Punda Maria
291 (Fig3c; Table S3). However, this spatial pattern was only significantly different for models
292 fit to the CDC data ($\beta_{1,1}$ for Punda Maria vs. Malelane = -1.88, p-value = 0.002). Unlike the

293 CDC trap, the BG and net trap were influenced by weather conditions (Table S3). The BG
294 and net trap collected higher numbers in warm, low wind conditions (Fig 3c). The net trap
295 also collected higher numbers in low relative humidity conditions. Results based on counts
296 aggregated from multiple traps were similar to results based on counts from either the CDC
297 or net trap alone (Fig 3c; Table S3).

298 *Mosquito community composition was consistent between the net and CDC trap but not the*
299 *BG trap*

300 Community composition was similar between the net and CDC trap, but not for the
301 BG trap (ANOSIM overall R = 0.126, p-value = 0.04; pairwise p-values: net vs. CDC p-value =
302 0.894; net vs. BG p-value = 0.023; CDC vs. BG p-value = 0.009). NMDS ordinations of traps
303 in species space reflect this relationship although some variation across regions of the park
304 does exist (Fig S2; Table S4). However, we did not find evidence for species-specific bias
305 between any of the traps, suggesting that differences in community composition in the BG
306 trap are driven by the relatively lower abundance collected in the trap (Fig 4; Fig S3). For
307 common species collected in the net vs. CDC trap, the number of individuals collected was
308 not significantly different between the traps (Fig 4a; No hypothesis tests for individual
309 species were significant). Rare species also did not show trap biases and include *Ae.*
310 *aerarius, Ae. metallicus, Ae. unidentatus, An. maculipalpis, An. ziemanni, Cx. antennatus, Cx.*
311 *bitaenorhynchus, Cx. nebulosus, Lutzia tigripes, Mansonia africana,* and *Uranotaenia balfouri*
312 (Fig S4). For comparisons with the BG trap, the net and CDC traps both collected higher
313 numbers of individuals, but there were no species or genus shifts driving this pattern (Fig
314 4b-c; Fig S3).

315 Although trap choice influences estimates of community composition (e.g.
316 community richness, ordinations), hierarchical cluster analysis suggests that may be less
317 important for comparisons between regions (Fig 5; Fig S5). Regional patterns in mosquito
318 communities were consistent across trap types, with samples from Malelane and Satara
319 being more similar than samples from Shingwedzi. Mosquito communities estimated from
320 the CDC and net trap were clustered by region (Fig S5), indicating that communities within
321 a region were more closely related to each other than communities between regions
322 regardless of the net or CDC trap. In contrast, samples from the BG trap were clustered
323 separately from the samples from the net and CDC trap due to the overall lower
324 abundances in the BG trap (Fig 5). We, therefore, describe the regional patterns of disease
325 vectors based on data from all traps together (Fig 6). Rift Valley fever vectors were most
326 common in Satara while West Nile virus and Sindbis vectors were more common in
327 Shingwedzi and Punda Maria. We did not find known vectors for Wesselsbron (*Ae. caballus,*
328 *Ae. circumluteolus*) within the park.
329

330 **Discussion**

331 Mosquito trapping is used for disease surveillance, biodiversity surveys, and
332 nuisance-reduction. In light of these multiple, non-exclusive aims, this study compared
333 traps based on four potential goals: collecting large numbers of mosquitoes; estimating
334 mosquito community composition, including vector or rare species; characterizing the
335 spatial patterns of abundance; and characterizing the spatial patterns of community
336 composition. We expected trade-offs among these aims, for example with traps specializing
337 on one vector to be less successful in estimating community richness (and vice versa, e.g.

338 [45]). In contrast to this expectation, our results indicated that the net and CDC trap
339 consistently performed best across multiple outcomes.
340 The CDC and net trap collected higher abundances and more unique species
341 compared to the BG and GAT traps, which collected no mosquitoes on most nights. This
342 result is different from trap comparison studies in Europe [7], the U.S. (BG: [13], GAT: [46]),
343 and South America (BG: [47], GAT: [48]), where both the BG and GAT traps have been
344 shown to perform well. One reason for their relatively low success within KNP could be the
345 diversity and types of species present within the park. The GAT trap has been designed to
346 capture container-breeding species, such as *Ae. aegypti* [48], and the BG trap performs well
347 in sampling *Ae. aegypti, Ae. albopictus*, and *Cx. pipiens* [7,49]. Although *Ae. aegypti* was
348 present in our study and previous studies [15], they were relatively rare (Table S1). The BG
349 trap's sampling efficiency for the two most-common species complexes in our dataset –
350 members of the *Ae. vexans* complex and *Cx. univitatus* complex – is either low [7] for the *Ae.*
351 *vexans* complex or previously unknown for the *Cx. univitatus* complex. An alternative
352 reason for the low success of the BG trap could be that it is negatively influenced by the
353 presence of alternative refugia and oviposition sites [50]. Habitat heterogeneity is likely to
354 be higher within KNP compared to other, primarily urban or suburban environments
355 where mosquito traps have been evaluated ([13, 46, 47], but see [7]). Additional
356 comparisons in urban environments in southern Africa are needed to distinguish these two
357 hypotheses.
358 By providing a detailed comparison between the net and CDC traps, our results also
359 suggest that estimates of community richness and diversity are comparable between them.
360 The CDC and net traps differed, however, in their inference about which regions had the

361 highest mosquito abundance because the net trap was more sensitive to weather
362 conditions. This difference is likely to be important for studies comparing vector
363 abundance across space or time and suggests that vector surveillance or control programs
364 should use caution in comparing patterns of abundance between studies that did not use
365 the same trap. However, the consistent patterns of community composition in the net and
366 CDC trap suggest that comparisons of vector communities between studies using the net
367 and CDC trap are possible.

368 The choice between the CDC and net trap should also consider other features of the
369 traps, such as ease of use and specimen quality. For morphological identification, the net
370 trap has the advantage that mosquito specimens can be collected with minimal damage,
371 which makes them easier to identify [14]. Therefore, studies requiring precise species
372 identification such as biodiversity assessments may prefer the net trap. However, for
373 sampling large numbers of sites or sites in remote locations, the CDC trap has an advantage
374 because the timing of when traps need to be cleared is more flexible compared to the net
375 trap, which has to be cleared at sunrise. These practical considerations may mean that the
376 CDC trap is better suited for large, comparative studies.

377

378 Conclusions

379 After assessing four different mosquito trapping methods in a natural savanna
380 ecosystem, we recommend the net trap, the CDC trap, or their combined use for outdoor
381 mosquito surveillance in southern Africa. These traps performed well based on four
382 evaluation criteria: collecting large numbers of mosquitoes; estimating mosquito
383 community composition, including vector or rare species; characterizing the spatial

patterns of abundance; and characterizing the spatial patterns of community composition. This suggests they are appropriate for both biodiversity surveys and vector surveillance. As such, this study provides a valuable proof-of-principle for characterizing the spatial patterns of non-vectors as well as vectors for multiple diseases.

Declarations

Ethics approval: Not applicable

Consent for publication: Not applicable

Availability of data and materials: The dataset supporting the conclusions of this article are available in the dryad digital repository and the free online mosquito databases, VectorMap (http://vectormap.si.edu).

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References

1. Meyer SDB, Ritchie SA, Laurance SGW. Mosquito communities and disease risk influenced by land use change and seasonality in the Australian tropics. Parasit Vectors. 2016;9:387.

2. Janko MM, Irish SR, Reich BJ, Peterson M, Doctor SM, Mwandagalirwa MK, et al. The links between agriculture, *Anopheles* mosquitoes, and malaria risk in children younger than 5 years in the Democratic Republic of the Congo: a population-based, cross-sectional, spatial study. Lancet Planet Health. 2018;2:e74–82.

3. Diuk-Wasser MA, Brown HE, Andreadis TG, Fish D. Modeling the spatial distribution of mosquito vectors for West Nile virus in Connecticut, USA. Vector Borne Zoonotic Dis. 2006;6:283–95.

4. Galardo AKR, Zimmerman RH, Lounibos LP, Young LJ, Galardo CD, Arruda M, et al. Seasonal abundance of anopheline mosquitoes and their association with rainfall and malaria along the Matapí River, Amapá, Brazil. Med Vet Entomol. 2009;23:335–49.

5. Dennett JA, Vessey NY, Parsons RE. A comparison of seven traps used for collection of *Aedes albopictus* and *Aedes aegypti* originating from a large tire repository in Harris County (Houston), Texas. J Am Mosq Control Assoc. 2004;20:342–9.

428 6. Hoel DF, Kline DL, Allan SA. Evaluation of six mosquito traps for collection of *Aedes*
429 *albopictus* and associated mosquito species in a suburban in north central Florida. J Am Mosq
430 Control Assoc. 2009;25:47–57.

431 7. Lühken R, Pfitzner WP, Börstler J, Garms R, Huber K, Schork N, et al. Field evaluation of
432 four widely used mosquito traps in Central Europe. Parasit Vectors. 2014;7:268.

433 8. L'Ambert G, Ferre J-B, Schaffner F, Fontenille D. Comparison of different trapping methods
434 for surveillance of mosquito vectors of West Nile virus in Rhone Delta, France. J Vector Ecol.
435 2012;37:269–75.

436 9. Jupp PG, McIntosh BM, Nevill EM. A survey of the mosquito and culicoides faunas at two
437 localities in the Karoo region of South Africa with some observations on bionomics.
438 Onderstepoort J Vet Res. 1980;47:1–6.

439 10. Crepeau TN, Healy SP, Bartlett-Healy K, Unlu I, Farajollahi A, Fonseca DM. Effects of
440 Biogents Sentinel Trap field placement on capture rates of adult Asian tiger mosquitoes, Aedes
441 albopictus. PLoS One. 2013;8:e60524.

442 11. Kraemer MUG, Sinka ME, Duda KA, Mylne AQN, Shearer FM, Barker CM, et al. The
443 global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus*. eLife.
444 2015;4:e08347.

445 12. Hay SI, Sinka ME, Okara RM, Kabaria CW, Mbithi PM, Tago CC, et al. Developing Global
446 Maps of the Dominant *Anopheles* Vectors of Human Malaria. PLoS Med. 2010;7:e1000209.

447 13. Farajollahi A, Kesavaraju B, Price DC, Williams GM, Healy SP, Gaugler R, et al. Field
448 Efficacy of BG-Sentinel and Industry-Standard Traps for *Aedes albopictus* (Diptera: Culicidae)
449 and West Nile Virus Surveillance. J Med Entomol. 2009;46:919–25.

450 14. Jupp PG. Mosquitoes of southern Africa. P.O.Box 178 Hartebeespoort 0216, South Africa:
451 Ekogilde Publishers; 1996.

452 15. Cornel AJ, Lee Y, Almeida APG, Johnson T, Mouatcho J, Venter M, et al. Mosquito
453 community composition in South Africa and some neighboring countries. Parasit Vectors.
454 2018;11:331.

455 16. Sikaala CH, Killeen GF, Chanda J, Chinula D, Miller JM, Russell TL, et al. Evaluation of
456 alternative mosquito sampling methods for malaria vectors in Lowland South - East Zambia.
457 Parasit Vectors. 2013;6:91.

458 17. Govella NJ, Chaki PP, Mpangile JM, Killeen GF. Monitoring mosquitoes in urban Dar es
459 Salaam: Evaluation of resting boxes, window exit traps, CDC light traps, Ifakara tent traps and
460 human landing catches. Parasit Vectors. 2011;4:40.

461 18. Maharaj R, Moonasar D, Baltazar C, Kunene S, Morris N. Sustaining control: lessons from
462 the Lubombo spatial development initiative in southern Africa. Malar J. 2016;15:409.

463 19. Sharp BL, Kleinschmidt I, Streat E, Maharaj R, Barnes KI, Durrheim DN, et al. Seven years
464 of regional malaria control collaboration Mozambique, South Africa, and Swaziland. Am J Trop
465 Med Hyg. 2007;76:42–7.

466 20. World Health Organization. Global Health Observatory (GHO) data. Malaria country
467 profiles. [Internet]. Available from: https://www.who.int/malaria/publications/country-
468 profiles/profile_zaf_en.pdf?ua=1

469 21. Burke A, Dandalo L, Munhenga G, Dahan-Moss Y, Mbokazi F, Ngxongo S, et al. A new
470 malaria vector mosquito in South Africa. Sci Rep. 2017;7:43779.

471 22. Coetzee M, Craig M, le Sueur D. Distribution of African malaria mosquitoes belonging to
472 the *Anopheles gambiae* complex. Parasitol Today. 2000;16:74–7.

473 23. Coetzee M, Hunt RH, Wilkerson R, Torre AD, Coulibaly MB, Besansky NJ. *Anopheles*
474 *coluzzii* and *Anopheles amharicus*, new members of the *Anopheles gambiae* complex. Zootaxa.
475 2013;3619:246–74.

476 24. Coetzee M, Fontenille D. Advances in the study of *Anopheles funestus*, a major vector of
477 malaria in Africa. Insect Biochem Mol Biol. 2004;34:599–605.

478 25. Jupp P. Mosquitoes as vectors of human disease in South Africa. South Afr Fam Pract.
479 2005;47:68–72.

480 26. Braack L, Gouveia de Almeida AP, Cornel AJ, Swanepoel R, de Jager C. Mosquito-borne
481 arboviruses of African origin: review of key viruses and vectors. Parasit Vectors. 2018;11:29.

482 27. Coetzee M, Hunt R, Braack L, Davidson G. Distribution of mosquitos belonging to the
483 *Anopheles gambiae* complex, including malaria vectors, south of latitude 15-degrees-S. South
484 Afr J Sci. 1993;89:227–31.

485 28. Charlwood JD. Some like it hot: a differential response to changing temperatures by the
486 malaria vectors *Anopheles funestus* and *An. gambiae* s.l. Peerj. 2017;5:e3099.

487 29. Kabaghe AN, Chipeta MG, Gowelo S, Mburu M, Truwah Z, McCann RS, et al. Fine-scale
488 spatial and temporal variation of clinical malaria incidence and associated factors in children in
489 rural Malawi: a longitudinal study. Parasit Vectors. 2018;11:129.

490 30. Hammami P, Tran A, Kemp A, Tshikae P, Kgori P, Chevalier V, et al. Rift Valley fever
491 vector diversity and impact of meteorological and environmental factors on Culex pipiens
492 dynamics in the Okavango Delta, Botswana. Parasit Vectors. 2016;9:434.

493 31. Pickett STA, Cadenasso ML, Benning TL. Biotic and abiotic variability as key determinants
494 of savanna heterogeneity at multiple spatiotemporal scales. The Kruger Experience: Ecology and
495 Management of Savanna Heterogeneity. Washington: Island Press; 2003. p. 22–40.

496 32. Ciota AT, Matacchiero AC, Kilpatrick AM, Kramer LD. The effect of temperature on life
497 history traits of Culex mosquitoes. J Med Entomol. 2014;51:55–62.

498 33. Hamer GL, Anderson TK, Donovan DJ, Brawn JD, Krebs BL, Gardner AM, et al. Dispersal
499 of adult *Culex* mosquitoes in an urban West Nile virus hotspot: A mark-capture study
500 incorporating stable isotope enrichment of natural larval habitats. PLoS Negl Trop Dis.
501 2014;8:e2768.

502 34. Muir LE, Kay BH. *Aedes aegypti* survival and dispersal estimated by mark-release-recapture
503 in northern Australia. Am J Trop Med Hyg. 1998;58:277–82.

504 35. Medeiros MCI, Boothe EC, Roark EB, Hamer GL. Dispersal of male and female *Culex*
505 *quinquefasciatus* and *Aedes albopictus* mosquitoes using stable isotope enrichment. PLoS Negl
506 Trop Dis. 2017;11:e0005347.

507 36. Verdonschot PFM, Besse-Lototskaya AA. Flight distance of mosquitoes (Culicidae): A
508 metadata analysis to support the management of barrier zones around rewetted and newly
509 constructed wetlands. Limnologica. 2014;45:69–79.

510 37. Gillies T, Coetzee M. A supplement of the Anophelinae of Africa south of the Sahara
511 (Afrotropical Region): Publications of the South African Institute for Medical Research.
512 1987;55:1–143.

513 38. Koekemoer LL, Kamau L, Hunt RH, Coetzee M. A cocktail polymerase chain reaction assay
514 to identify members of the *Anopheles funestus* (Diptera: Culicidae) group. Am J Trop Med Hyg.
515 2002;66:804–11.

516 39. Venter FJ, Scholes RJ, Eckhardt HC. The abiotic template and its associated vegetation
517 pattern. The Kruger Experience: ecology and management of savanna heterogeneity.
518 Washington: Island Press; 2003. p. 83–129.

519 40. R Development Core Team. R: A language and environment for statistical computing.
520 Vienna, Austria: R Foundation for Statistical Computing; 2014.

521 41. Bates D, Maechler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. J
522 Stat Softw. 2015;67:1–48.

523 42. Anderson MJ, Walsh DCI. PERMANOVA, ANOSIM, and the Mantel test in the face of
524 heterogeneous dispersions: What null hypothesis are you testing? Ecol Monogr. 2013;83:557–74.

525 43. Legendre P, Gallagher ED. Ecologically meaningful transformations for ordination of species
526 data. Oecologia. 2001;129:271–80.

527 44. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, et al. vegan:
528 Community Ecology Package. R package version 2.5-3 [Internet]. 2018. Available from:
529 https://cran.r-project.org/package=vegan

530 45. Brown R, Hing CT, Fornace K, Ferguson HM. Evaluation of resting traps to examine the
531 behaviour and ecology of mosquito vectors in an area of rapidly changing land use in Sabah,
532 Malaysian Borneo. Parasit Vectors. 2018;11:346.

533 46. Burkett DA, Kelly R, Porter CH, Wirtz RA. Commercial mosquito trap and gravid trap
534 oviposition media evaluation, Atlanta, Georgia. J Am Mosq Control Assoc. 2004;20:233—238.

535 47. Degener CM, Eiras ÁE, Ázara TMF, Roque RA, Rösner S, Codeço CT, et al. Evaluation of
536 the Effectiveness of Mass Trapping With BG-Sentinel Traps for Dengue Vector Control: A
537 Cluster Randomized Controlled Trial in Manaus, Brazil. J Med Entomol. 2014;51:408–20.

538 48. Eiras AE, Buhagiar TS, Ritchie SA. Development of the gravid *Aedes* trap for the capture of
539 adult female container-exploiting mosquitoes (Diptera: Culicidae). J Med Entomol.
540 2014;51:200–9.

541 49. Meeraus WH, Armistead JS, Arias JR. Field comparison of novel and gold standard traps for
542 collecting *Aedes albopictus* in northern Virginia. J Am Mosq Control Assoc. 2008;24:244–8.

543 50. Ball TS, Ritchie SR. Sampling biases of the BG-Sentinel trap with respect to physiology,
544 age, and body size of adult *Aedes aegypti* (Diptera: Culicidae). J Med Entomol. 2010;47:649–56.

Figures

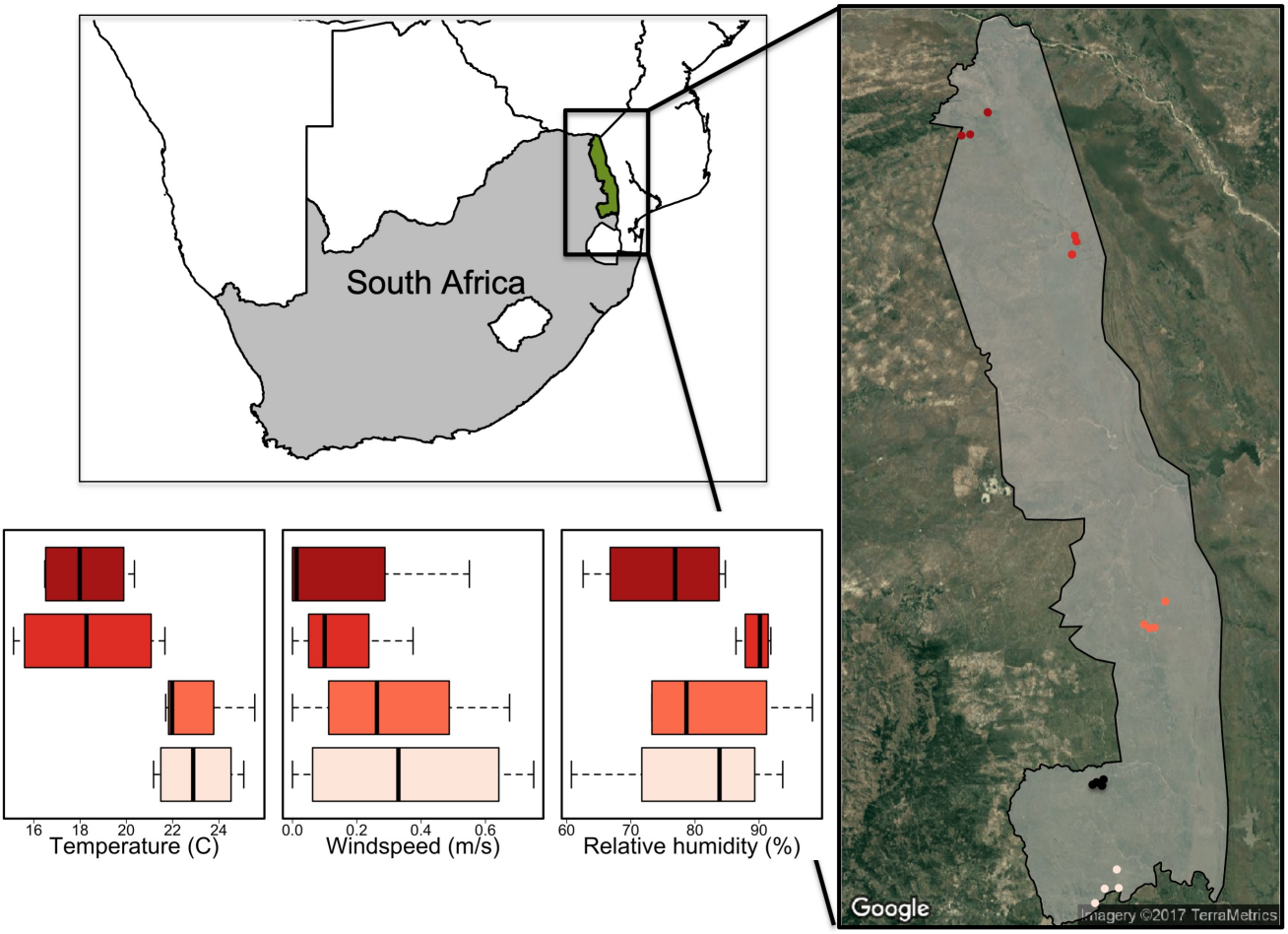
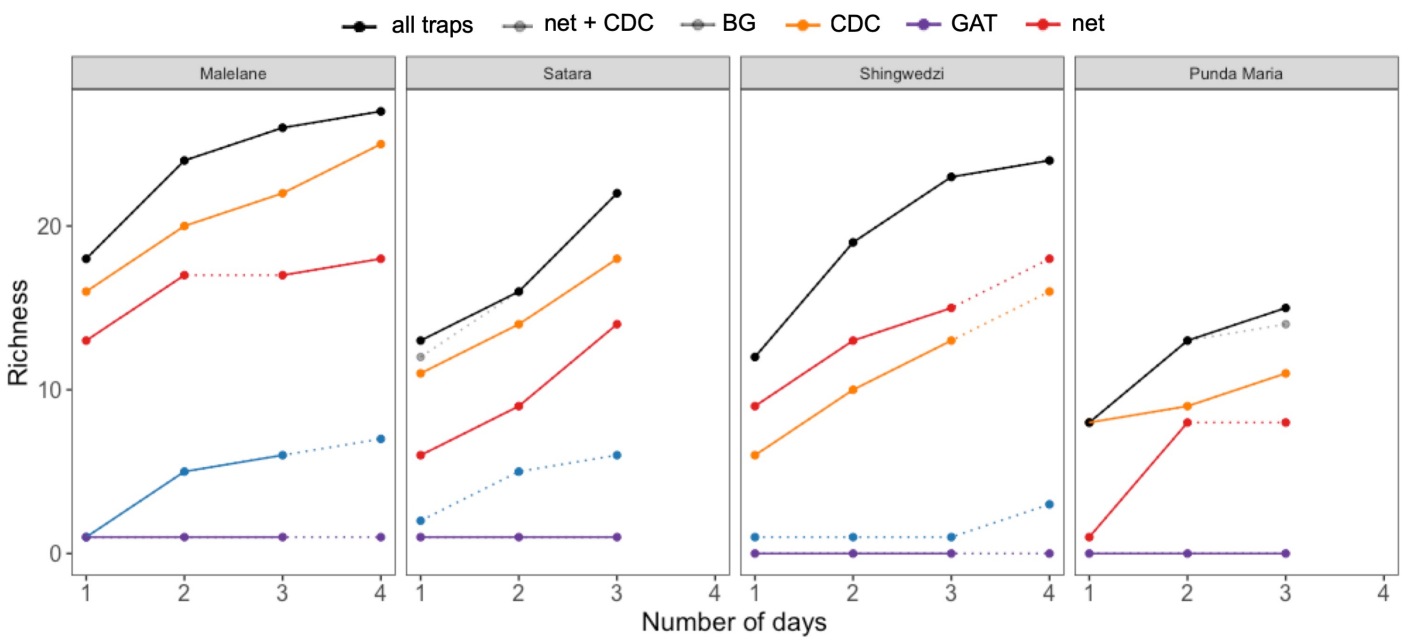


Fig 1. Map of the spatial locations and weather conditions of trapping sites within KNP. Colors represents the five regions where trapping occurred. From south to north, these include the Malelane, Skukuza (no weather data), Satara, Shingwedzi, and Punda Maria sections. Each dot in the map represents a unique water body sampled. Regions of the park were characterized by distinct weather patterns (Table S1).



553

554 Fig 2. Richness (number of unique species) was sensitive to sampling effort and trap type.

555 Richness values in each region were aggregated across sites; figure S1 displays data for

556 each site within a region. Solid lines indicate that no traps were compromised at any site,

557 dashed lines indicate that one trapping site was not collected. Sites within the Satara and

558 Punda Maria region were only sampled for three nights due to rain.

559

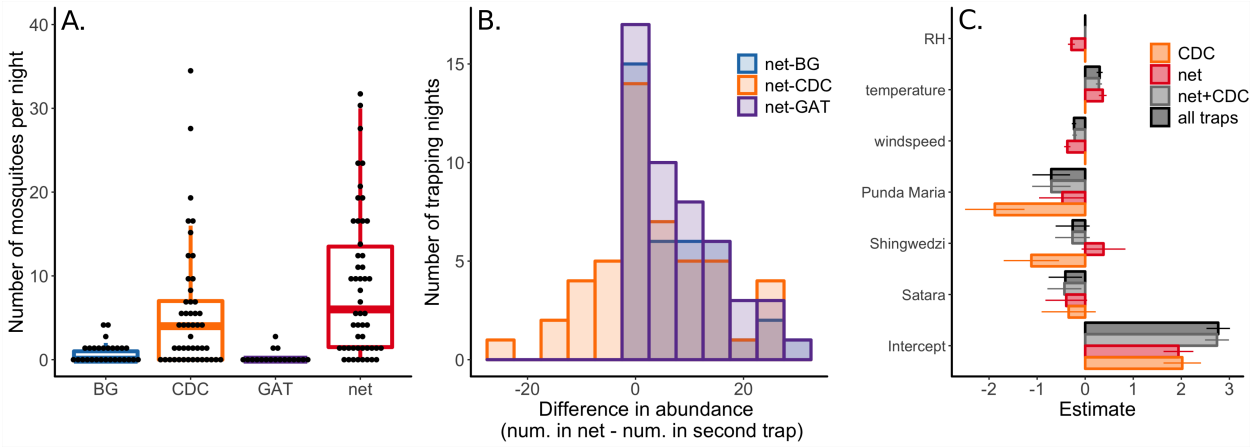


Fig 3. Mosquito abundance. (A) The number of mosquitoes captured by trap type; dots represent the number captured on each night at each site. (B) Histograms showing the difference in the number of mosquitoes sampled between trap types paired by sampling site. (C) Regression parameter estimates and standard errors from statistical models characterizing the median number of mosquitoes sampled at sites relative to Malelane and compared to the mean relative humidity (RH), temperature, or wind speed conditions. Colors indicate whether data used for model fitting was based on one trap or aggregated from multiple traps. Table S3 defines the parameter estimates and hypothesis tests.

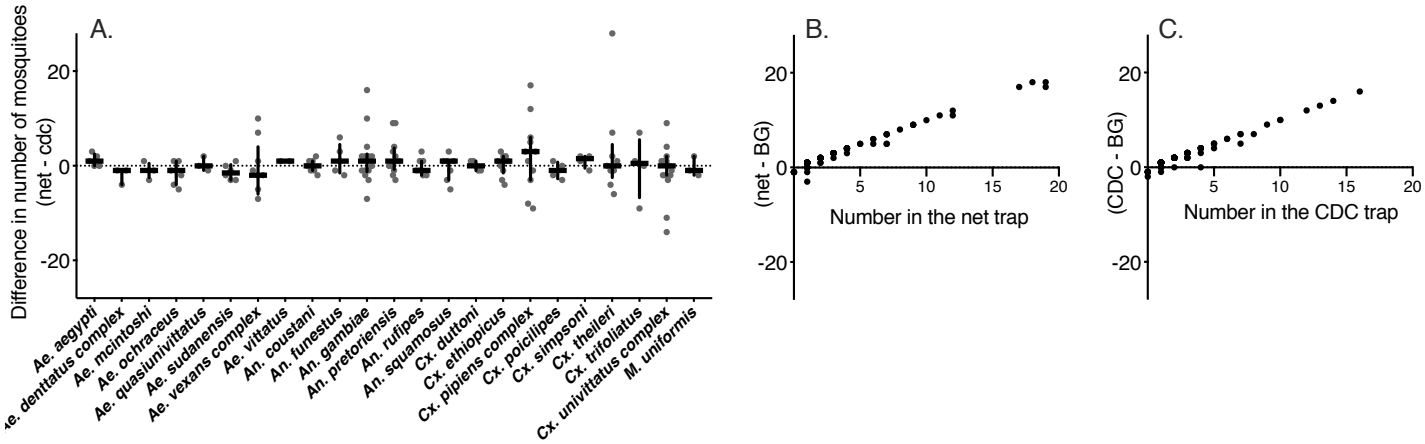
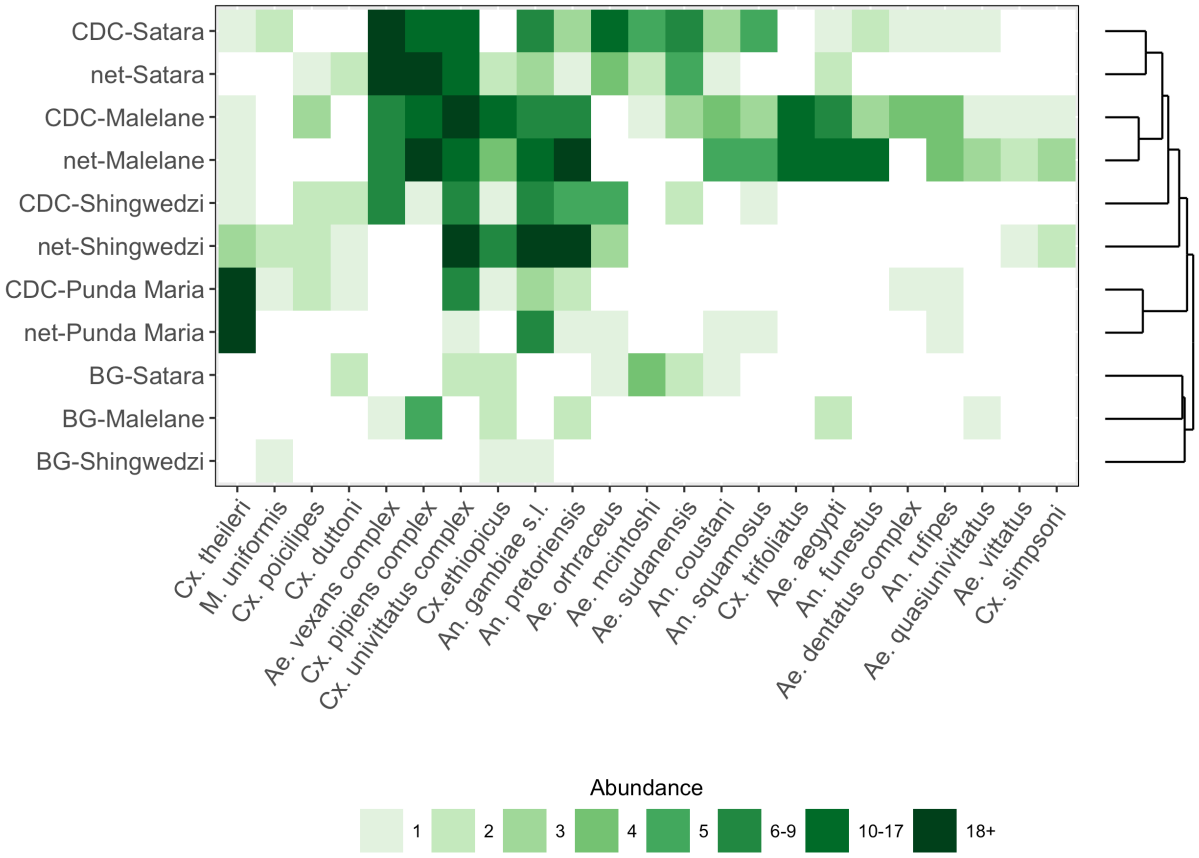


Fig 4. There were no species-specific differences between (A) the net vs. cdc trap, while species-specific differences between (B) the net vs. BG trap and the (C) CDC vs. BG trap were driven by overall abundance in the net or CDC trap. Dots represent the difference in the number of mosquitoes collected between pairs of traps based on the total number of mosquitoes sampled across nights at each site. (A) Lines represent the median and interquartile range of the data. Data displayed do not include sites where no individuals of a given species were collected in either trap, but results remain consistent regardless of whether these sites are or are not included. No hypothesis test for the individual species was significant (p-values = 0.181, 0.174, 0.345, 0.143, 1, 0.134, 0.476, 0.346, 1, 0.498, 0.360, 0.152, 0.796, 0.931, 1, 0.719, 0.372, 0.423, 0.265, 0.725, 1, 0.850, 1). See Figure S3 for species-specific comparisons with the BG trap and Table S4 for a summary table for the BG trap.



585

586 Figure 5. Communities collected in the net and CDC trap are clustered by region. Traps and

587 regions are ordered according to the tree produced by clustering (Fig S5). Colors represent

588 species abundance, with color bins defining the 30th to 90th percentile in increments of 10.

589

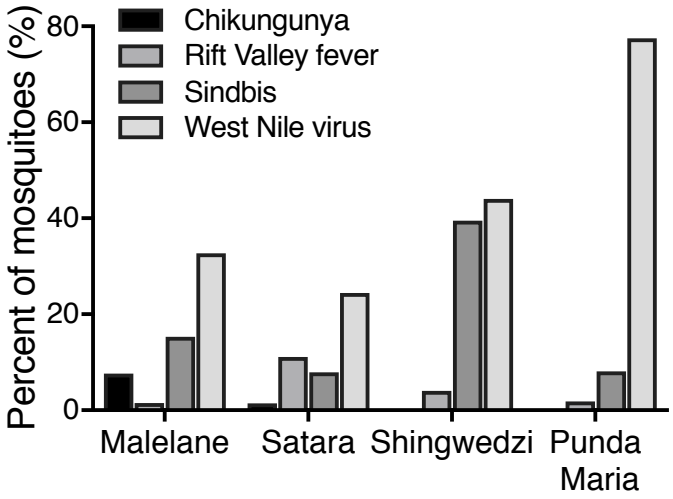


Fig 6. The percentage of mosquitoes identified as primary vectors in each region.