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

which are endemic to the region [14]. Additional studies evaluating trapping methods are needed to evaluate if traps designed for other locations and species perform equally well in the rich communities in southern Africa. For example, a recent comparison of four traps in Germany found that the Biogents Sentinel trap (BG trap) collected the highest number of individuals in urban and snowmelt forest environments where *Culex* species predominate. In contrast, the Centers for Disease Control miniature light trap (CDC trap) collected the most mosquitoes in floodplain environments where *Aedes vexans* predominate, suggesting that the preferred trapping device may vary by habitat and community composition [7]. Qualitative evaluations in southern Africa suggest different traps likely collect different subsets of the mosquito community [15]. However, the two studies systematically evaluating trapping methods in southern Africa focus only on house-based trapping in residential areas [16,17].

An evaluation of trapping methods is also needed to influence the design of future mosquito surveillance efforts. Such efforts could provide baseline information for public health interventions by identifying hotspots with a high abundance of vector species [18,19]. There were more than 26,000 estimated cases of malaria in southern Africa in 2017 (Botswana, Namibia, South Africa, Lesoto, Swaziland) [20]; key malaria vectors include members of the *Anopheles gambiae* complex *(An. arabiensis, An. gambiae, An. merus)* [21–23] and the *An. funestus* complex [24]. Mosquito-borne livestock infections are also a concern, as outbreaks of West Nile virus, Rift Valley fever, Sindbis, and Wesselsbron occur [25,26]. Key vectors for these viral infections include, *Aedes caballus, Ae. circumluteolus, Ae. dentatus, Ae. juppi, Ae. mcintoshi, Ae. ochraceus, Culex pipiens, Cx. quinquefasciatus, Cx. univittatus,* and *Cx. theileri* (based on suspected or known prime

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

In this study, we assessed four commonly used trapping methods for adult mosquito data collection in natural savanna ecosystems. We compared estimates of abundance and community composition from four traps: 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 also evaluated the importance of trapping method for inferring the regional distribution of mosquito abundance, mosquito community composition, and the distribution of key disease vectors.

Methods

Study location

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

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

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

To assess if different traps provide different estimates of community composition, we first evaluated if certain species were particularly attracted to one trap over the other. We assessed species-specific trap bias by calculating the difference in the number of individuals for each species sampled between each pair of traps collected at a site over all nights of trapping. Because traps were paired at each site, we tested for differences between the traps using a Wilcoxon signed-rank test and a Bonferroni correction for multiple comparisons. We only compared the species-specific trap bias of common species, 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)

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

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

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

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

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

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

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

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

Conclusions

After assessing four different mosquito trapping methods in a natural savanna ecosystem, we recommend the net trap, the CDC trap, or their combined use for outdoor mosquito surveillance in southern Africa. These traps performed well based on four evaluation criteria: collecting large numbers of mosquitoes; estimating mosquito 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).

Competing Interests: The authors declare that they have no competing interests.

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Author contribution statement: EG, BB, MS conceived and designed the analysis; EG, BB, DG, MS collected the data; EG, MG, MS conducted identifications; EG, PB, MS performed analyses; all authors wrote the paper.

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Figures

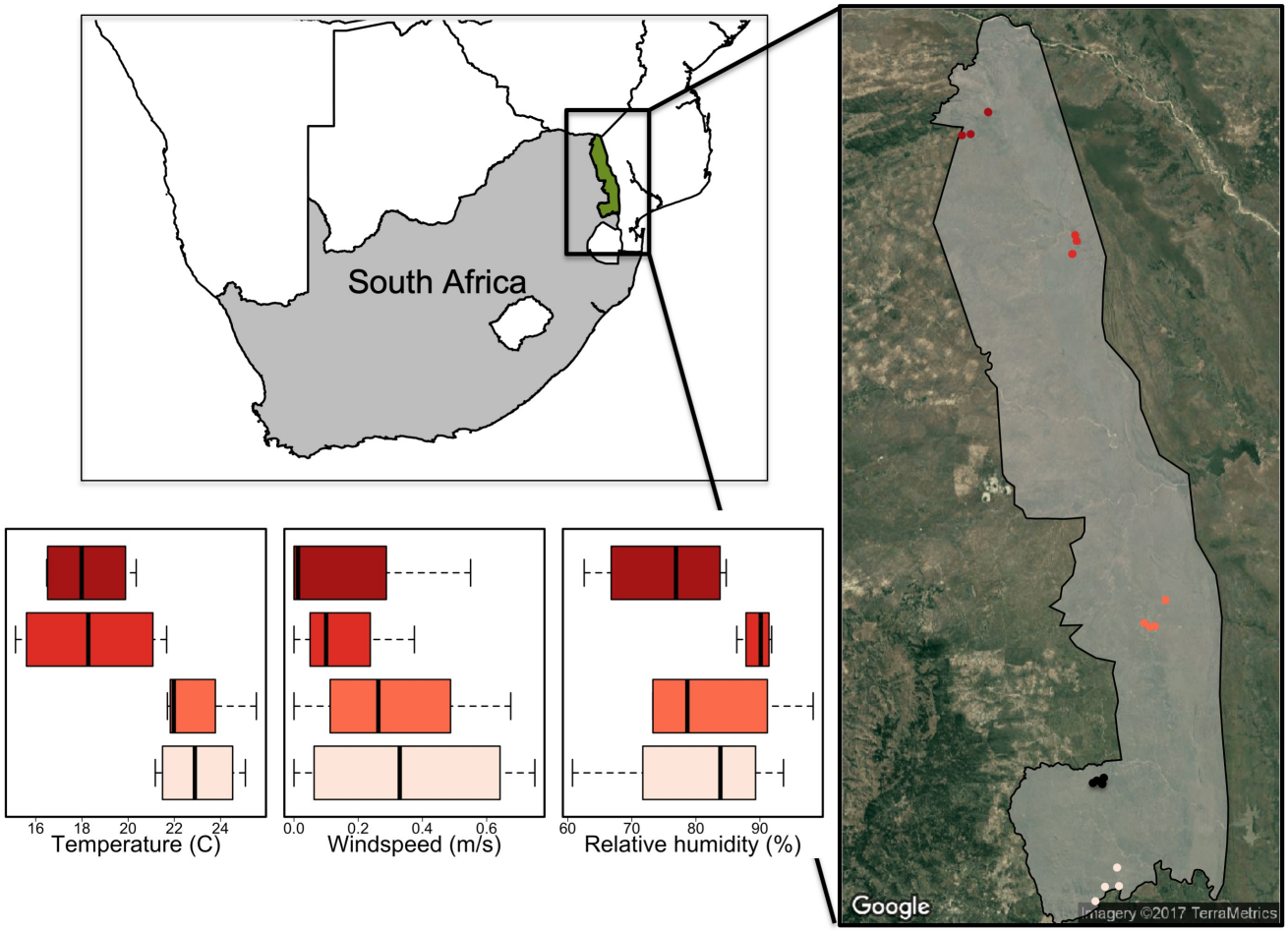
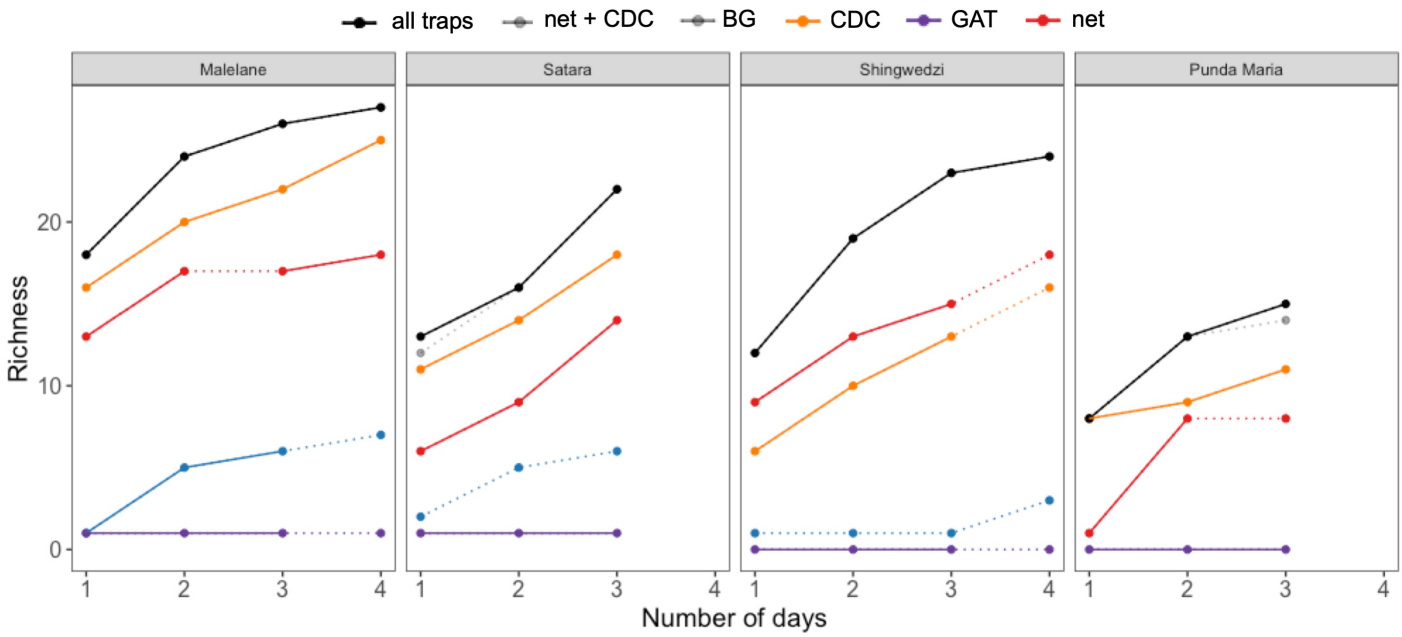


Fig 1. Map of the spatial locations and weather conditions of trapping sites within KNP. Colors represents the five regions where trapping occurred. From south to north, these include the Malelane, Skukuza (no weather data), Satara, Shingwedzi, and Punda Maria sections. Each dot in the map represents 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.

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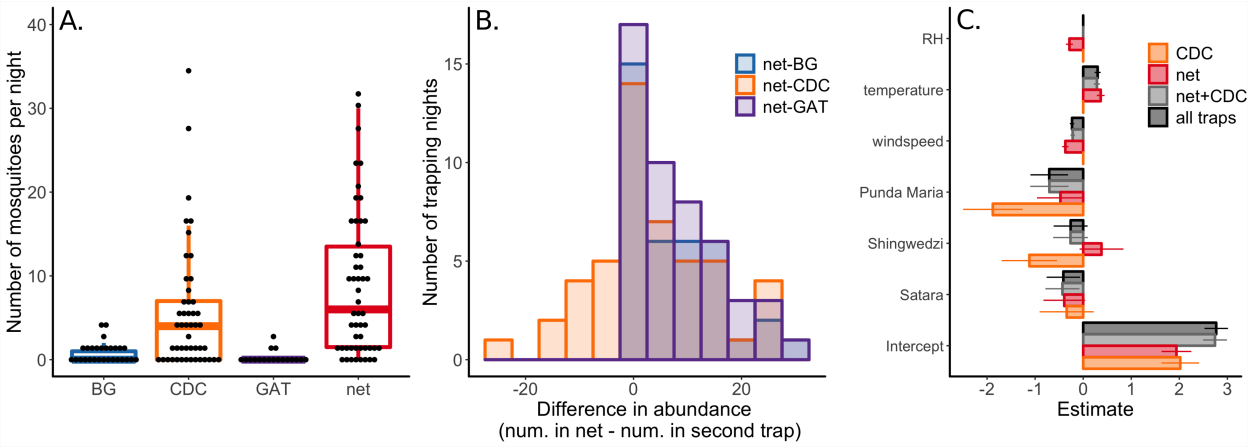
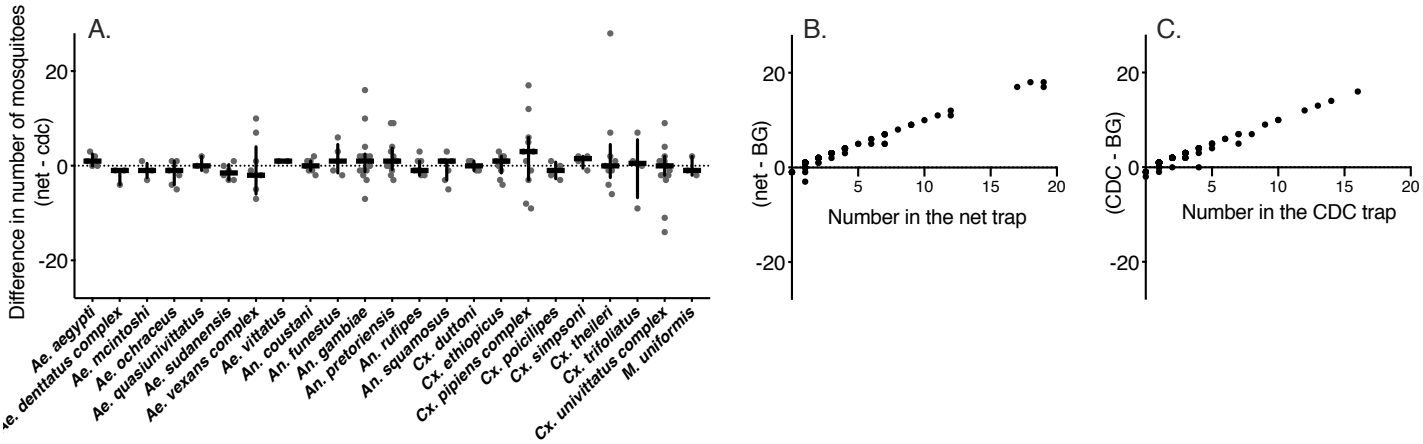


561

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

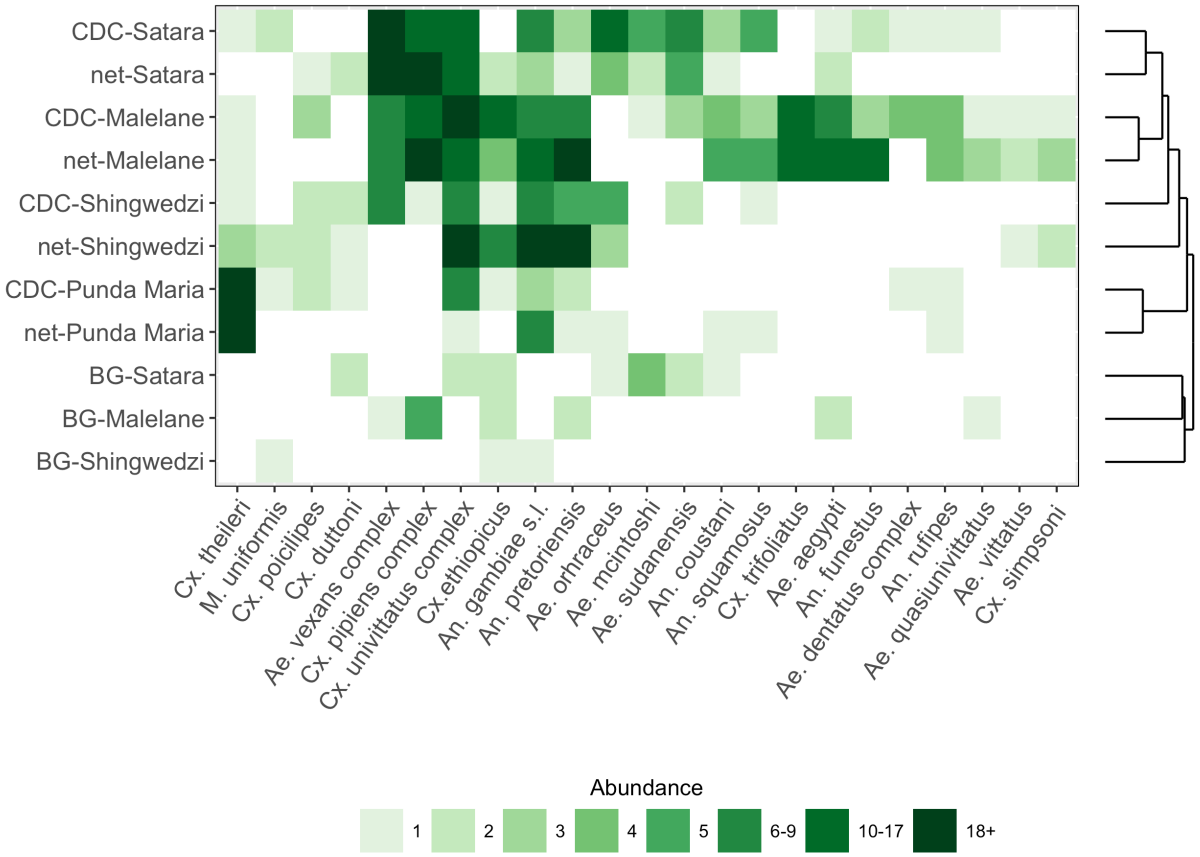
570

571



572

573 Fig 4. There were no species-specific differences between (A) the net vs. cdc trap, while
574 species-specific differences between (B) the net vs. BG trap and the (C) CDC vs. BG trap
575 were driven by overall abundance in the net or CDC trap. Dots represent the difference in
576 the number of mosquitoes collected between pairs of traps based on the total number of
577 mosquitoes sampled across nights at each site. (A) Lines represent the median and
578 interquartile range of the data. Data displayed do not include sites where no individuals of
579 a given species were collected in either trap, but results remain consistent regardless of
580 whether these sites are or are not included. No hypothesis test for the individual species
581 was significant (p-values = 0.181, 0.174, 0.345, 0.143, 1, 0.134, 0.476, 0.346, 1, 0.498, 0.360,
582 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
583 species-specific comparisons with the BG trap and Table S4 for a summary table for the BG
584 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.

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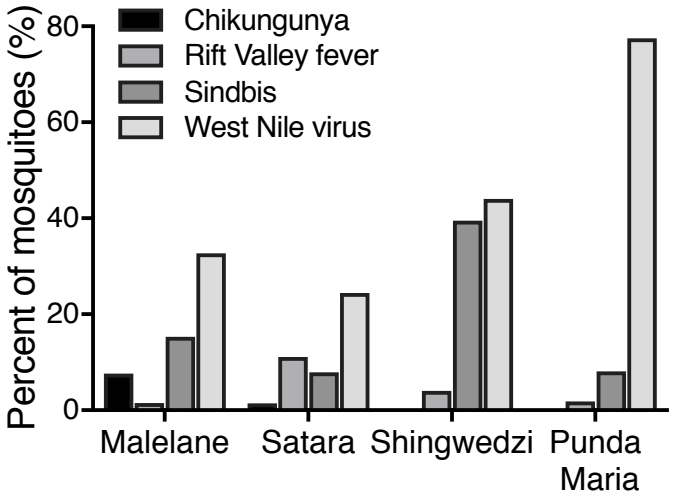
590



591

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

593