Artificial resting sites: An alternative sampling method for adult mosquitoes

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Abstract
Mosquito collections are commonly conducted with baited traps predominantly attracting host-seeking females. In contrast, resting sites are generally colonized by a broader range of the mosquito population, including a higher proportion of males and blood-engorged females. This study evaluates the sampling success of different artificial resting sites, attached to a deciduous or coniferous tree at different heights. As standard sampling method, carbon dioxide-baited Biogents Sentinel traps (BG traps) were operated in parallel. BG traps caught a higher number of specimens compared to the resting sites. However, the proportion of blood-engorged females and males was higher in resting sites. More Culiseta spp. specimens were collected in resting sites compared to BG traps, but less Aedes spp. specimens. In general, fewer specimens and species were recorded in small resting sites and at top height level compared to medium or large resting sites at medium or ground level. The proportion of males was highest at the ground, while the proportion of engorged females was highest at medium and top level. Due to the higher proportion of blood-engorged females, artificial resting sites are especially useful for studies of host-feeding patterns or xenosurveillance. Low costs and efforts allow a cost-effective increase of the number of resting sites per sampling site to collect more mosquitoes.

KEYWORDS
monitoring, mosquito, resting behaviour, surveillance

INTRODUCTION

According to the World Health Organization, vector-borne pathogens are responsible for 17% of infectious diseases worldwide causing more than 700,000 deaths every year (WHO, 2020). Thereby, mosquitoes are the most important vector group, for example, for parasites (e.g. Plasmodium spp., Filaria spp.) or viruses (e.g. dengue virus, chikungunya virus). Knowledge on the distribution and density of mosquitoes is an important information to understand and control the circulation of mosquito–borne pathogens.

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Surveillance studies for mosquitoes and associated pathogens are mostly conducted with carbon-dioxide-baited traps (L’Ambert et al., 2012; Lühken et al., 2014). Additional attractants mimicking host cues (e.g. carbon dioxide or olfactory attractants) are commonly added. However, these traps predominantly bait host-seeking females, that is, a high proportion of the trapped specimens can be expected to be non-engorged females (Pezzin et al., 2016). Hence, large numbers of mosquitoes have to be collected to detect human or veterinary pathogens.

Although carbon dioxide-baited traps catch a wide range of mosquito species (Lühken et al., 2014), several alternative trapping methods are applied to collect adult mosquitoes (e.g. gravid traps or human landing catches). These methods vary in their targeted species composition and attract different proportions of the mosquito population (L’Ambert et al., 2012; Panella et al., 2011; Williams & Gingrich, 2007), for example, gravid traps are suitable to collect container-breeding female mosquitoes seeking for breeding sites (Hoel et al., 2011; Reiter et al., 1995; Williams & Gingrich, 2007). Another common method is the sampling of resting mosquitoes. Mosquitoes can be collected from natural (e.g. tree holes) or man-made resting sites (e.g. cellars, barns or pit shelters) (Bhati et al., 1989; Kweka & Mahende, 2009). However, the installation of artificial resting sites allows a standardized sampling within surveillance programmes and is beneficial, when natural resting sites are missing or difficult to find (Edman et al., 1968).

In comparison to carbon-dioxide-baited traps, mosquito collections with artificial resting sites are generally smaller in quantity of specimens and species (L’Ambert et al., 2012; Panella et al., 2011; Williams & Gingrich, 2007). Samples from artificial resting sites are considered more standardized compared to backpack aspiration in the understory vegetation (Brown et al., 2018) and have a higher proportion of gravid and engorged specimens (Brugman, 2016; Sauer et al., 2020), increasing the probability of detection of mosquito-borne pathogens. Furthermore, different studies demonstrated that collections from resting sites allow the trapping of species with a host preference for amphibians, birds or reptiles [e.g. *Culex territans* (Burkett-Cadena et al., 2008) or *Culiseta morsitans* (Edman et al., 1968; Sauer et al., 2020)], which are usually underrepresented in samples with other trapping methods. In contrast, species of the genus *Aedes* rather rest in the understory vegetation and are rarely caught in artificial resting sites (Bidlingmayer, 1971; Burkett-Cadena et al., 2008; Gusciora, 1971; Ibey & Apperson, 1992).

 Different factors are considered to affect the sampling efficiency from artificial resting sites, while a systematic evaluation of different factors is missing. Herein, we thus studied three different factors, which might influence the sampling efficiency: Size of the resting site, height level and environment of resting site installation (here: Tree type). This study was conducted in rural areas with a high number of mosquito breeding sites (e.g. water drinking troughs, ditches, etc.). One deciduous and one coniferous tree per sampling site were selected for the installation of resting sites. These are known to provide different environmental conditions, for example, microclimate or light regime (İrmak et al., 2018). Although the exact relationship of these factors is mostly unknown, various studies highlight their relevance for the resting site selection of mosquitoes.

**FIGURE 1** Experimental setup per sampling site. Trapping per sampling site was conducted with one Biogents Sentinel trap and 18 artificial resting sites. Resting sites of three sizes (0.4, 76 and 162 L) were attached to one coniferous and one deciduous tree at three heights (0, 1.5, 4.5 m). Vector graphics for vegetation structures from pixabay.com
(Paijmans & Thomas, 2011; Sauer et al., 2021). Following previous studies (Komar et al., 1995; Morris, 1981; Ritchie et al., 2013), we hypothesize that the number of specimens will increase with size and decreasing height level of the artificial resting sites, because larger resting sites might be better recognized by mosquitoes, and resting sites on the ground are less exposed to harsh climatic conditions (Bidlingmayer, 1971; Edman et al., 1968; Service, 1980).

MATERIALS AND METHODS

Mosquito sampling and identification

In 2016 and 2017, mosquito sampling was conducted at a total of 16 sampling sites (8 per year) in North-West Germany (Figure S1, Supporting Information). All sampling sites were located in rural regions surrounded by meadows with cattle and horses. Several artificial and temporary water bodies were available at each sampling site, for example, water drinking troughs or water-filled flowerpots.

At each sampling site, resting sites of three different sizes (162, 76, 0.4 L) were attached at three different heights (0, 1.5 and 4.5 m) to one coniferous and one deciduous tree. In addition, one carbon dioxide-baited Biogents Sentinel trap (BG trap; Biogents, Regensburg, Germany) was used per sampling site (Figure 1). At each site, both trees and the BG trap were not more than 20 m apart from each other. This reduced the impact of the surrounding land-use or microclimate conditions and enabled sampling of a closely defined mosquito population. Pop-up garden bags (Relaxdays, Halle, Germany) of two different sizes were used as large- and medium-sized artificial resting sites: 162 L (height: 63 cm, diameter: 56 cm) and 76 L (height: 44 cm, diameter: 46 cm) (Figure S2). Black plastic cups (0.4 L, height: 9 cm, diameter: 7.5 cm) made from downpipes (Fallrohr NW 80 RAL 8028 anthrazit 2 m, Grüna, Germany) were attached as small resting sites.

In addition, the compass orientation (8 levels: North; North-East; East; North-West; West; South; South-East; South-West) and angle of declination (range: −100 to 12.5) were recorded for each artificial resting site, which differed depending on the natural characteristics of the trees, for example, distribution of branches to attach the resting sites.

Mosquito sampling from the resting sites was conducted biweekly from April to October (2016, 2017) with a self-made aspirator similar to the Prokopack aspirator (Vazquez-Prokopec et al., 2009), which is known to perform equivalent to the Centres for Disease Control and Prevention

| TABLE 1 | Mosquitoes collected from artificial resting sites |
|---------|--------------------------------------------------|
| Species/taxa | Total number collected specimens including the Biogents traps (Table 2) | Number collected from artificial resting sites (female/males) | Engorged females (%) | Mean catch per artificial resting site |
|-----------|------------------------------------------------|-------------------------------------------------|----------------|----------------------------------|
| Culex p. pipiens s.l./torrentium | 16 471 | 1170 (1156/14) | 17.3 | 2.34 ± 0.07 |
| Culiseta annulata | 1231 | 1120 (595/525) | 34.1 | 2.24 ± 0.23 |
| Culex p. pipiens | 1103 | 1081 (0/1081) | 0 | 2.16 ± 0.06 |
| Culiseta morsitans | 998 | 974 (554/420) | 9.4 | 1.94 ± 0.61 |
| Anopheles maculipennis s.l. | 278 | 178 (99/79) | 25.3 | 0.35 ± 0.61 |
| Aedes vexans | 161 | 2 (1/1) | 0 | 0.004 ± 0.06 |
| Aedes rusticus | 77 | 0 | — | — |
| Anopheles plumbeus | 51 | 37 (10/27) | 5.4 | 0.074 ± 0.06 |
| Aedes punctor | 36 | 1 (1/0) | 0 | 0.002 ± 0.08 |
| Aedes cantans | 35 | 3 (2/1) | 0 | 0.006 ± 0.08 |
| Culex territans | 33 | 33 (20/13) | 0 | 0.067 ± 0.45 |
| Coquillettidia richardi | 25 | 8 (5/3) | 40.0 | 0.016 ± 0.24 |
| Anopheles claviger/petragnani | 23 | 2 (2/0) | 0 | 0.004 ± 0.06 |
| Culex spp. | 19 | 14 (2/12) | 50.0 | 0.028 ± 0.18 |
| Aedes spp. | 14 | 1 (1/0) | 100.0 | 0.002 ± 0.05 |
| Culex torrentium | 13 | 13 (0/13) | 0 | 0.026 ± 0.16 |
| Aedes caspius | 9 | 1 (1/0) | 100.0 | 0.002 ± 0.08 |
| Aedes communis | 5 | 1 (0/1) | 0 | 0.002 ± 0.08 |
| Aedes sticticus | 3 | 1 (1/0) | 0 | 0.002 ± 0.08 |
| Aedes geruniculatus | 3 | 0 | — | — |
| Aedes cinereus/geminus | 1 | 0 | — | — |
| Aedes annulipes | 1 | 0 | — | — |
| Sum | 20 590 | 4640 (2450/2190) | — | — |

Note: Additional information is given on the number of females/males, the percentage of engorged females and mean catch of specimens per trap for both trapping methods ± standard error.
backpack aspirator in field studies (Maia et al., 2011). BG traps were run biweekly for 24 h in the week before the sampling of the resting sites to reduce direct interference between the two sampling methods. Samples were brought to the laboratory where mosquitoes were killed by freezing

\[ C_0 \quad C_{14} \] )]. All mosquito specimens were selected, enumerated, differentiated by sex and identified to lowest taxonomic level possible by using the identification key of Becker et al. (2010).

Statistical analysis

In order to account for sampling site-specific differences, the total number of mosquito specimens and the number of female mosquitoes for each artificial resting site was standardized relative to the samples with the carbon-dioxide-baited BG trap. Accordingly, for each sampling site, specimens per artificial resting site were divided by the sum of specimens per artificial resting site and BG trap. In addition, for each resting site per sampling site, we calculated (1) the proportion of overlapping taxa (collected with both sampling methods) and non-overlapping taxa (only collected from the resting site) relative to the BG trap, (2) the proportion of blood-fed females and (3) the proportion of males. The impact of resting site size (three levels: 0.4, 76, 162 L), height (three levels: 0, 1.5, 4.5 m), tree type (two levels: deciduous or coniferous trees), compass orientation (8 levels: North; North-East; East; North-West; West; South; South-East; South-West), angle of declination (range: \(-100 \) to 12.5) were assessed as fixed factors with a binomial generalized linear model (glm) in R (R Development Core Team, 2011). In addition, the interactions terms ‘tree type: height’ and ‘size: height’ were together included in the glms, but removed from the full model as both terms were not statistically significant in any of the models. The amount of deviance accounted by each glm were calculated as

\[ D^2 = \frac{\text{Null deviance} - \text{Residual deviance}}{\text{Null deviance}}. \]

For the individual factors, multiple comparisons were made with the glht-function in the multcomp package (Hothorn et al., 2008). Data manipulation and visualization were conducted with the R packages magrittr (Bache & Wickham, 2014), plyr (Wickham, 2011) and tidyr (Wickham & Henry, 2017) and ggplot2 (Wickham, 2009).

RESULTS

In total, 20,592 specimens were collected, 15,950 mosquito specimens (77.5% of all specimens) and 4,642 specimens (22.5%) in the 16 BG traps and in 288 artificial resting sites (Tables 1 and 2). Height
and size had a statistically significant impact on the proportional number of collected total specimens and females per resting site relative to the parallel operated BG trap (Figure 2, Tables 1 and 2, $D^2$ for both glm$s = 0.32$). In contrast, the number of collected mosquitoes was not statistically influenced by type of tree, compass orientation or angle of declination (Table S1). For the total number of mosquito specimens, statistically significant fewer mosquitoes were collected in the top and in the smaller artificial resting sites (height: low–high: $Z = -1.6053$, $p < 0.001$, medium–high: $Z = -1.3006$, $p = 0.00181$, medium–low: $Z = 0.3046$, $p = 0.46109$, size: large–small: $Z = -2.3158$, $p < 0.001$; medium–small: $Z = -2.2096$, $p < 0.001$, medium–large: $Z = -0.1062$, $p = 0.878$). Neither artificial resting sites exposed at low and medium height nor artificial resting sites of medium and large size differed significantly in the number of mosquito specimens. The same was observed if only data of female mosquitoes were analysed (Figure S3, Table S1).

In total, 18 taxa of 5 genera were detected. In both trapping methods *Culex pipiens* s.l./*torrentium* represented the largest fraction of taxa being collected. The total number of specimens was much higher in BG traps (15 323 specimens, 96.1% of total) than in artificial resting sites (2265 specimens, 48.8%). Taxa collected less frequently were *Cs. annulata* (1120, 24.1%), *Cs. morsitans* (974, 21.0%) and *An. maculipennis* s.l (178, 3.8%) for resting sites and *Aedes vexans* (159, 1.0%), *Cs. annulata* (110, 0.7%) and *An. maculipennis*
s.l. (100, 0.6%) for BG traps (Table 2). All in all, artificial resting sites collected a small proportion of the total number of taxa present than the BG traps did [mean proportion of overlapping taxa: 35.6%, 95% confidence interval (CI): 30.9–33.3%], which was statistically affected by size and height, but not by other factors. The medium and larger artificial resting sites at the medium or ground height level showed a higher overlap with the species composition of the BG traps compared to the small resting sites or the top height level (height level: low–high: Z = 0.5868, p ≤ 0.001; medium–high: Z = 0.5150, p ≤ 0.001, medium–low: Z = −0.0719, p = 0.8413, size: large–small: Z = 0.8166, p ≤ 1e−05; medium–small: Z = 0.7914, p ≤ 0.001, medium–large: Z = −0.0252, p = 0.973; Figure 3; D² for glm = 0.21).

Notably, Aedes species were underrepresented in artificial resting sites (10 specimens, 0.2% of all collected specimens) compared to 335 specimens in the BG traps (2.1%). Moreover, four Aedes taxa were present in the BG traps only: Ae. rusticus, Ae. cinereus/geminus, Ae. annulipes, Ae. geniculatus. In contrast, Cx. territans was exclusively collected by means of artificial resting sites (the mean proportion of these non-overlapping taxa was 15.5, 95% CI: 10.2–12.9%; Figure S4). Compared to small resting sites, a higher proportion of non-overlapping taxa was collected in the large and medium-sized artificial resting sites (large–small: Z = 1.46758, p = 0.009; medium–small: Z = 1.48564, p = 0.009; medium–large: Z = 0.01806, p = 0.997; D² for glm = 0.12). This is caused by a higher proportion of Cs. annulata and

**FIGURE 4** Percentage of male specimens (95% confidence interval) of the total number of collected mosquito species per resting site and year over all sampling areas (n = 16). Each of the three sizes of resting sites were installed in a coniferous and deciduous tree at three different heights.

**FIGURE 5** Percentage blood-fed specimens (95% confidence interval) of the total number of collected mosquito females per resting site and year per sampling areas (n = 16). Each of the three sizes of resting sites were installed in a coniferous and deciduous tree at three different heights.
Cs. morsitans for resting sites (2094 specimens, 45.1%) compared to BG traps (135 specimens, 0.8%), and Cx. territans which was collected in resting sites only.

Male mosquitoes were rarely collected by carbon dioxide-baited traps (0.24% of all collected specimens in BG traps). In contrast, the gender ratio from the resting sites was almost balanced and consisted of 48.7% males. Regarding the analysed factors, height only had a statistically significant impact (low-high: \(Z = 0.5937, p = 0.0113\); medium-high: \(Z = 0.2237, p = 0.5206\); medium-low: \(Z = -0.3700, p = 0.1387; \) Figure 4; \(D^2\) for glm = 0.07) on the proportion of males, with lower proportions in the top (mean: 36.7%, 95% CI: 28.2–45.2%) compared to the ground (mean: 50.9%, 95% CI: 45.6–56.2%)

Artificial resting sites collected considerably more engorged females (19.6%) than BG traps (0.4%). As observed for the proportion of males, only height had a statistically significant impact on the proportion of engorged females (low-high: \(Z = 1.3540, p = 0.0001\); medium-high: \(Z = -0.3955, p = 0.2614\); medium-low: \(Z = 0.9585, p = 0.0037; \) Figure 5; \(D^2\) for glm = 0.16). Medium and top height had a higher proportion of blood-fed specimens (mean 15.3%, 95% CI: 11.7–18.8%) and mean 22.7%, 95% CI: 14.7–30.8%), but no statistically significant differences was observed between the low and medium height (mean 7.8, 95% CI: 5.37–10.1%)

**DISCUSSION**

This study systematically compared the number of mosquito specimens and species collected in artificial resting sites and carbon dioxide-baited traps. The study was carried out in North-Western Germany, using resting sites of different sizes attached at different heights in deciduous and in coniferous trees. The number of specimens and species differed according to height level and size of the artificial resting sites only. Further factors included in this study (type of tree, compass orientation and angle of declination) had no statistically significant effect. As reported by previous authors (Morris, 1981; Ritchie et al., 2013), small resting sites and artificial resting sites at the top height level collected fewer specimens and species than larger artificial resting sites at medium or ground level. In addition, the species composition of medium to large artificial resting sites attached at low or medium height was more similar to the carbon dioxide traps. Dark colour is an effective attractant for mosquitoes (Bidlingmayer, 1971; Edman et al., 1968), which might explain why larger resting sites with a larger dark opening are better recognized by mosquitoes. As hypothesized by Komar et al. (1995), the higher number of mosquito specimens at ground levels could be driven by climatic conditions, which should be harsher for resting sites at higher levels, where they are, for example, exposed to wind (Service, 1980). Further studies, representing a finer gradation of resting site sizes and height levels should improve our understanding of height preferences of mosquitoes and process guiding species-specific selection.

In general, there is a lack of knowledge on the influence of inclination angles, compass orientation or type of tree on resting site selection, which all did not have a statistically significant effect in this study. Downward directions increase the shaded area within the pop-up bags and thereby attract higher numbers of mosquito specimens in the resting sites compared to sideways installation (Sauer et al., 2020). However, the inclination of the resting sites in our study was mostly downwards, that is, did not cover a broad range of inclinations. Few studies detected a positive effect when resting sites were facing east (Edman et al., 1968) or towards swamps (Morris, 1981). In agreement with another study in North-Western Germany (Sauer et al., 2020), the here presented study also did not find a statistically significant impact of compass direction. Trees offer shade and protection from unfavourable weather events like strong wind or rain, but this study did not find any relevance of tree type. The resting sites were not attached in forests, but in single trees or smaller tree groups with relative sparse canopy. Artificial resting sites are considered particularly suitable, when there is a lack of alternative resting sites for mosquitoes (Edman et al., 1968; Pezzin et al., 2016), but the specific impact of land-use on their sampling efficiency is largely unknown.

On average, the number of mosquito specimens collected with an individual artificial resting site was 20 times lower compared to the parallel run BG trap, matching the results of other studies using carbon dioxide as an attractant (Panella et al., 2011; Ritchie et al., 2013; Williams & Gingrich, 2007). Collections with resting sites represent a temporal snapshot only of the resting mosquito population. Mosquitoes can enter and leave the resting site at any time and their resting behaviour is further influenced by different environmental conditions, for example, temperature or light (Panella et al., 2011; Thomson, 1938). Glue (Brown et al., 2018; Degefa et al., 2019), fans (Panella et al., 2011) or lids (Burkett-Cadena, 2011) might be useful to increase the sampling efficiency of artificial resting sites.

The species composition differed strongly between both sampling methods in this study. In particular, only few specimens of the genus *Aedes* were sampled from the artificial resting sites (Brown et al., 2018; Gusciora, 1971), resulting in a lower number of species compared to collections with the carbon dioxide traps. In contrast, *Cs. morsitans* (Becker et al., 2010; Morris, 1981), *Cs. annulata* or *An. maculipennis* s.l. were more frequently detected with artificial resting sites. Moreover, Cx. territans, which acquires bloodmeals from amphibian hosts (Crans, 1970), was detected exclusively in artificial resting sites, but not in BG traps. This is in concordance with previous studies, comparing different trapping methods for mosquitoes (Pezzin et al., 2016; Sauer et al., 2020; Williams & Gingrich, 2007). Trout et al. (2007) described species of the genus *Aedes* (*Aedes albopictus* and *Ae. vexans*) with resting preferences below 4 m in vegetation. *Aedes* species seem to prefer understory vegetation rather than artificial resting sites (Bidlingmayer, 1971; Burkett-Cadena et al., 2008; Doyle et al., 2009; Irby & Apperson, 1992).

Irrespective of the differences in the species composition, artificial resting sites collect a different proportion of the mosquito population compared to carbon dioxide-baited traps with a considerable higher proportion of males and engorged females. Male mosquito specimens are generally not in the focus of mosquito sampling campaigns as they have a low relevance for the surveillance of pathogens. Nevertheless, for several taxa only the males allow reliable morphological differentiation of
species (e.g. Ae. cincerus and Ae. geminus) (Schaffner et al., 2001). In addition, the collection of both, females and males, give comprehensive insights into mosquito population structures, for example, male mosquitoes are known to provide information on mosquito mass occurrence, allowing targeted control measurements (Fay & Prince, 1968; Sawadogo et al., 2017). As demonstrated before (Brugman, 2016; Sauer et al., 2020), resting sites also attract a considerable higher proportion of engorged females compared to carbon dioxide-baited traps. Blood-fed females are of specific interest for the xenosurveillance (Drummond et al., 2020; Tomazatos et al., 2019) and to understand the interaction between vectors, hosts and pathogens through the identification of host species. A higher proportion of engorged females for resting sites at upper height levels might be explained by ornithophilic host-seeking females at the top, like it was shown for resting Cx. pipiens s.l. and Culex restuans in proximity to bird roots (Panella et al., 2011). In general, traps at higher levels were found to increase the capture success for ornithophilic mosquitoes (Tempelis et al., 1965).

Artificial resting sites have many operational advantages in comparison to conventional mosquito traps (Burkett-Cadena, 2011; Degefa et al., 2019; Panella et al., 2011; Sauer et al., 2020). The pop-up garden bags we used are inexpensive, non-insecticidal, easy to store and transport. In the field, they can be easily attached to trees or on the ground, for example, using cable ties, tent pegs or stones. Due to the weather-resistant material, the pop-up garden bags can remain in the field throughout the complete trapping season. During the term of this biennial study, not a single resting site had to be replaced. Furthermore, resting sites do not require the use of bait (e.g. light or carbon dioxide), which can break down or be used up. In addition, the usage of resting sites as trapping device saves at least 50% of travel time, because the sampling devices do not have to be switched on or off as it is the case for commonly used carbon dioxide traps. This saves both, financial expenses (e.g. for carbon dioxide) and time. To maximize the sampling success, the number of artificial resting sites per sampling site can be easily increased. Our results suggest that approximately 20 resting sites allow the collection of the same number of specimens as one standard carbon dioxide-baited trap.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHORS’ CONTRIBUTIONS

Conceived and designed the study: LJ, ET, JSC, EK and RL. Collected the data: LJ, FS, RL. Analysed the data: LJ and RL. Drafted the manuscript: LJ, EK and RL. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data supporting the conclusions of this article are included within the article and its additional files.

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REFERENCES

Bache, S.M. & Wickham, H. (2014) Magrittr: a forward-pipe operator for R. R package version, 1(1).
Becker, N., Petric, D., Boase, C., Zgomba, M., Dahl, C. & Kaiser, A. (2010) Mosquitoes and their control. Heidelberg: Springer.
Bhatt, R., Warma, R., Yadavi, R. & Warma, V. (1989) Resting of mosquitoes in outdoor pit shelters in Kheda district, Gujarat. Indian Journal of Malariology, 26, 75–81.
Bidlingmayer, W. (1971) Mosquito flight paths in relation to the environment. Illumination levels, orientation, and resting areas. Annals of the Entomological Society of America, 64, 1121–1131.
Brown, R., Hing, C.T., Fornace, K. & Ferguson, H.M. (2018) Evaluation of resting traps to examine the behaviour and ecology of mosquito vectors in an area of rapidly changing land use in Sabah, Malaysian Borneo. Parasites & Vectors, 11, 346.
Brugman, V. (2016) Host selection and feeding preferences of farm-associated mosquitoes (Diptera: Culicidae) in the United Kingdom. PhD Thesis, University of London.
Burkett-Cadena, N.D. (2011) A wire-frame shelter for collecting resting mosquitoes. Journal of the American Mosquito Control Association, 27, 153–155.
Burkett-Cadena, N.D., Eubanks, M.D. & Unnasch, T.R. (2008) Preference of female mosquitoes for natural and artificial resting sites. Journal of the American Mosquito Control Association, 24, 228.
Crans, W.J. (1970) The blood feeding habits of Culex territans Walker. Mosquito News, 30, 445–447.
Degefa, T., Yewhalaw, D., Zhou, G., Lee, M.-C., Atieli, H., Githeko, A.K. et al. (2019) Evaluation of the performance of new sticky pots for outdoor resting malaria vector surveillance in western Kenya. Parasites & Vectors, 12, 1–14.
Doyle, M.A., Kline, D.L., Allan, S.A. & Kaufman, P.E. (2009) Efficacy of residual bifenthrin applied to landscape vegetation against Aedes albopictus. Journal of the American Mosquito Control Association, 25, 179–184.
Drummond, C., Gebhardt, M.E., Sáenz Robles, M.T., Carpi, G., Hoyer, I., Pastusiak, A. et al. (2020) Stability and detection of nucleic acid from viruses and hosts in controlled mosquito blood feeds. PLoS One, 15, e0231061.
Edman, J.D., Evans, F. & Williams, J.A. (1968) Development of a diurnal resting box to collect Culex melanura (co.). The American Journal of Tropical Medicine and Hygiene, 17, 451–456.
Fay, R. & Prince, W. (1968) A trap based on visual responses of adult mosquitoes. Mosquito News, 28, 1–7.
Gusciora, W. (1971) The resting box technique for the sampling of Culiseta melanura (Coquillet), Proceeding of the Annual Meeting of the New Jersey Mosquito Extermination Association, 48, 122–125.

Hoel, D.F., Obenauer, P.J., Clark, M., Smith, R., Hughes, T.H., Larson, R.T. et al. (2011) Efficacy of ovitraps colors and patterns for attracting Aedes albopictus at suburban field sites in north-Central Florida. Journal of the American Mosquito Control Association, 27, 245–251.

Hothorn, T., Bretz, F. & Westfall, P. (2008) Simultaneous inference in general parametric models. Biometrical Journal: Journal of Mathematical Methods in Biosciences, 50, 346–363.

Howard, J.J., Oliver, J. & Kramer, L.D. (2011) Assessing the use of diurnal resting shelters by Culiseta melanura (Diptera: Culicidae). Journal of Medical Entomology, 48, 909–913.

Irby, W.S. & Apperson, C.S. (1992) Spatial and temporal distribution of resting female mosquitoes (Diptera: Culicidae) in the coastal plain of North Carolina. Journal of Medical Entomology, 29, 150–159.

Irmak, M.A., Yilmaz, S., Mutlu, E. & Yilmaz, H. (2018) Assessment of the effects of different tree species on urban microclimate. Environmental Science and Pollution Research, 25, 15802–15822.

Kumar, N., Pollack, R. & Spielman, A. (1995) A nestable fiber pot for sampling resting mosquitoes. Journal of the American Mosquito Control Association, 11, 463–467.

Kweka, E.J. & Mahande, A.M. (2009) Comparative evaluation of four mosquito sampling methods in rice irrigation schemes of lower Moshi, northern Tanzania. Malaria Journal, 8, 149.

L’Ambert, G., Ferré, J.B., Schaffner, F., Angel, G., Geoffroy, B., Hervy, J.-P., Rhaiem, A. & Brunhes, J. (2011) Les moustiques d’Europe: logiciel d’identification et d’enregistrement – The mosquitoes of Europe: an identification and training programme, Montpellier, France.

Morris, C. (1981) A structural and operational analysis of diurnal resting shelters for mosquitoes (Diptera: Culicidae). Journal of Medical Entomology, 18, 419–424.

Paaijmans, K.P. & Thomas, M.B. (2011) The influence of mosquito resting behaviour and associated microclimate for malaria risk. Malaria Journal, 10, 183.

Panella, N.A., Crockett, R.J.K., Biggerstaff, B.J. & Komar, N. (2011) The Centers for Disease Control and Prevention resting trap: a novel device for collecting resting mosquitoes. Journal of the American Mosquito Control Association, 27, 323.

Pezzin, A., Sy, V., Puggioli, A., Veronesi, R., Carrieri, M., Maccagnani, B. et al. (2016) Comparative study on the effectiveness of different mosquito traps in arbovirus surveillance with a focus on WNV detection. Acta Tropic, 153, 93–100.

R Development Core Team. (2011) R: A language and environment for statistical computing. Vienna, Austria: R Development Core Team. http://www.R-project.org/

Reiter, P., Amador, M.A., Anderson, R.A. & Clark, G.G. (1995) Dispersal of Aedes aegypti in an urban area after blood feeding as demonstrated by rubidium-marked eggs. The American Journal of Tropical Medicine and Hygiene, 52, 177–179.

Ritchie, S.A., Cortis, G., Paton, C., Townsend, M., Shroyer, D., Zborowski, P. et al. (2013) A simple non-powered passive trap for the collection of mosquitoes for arbovirus surveillance. Journal of Medical Entomology, 50, 185–194.

Sauer, F., Grave, J., Lühken, R. & Kiel, E. (2021) Habitat and microclimate affect the resting site selection of mosquitoes. Medical and Veterinary Entomology, 35, 379–388.

Sauer, F.G., Jaworski, L., Lühken, R. & Kiel, E. (2020) Impacts of sampling rhythm and exposition on the effectiveness of artificial resting shelters for mosquito collection in northern Germany. Journal of Vector Ecology, 45, 137–141.

Sawadogo, S.P., Niang, A., Bilgo, E., Millogo, A., Maiga, H., Dabire, R.K. et al. (2017) Targeting male mosquito swarms to control malaria vector density. PLoS One, 12, e0173273.

Schaffner, F., Angel, G., Geoffroy, B., Hervy, J.-P., Rhaeam, A. & Brunhes, J. (2001) Les moustiques d’Europe: logiciel d’identification et d’enseignement– The mosquitoes of Europe: an identification and training programme, Montpellier, France.

Schreiber, E., Walton, W. & Mullu, M. (1993) Mosquito utilization of resting sites at an urban residence in southern California. Bulletin of the Society of Vector Ecologists, 18, 152–159.

Service, M. (1980) Effects of wind on the behaviour and distribution of mosquitoes and blackflies. International Journal of Biodeterioration, 24, 347–353.

Tempelis, C., Reeves, W., Bellamy, R. & Lofy, M. (1965) A three-year study of the feeding habits of Culex tarsalis in Kern County, California. The American Journal of Tropical Medicine and Hygiene, 14, 170–177.

Thomson, R.M. (1938) The reactions of mosquitoes to temperature and humidity. Bulletin of Entomological Research, 29, 125–140.

Tomazatos, A., Jansen, S., Pfister, S., Török, E., Maranda, I., Horváth, C. et al. (2019) Ecology of West Nile Virus in the Danube Delta, Romania: phylogeography, xenosurveillance and mosquito host-feeding patterns. Viruses, 11, 1159.

Trott, R., Brown, G., Potter, M. & Hubbard, J. (2007) Efficacy of two pyrethroid insecticides applied as barrier treatments for managing mosquito (Diptera: Culicidae) populations in suburban residential properties. Journal of Medical Entomology, 44, 470–477.

Vazquez-Prokopec, G.M., Galvin, W.A., Kelly, R. & Kitron, U. (2009) A new, cost-effective, battery-powered aspirator for adult mosquito collections. Journal of Medical Entomology, 46, 1256–1259.

WHO (2020) Vector-borne diseases, 2020. https://www.who.int/en/news-room/fact-sheets/detail/vector-borne-diseases

Wickham, H. (2009) ggplot2: elegant graphics for data analysis. New York: Springer.

Wickham, H. (2011) The split-apply-combine strategy for data analysis. Journal of Statistical Software, 40. 1–29.

Wickham, H. & Henry, L. (2017) tidyr: easily tidy data with tidyr functions. https://CRAN.R-project.org/package=tidyr, 1. 1.

Williams, G.M. & Gingrich, J.B. (2007) Comparison of light traps, gravid traps, and resting boxes for West Nile virus surveillance. Journal of Vector Ecology, 32, 285–291.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher’s website.

Figure S1 Sampling sites in in North-West Germany; 2016 in grey and 2017 in black.

Figure S2 Section of the experimental setup showing a coniferous tree with different-sized artificial resting sites attached close to the ground and at about 1.5 m above the ground.

Figure S3 Mean percentage (95% confidence interval) of the total number of collected female mosquitoes per resting site and year compared to one parallel running Biogents Sentinel trap per sampling area (n = 16). Each resting site in three sizes were attached in a coniferous and deciduous tree at three different heights.
Figure S4 Mean percentage (95% confidence interval) of the total number of non-overlapping taxa per resting site and year compared to one parallel running Biogents Sentinel trap per sampling area (n = 16). Each resting site in three sizes were attached in a coniferous and deciduous tree at three different heights.

Table S1 Estimated regression parameters, standard errors, t-values and p-values for the binomial generalized linear model.

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