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Longitudinal field evaluation of outdoor *Anopheles* and non-*Anopheles* host-seeking in response to a volatile pyrethroid spatial emanator (SE) product among forest-dwelling indigenous residents of Sumatra, Indonesia

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Abstract

Background Interventions against adult *Anopheles* mosquitoes responsible for malaria transmission have traditionally been aimed at indoor spaces and biting behaviours. However, no globally recommended intervention exists which directly interrupt or target outdoor biting behaviours. A volatile pyrethroid spatial emanator (SE) containing transfluthrin aims to address this gap in protection via a simple-to-use, readily deployable device to provide multiple weeks of protection. The device was tested in open-walled households of the forest-dwelling Orang Rimba people in Sumatra, Indonesia, over the course of sixteen weekly entomological visits.

Methods Double-net traps were used for all mosquito collections. Collections occurred near Bukit Duabelas National Park in central Sumatra, an area characterized by secondary forests undergoing widespread conversion to palm and rubber plantations. Four collections occurred per collection night within ten geographically separated small familial groups for a total of 40 trap-nights per week. Groups were assigned the SE or a control device after a seven-week baseline trapping period. Devices were replaced every four weeks. Results were compared using generalized linear models, incorporating treatment, weather, and landscape parameters as fixed effects, with date and location included as random effects.

Results *Anopheles* mosquitoes were captured on 63.2% of all collection nights. Overall nightly *Anopheles* host-seeking activity was lower in the presence of SE devices (RR: 0.29 [0.19–0.45], $p < 0.001$). Non-*Anopheles* mosquitoes experienced a smaller nightly decline in behaviour (RR: 0.78 [0.64–0.93], $p = 0.007$). The age of the device (1 month) did not impact modeled efficacy. *Anopheles* host-seeking activity was also positively correlated with humidity, topographic wetness, and local human structure density.

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Conclusions The SE device evaluated in this field trial was effective in reducing outdoor human exposure to *Anopheles* and non-*Anopheles* mosquito host-seeking activity. The effect was not found to depend upon the age of the device, suggesting that the protection was persistent over the 4-week replacement period during this study. There was an association between hour of collection and intervention efficacy, suggesting the possibility of species-specific effects which were not further investigated. The SE device is a promising, low-cost, easily deployable, and distributable intervention that reduces exposure to mosquitoes with consequent impacts on transmission in outdoor environments.

Background

Vector control measures have been highly useful in global efforts to control malaria, most notably long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS) [1]. These interventions target specific endophagic (indoor feeding) and endophilic (indoor resting) behaviours—the primary behaviours of important vectors such as *Anopheles gambiae* sensu stricto (*s.s.*)—to interrupt the transmission cycle. In many settings, molecular identification has revealed high *Anopheles* species diversity and diverse biting behaviours [2–6]. Additionally, mosquito population structure and behaviour has been recorded to shift following intervention deployment [7–14]. This diversity in species and their associated plastic bionomic traits highlights both the limitations of present strategies and the existing gaps in protection requiring novel vector control measures to target these species and behaviours that may function outside the protection of indoor-focused interventions. These indoor interventions can still contribute to transmission reduction in outdoor biting species, but are insufficient for migrant and mobile populations, or forest-dwelling communities, among other cases where LLINs and IRS either do not function or cannot be optimally used [15]. In this study, a volatile pyrethroid spatial repellent (SE) designed to inhibit mosquito biting was evaluated among an indigenous group at heightened malaria risk living in a remote forest setting in central Sumatra, Indonesia.

Indonesia, with a decentralized health system, is a large, diverse country with a range of transmission settings, including areas implementing localized malaria elimination programs [16, 17]. The country contains an abundance of *Anopheles* diversity between and within sites, with rich species compositions that, in many locations, include species capable of displaying behaviours which circumvent the existing indoor interventions [4, 18, 19]. The study site was in central Sumatra, surrounding the Bukit Duabelas National Park, in an area of tropical rainforest which has been largely converted to palm and rubber plantations with some small pockets of secondary growth forest. Many of the local species display zoophilic tendencies, including some species found near the study site that exhibit opportunistic feeding behavior—*Anopheles aconitus, Anopheles barbirostris, Anopheles nigerrimus*, among others—which have been incriminated as malaria vectors locally or elsewhere in Indonesia [4, 5, 18–20]. The area is populated by the Orang Rimba, the local indigenous group who live in and near forests which have undergone large-scale conversion to palm and rubber plantations in recent decades [21]. They are mobile, frequently relocating their camps as needed and, therefore, tend to live in light, temporary open-walled structures (Fig. 1). They are likely at heightened risk of malaria exposure—possibly including zoonotic forms—due to their residence within the forest and proximity to larval habitats [22]. Malaria incidence is low at a provincial level, with an annual incidence rate of 0.01 per 1000 people, but malaria elimination efforts have had mixed success on a district level, with only 63.6% of districts reporting elimination in 2021 [23]. IRS is also not feasible in this setting due to their open-walled structures, although most surveyed participants reported using bed nets [Syafruddin, Pers. Comm.]. The Orang Rimba represents a population which may not be well-covered by the existing interventions and formed the basis for a small-scale longitudinal field trial to quantify the efficacy of the SE intervention in a field setting using entomological outcomes.

The SE device, a proprietary design by Widder Bros Inc., was a prototype of the PIC® BITEBARRIER® that uses a transfluthrin active ingredient which has been demonstrated in previous studies to be effective in interrupting host-seeking, possibly via mortality and other effects on landing and biting behaviour, in laboratory and wild *Anopheles* and other vector species [24] [Syafruddin Pers. Comm., Widder Pers. Comm.]. This includes effects against mosquitoes with metabolic resistance to other pyrethroids [25–27]. Most transfluthrin products to date have been tested against *Anopheles* vectors outside of Southeast Asia [5, 28–34]. A large-scale field trial of an indoor transfluthrin spatial repellent product was conducted on Sumba Island, Indonesia, while species-specific efficacy against local vectors *Anopheles dirus* and *Anopheles minimus* was demonstrated in a semi-field environment in Vietnam

Fig. 1 Typical structures and local environment inhabited by Orang Rimba study participants in the study area in Jambi, Indonesia

and Thailand and in the field in Cambodia [35–39]. These studies involved passive transfluthrin devices, while a field trial testing actively burning transfluthrin coils demonstrated protection against malaria infection and possible synergistic effects with LLINs [40]. The SE intervention is designed to passively release transfluthrin, creating a protective zone that inhibits mosquito biting for individuals within that space. This mode of action could influence mosquito behaviours that draw them within the domestic or peridomestic space (depending on where the device is deployed). To evaluate the impact of the SE on *Anopheles*—and other mosquito species—host-seeking behaviour, the devices were deployed in target communities and activity was compared to activity in a control arm receiving placebo interventions. The host-seeking rate was compared between treatment and control clusters to quantify the entomological protection associated with SE use during the study period.

Methods

Ethics approval

This study was approved by the Ethics Committee for Medical Research, Faculty of Medicine, University of Hasanuddin, Makassar, Indonesia, and the University of Notre Dame Institutional Review Board (Protocol #: 18-05-4675).

SE product description and placement

The SE product is a volatile pyrethroid spatial repellent designed to be used in both indoor and outdoor settings. The design is proprietary and was a prototype version of the PIC® BITEBARRIER® product, which has a recommended lifetime of 21 days. Each device consisted of two thin 30 × 30 cm sheets hung via twine, operating through passive release of the transfluthrin active ingredient. Their exact placement varied by household, but in general devices were hung centrally at a height of approximately 1.5 m in participating households, away from active fires/kitchen areas or other areas which would interfere with the occupants' activities. Placebo devices were created by cutting sheets of substrate to the size of the SE device and were deployed to the same specifications as the SE devices.

Study location

The study was conducted in Jambi Province in central Sumatra, Indonesia. Study participants were recruited from an approximately 2000 square km area surrounding Bukit Duabelas National Park, a lowland tropical rainforest (Fig. 2). Much of the land surrounding the National Park has been converted to palm oil and rubber plantations, with some remaining pockets of secondary forest.

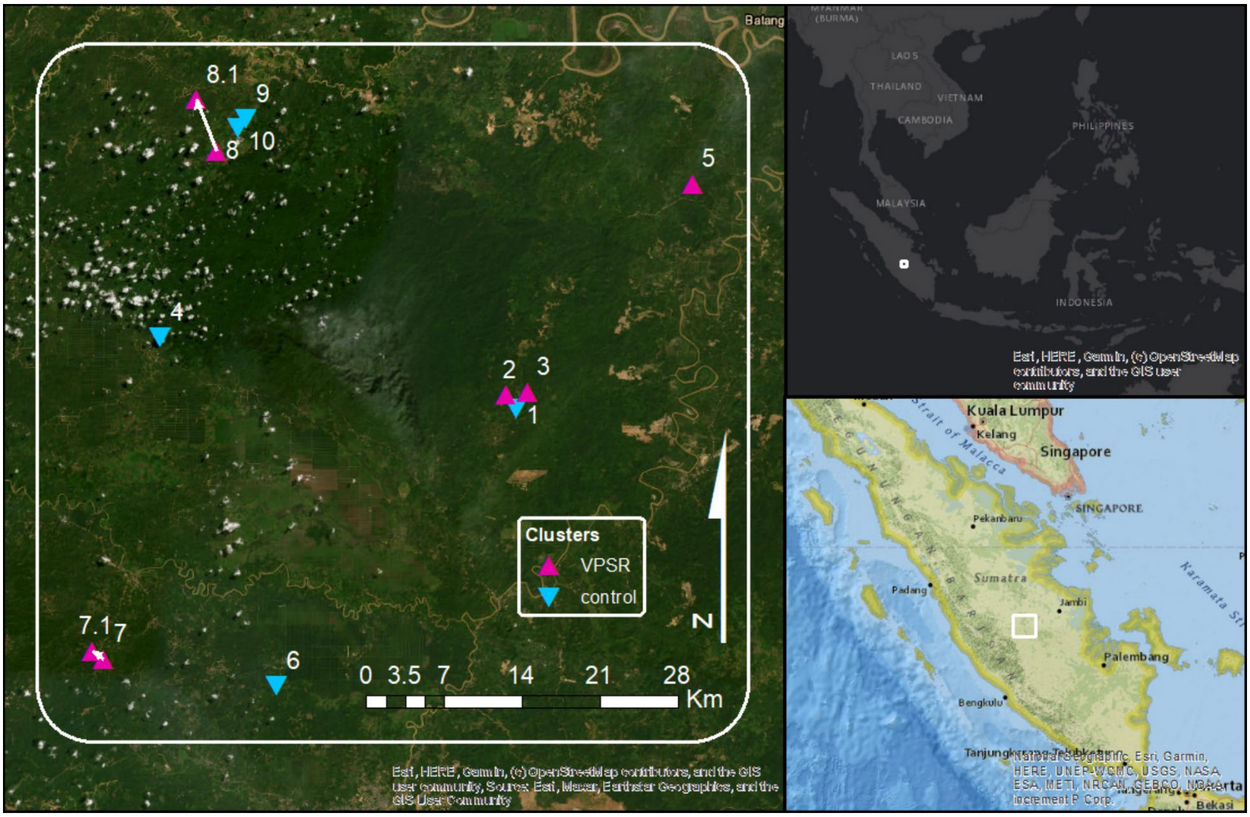

Fig. 2 Map of the study area displaying cluster locations. The national park is in the center of the study area, bordered closely by clusters 1–3, 4, and 8–10. The national park consists of secondary forest, while the surrounding forest has been converted to palm or rubber plantations. Clusters are denoted by their number, with an *arrow* connecting cluster sites for the two clusters which moved during the follow-up period of the study

Study population

Study participants were recruited from among the Indigenous Orang Rimba people living near the Bukit Duabelas National Park. Orang Rimba people are generally mobile, living in small familial groups localized to this region. They often sleep in temporary, lightly built structures, allowing them to move frequently in search of resources or work. Ten separate familial groups were selected for enrollment from the study area and assigned randomly into the treatment or control arm of the study. Every household within a group was given the assigned SE treatment or placebo control devices, and four households were selected from each for mosquito collections for a total of forty collection sites.

Mosquito collection

Double net traps (DNT) were used for all mosquito collections between 18:00 and 06:00 on designated collection nights [41]. Traps were set adjacent to structures with a hanging SE or placebo device. The trap consisted of one small tent inside a larger net, with a small gap underneath the larger net to allow mosquitoes to enter. Both tents were situated underneath a tarp roof to protect the structures from rain. Every hour, a second volunteer entered the larger net and collected trapped mosquitoes. These were counted by hour and preserved. Collectors rotated responsibilities hourly. Upon collection, mosquitoes were identified to genus and preserved in Eppendorf tubes with desiccant beads for future investigations.

Follow-up and product replacement

Baseline collections involving no SE or placebo devices occurred during November and December 2020 for a total of seven weeks. Clusters were assigned following baseline collections and SE and placebo products were installed before follow-up collections began in the second week of January 2021. Households were then followed for a total of sixteen weeks until the last week of April 2021. Mosquito collections were conducted in all sentinel households weekly, with a small degree of missed coverage during early baseline collection weeks. SE and placebo devices were replaced every four weeks during follow-up visits.

Site characterization

Clusters were characterized broadly (i.e. "yes" or "no") by the type of surrounding land cover. Remote sensing was employed to further characterize the study sites. A high spatial resolution (8.25 m) digital elevation model (DEMNAS) raster image released by the Indonesian Geospatial Information Agency was used for hydrologic analysis [42]. The DEM raster was processed through a pipeline calculating slope followed by flow direction and then flow accumulation (a model predicting the total amount of water flowing into each defined cell in the landscape raster) [43]. Topographic wetness index (TWI) was derived from these outputs by the formula:

$$ln\frac{a}{tanb}$$

where a is the local flow accumulation and b is the slope at each point in radians. The average TWI value within a radius of 200 m from each cluster center was included in statistical analysis. All hydrological processing was conducted in ArcMAP version 10.6 using the TauDEM toolbox [44].

The presence of human structures in the study area was quantified remotely using a variety of images. Google Earth provided a sufficiently high-resolution base map used for most structure identification. In cases of cloud coverage or low-resolution, a collection of high-resolution PLANET satellite imagery was referenced [45]. Imagery captured around the time of the study period was prioritized over older imagery, with older imagery only used to fill in gaps. The percentage of land covered by human structures within 200 m of each cluster center was included in analysis.

Data analysis

All data cleanup and analyses were performed in R version 4.2.1. Data cleanup and validation was performed with the 'dplyr' and 'tidyr' packages and visualized using the 'ggplot2' package. Models were created and assessed using the 'lme4' and 'arm' packages. The primary endpoint of DNT-based host seeking (a proxy for human landing, measured per hour and modeled per hour and per night) was modeled using generalized linear mixed effect models (glmer) incorporating SE status, age, spatial variables (TWI and human structure cover), and weather variables (temperature, relative humidity, and rainfall) as fixed effects. Collection date and cluster nested within location (Fig. 2) were included as random effects in nightly models, with household included as an additional random effect nested within cluster in hourly models. Models were tested for zero inflation and overdispersion after fitting, and models were selected by AIC. Hourly models were constructed with glmer models with a Poisson link function, while nightly models were constructed with a negative binomial link function due to overdispersion of comparable Poisson models. Presence vs absence was assessed using binomial mixed effect models. Fixed effect model coefficients are exponentiated and reported as risk ratios (RR) along with the standard deviation of random effect terms.

Results

Enrollment and cluster description

The ten clusters were located on a study area of approximately 2500 sq km, with a minimum distance of 1 km and a maximum distance of 68 km between any two clusters. Clusters were enrolled randomly into treatment and control arms and the first SE or placebo devices were distributed between January 10 and 15, 2021. The number of collection nights was consistent between clusters with slight variation due to weather or unforeseen circumstances leading to an unavailable collector. Study clusters were located within forests of varying types and mixtures. Palm oil plantations were the most common type of forest cover surrounding study clusters ($n=7$), followed by rubber plantations ($n=4$) and secondary forest ($n=3$). Six clusters resided in an area characterized exclusively by one type of plantation cover. Three clusters were characterized by a combination of plantation cover and secondary forest, and one cluster by a combination of both types of plantations (Fig. 2).

Mosquito host-seeking activity

A total of 27,509 mosquitoes were collected across all households over 876 total nights of mosquito collections, including 240 nights of baseline collections and 636 nights during the follow-up period (Table 1, S1 Table). Mosquitoes were captured on 98.2% of all nights ($n=860$), with a median of 21 mosquitoes per collection night when present. There was no significant difference in presence versus absence between baseline (97.5%, $n=234/240$) and follow-up (98.4%, $n=626/636$) collections ($p=0.63$), but a significant decrease in overall mosquito abundance, independent of intervention status, was observed as the study progressed (Table S2; Weekly Risk ratio (RR): 0.96 [0.94–0.98], $p<0.001$).

In total, 2450 mosquitoes (8.9%) were identified as members of the *Anopheles* genus. *Anopheles* were captured on 63.2% ($n=554$) of all collection nights at a median rate of three *Anopheles* captured per night when present. No significant difference attributed to collection period or visit was observed in *Anopheles* presence ($p=0.47$) or abundance (Table 2; $p=0.46$). General seasonal trends of *Anopheles* host-seeking activity are displayed in Fig. S1. Mosquitoes were captured at all hours of collection, with hourly biting rates generally

Table 1 Numbers are displayed as the total number of Anopheles with the number of collection nights in parenthesis. Sub-totals are provided for baseline and follow-up periods for treatment and placebo clusters. In the case of clustermovement, sub-locations are indicated in italics and parentheses on the left, with corresponding sub-location values listed in italics in the baseline and follow-up columns

Cluster	Total *An*. (# nights)	
	Baseline	Follow-up
Control		
1	32 (24)	31 (64)
4	59 (24)	226 (64)
6	265 (28)	394 (64)
9	34 (28)	299 (64)
10	37 (20)	173 (64)
Sum	427 (124)	1123 (320)
Treatment		
2	31 (20)	40 (64)
3	61 (24)	26 (64)
5	65 (20)	48 (60)
7	121 (24)	305 (64)
(1)	*121 (24)*	*73 (48)*
(2)	– (–)	*232 (16)*
8	46 (28)	157 (64)
(1)	*46 (28)*	*58 (28)*
(2)	– (–)	*0 (4)*
(3)	– (–)	*99 (32)*
Sum	324 (116)	576 (316)

observed to decrease over the course of the night (Fig. 3). This trend was significant across both treatment arms (Table 2; hourly RR: 0.89 [0.85–0.93], $p<0.001$), with capture rates shifting slightly towards later hours in the intervention period compared to the baseline (Table 2; hourly RR: 1.07 [1.02–1.12], $p=0.005$). Hourly trends for non-*Anopheles* species were similar (S2 Table).

The mean nightly temperature was 24.2 °C across all collection nights and did not vary between baseline and follow-up collection periods. The relative humidity also remained high with slight variation with a nightly mean of 97.7% RH. Rain occurred during 71.2% of collection nights ($n=171/240$) during the baseline period, with an average of 11.7 mm of rainfall on rainy nights. During the follow-up period, rain was observed on 60.2% of collection nights ($n=383/636$) and an average of 12.2 mm on rainy nights (Fig. S2).

Mosquito species

A total of 451 *Anopheles* specimens (18.4% of all collected) and 6518 non-*Anopheles* (26.0% of total) were identified to species. The most dominant *Anopheles* species was *Anopheles umbrosus* (42.4% of identified *Anopheles*), with the majority of the identified specimens (66.1%) belowing to the Umbrosus group. *Anopheles maculatus*, *Anopheles vagus*, and members of Group Hyrcanus and Group Leucosphyrus, among other species, were also identified (Table 3). Most identified non-*Anopheles* mosquitoes were *Culex* species (58.1%), followed by *Mansonia* species (25.1%) and others (Table 4). Species determinations were made for a subset of specimens (S4 Table).

Variation and movement of clusters

Anopheles host-seeking activity varied considerably among clusters, from a minimum of 31 and a maximum of 394 mosquitoes captured over 64 nights in control structures during the follow-up period (Table 1). Two intervention clusters moved during the intervention period while control clusters stayed in place (Fig. 4). Cluster 8 moved to a nearby new location before visit #5 before returning to their previous location prior to visit #6. In these two locations, they encountered 58 mosquitoes over 32 nights (1.8 per night). They proceeded to move to a location deeper in the forest prior to follow-up #9, where they remained until the end of the study. At this new location they encountered 99 mosquitoes over 32 nights (3.1 per night). This difference was not significant (RR: 1.17 [0.46–2.99], $p=0.74$). Mosquito collectors captured 73 *Anopheles* over 48 nights (1.5 per night) in cluster 7 between intervention visits #1 and #12. The cluster moved to a new location prior to visit #13, where 232 *Anopheles* were captured over the remaining 16 collection nights (14.5 per night; RR: 9.96 [4.41–22.5], $p<0.001$). These two clusters were assigned unique cluster identification codes during collection visits when they were not in their original location (Table 1).

SE efficacy

During the baseline period and prior to intervention or placebo deployment, a total of 427 *Anopheles* were captured over 124 collection nights in control structures (3.4 mosquitoes/night). Comparatively, 324 *Anopheles* were captured over 116 nights in treatment structures (2.8 mosquitoes/night; RR: 1.51 [0.80–2.87], $p=0.207$). During the follow-up period, 1123 *Anopheles* mosquitoes were collected over 320 nights in control structures (3.5 mosquitoes/night) compared to 576 over 316 nights in treatment structures (1.8 mosquitoes/night; RR: 0.29 [0.19–0.45], $p<0.001$) (Fig. 5). A significant interaction was observed between hourly activity and intervention status in the follow-up period, with hourly host seeking activity declining quicker in treated structures (hourly RR: 0.91 [0.85–0.97], $p=0.005$). All model coefficients are presented in Table 2. *Anopheles* host-seeking activity was

Table 2 Coefficients have been exponentiated for interpretation, and results are reported as rate ratio (RR) with 95% confidence intervals. The random effect terms are specified for each model, with crossed effects on separate lines and nested effects separated on the same line. Untransformed fixed effect coefficients and standard deviation for random effect terms are presented in Table S3. Coefficients were scaled and centered around study means. AIC and degrees of freedom for the full model are provided, with corresponding values for the null model provided in parenthesis

(A) Nightly HLC		**Modeled endpoint**	**(B) Hourly HLC**	
Negative binomial		**Model family**	**Poisson**	
Fixed effects				
1.85 [1.10–3.12]	0.021	(Intercept)	0.23 [0.13–0.41]	<0.001
1.15 [0.80–1.65]	0.461	Follow-up period	0.88 [0.58–1.34]	0.542
0.29 [0.19–0.45]	<0.001	Treatment (SE)—Follow-up	0.37 [0.25–0.56]	<0.001
1.51 [0.80–2.87]	0.207	Treatment (SE)—Baseline	1.52 [0.75–3.06]	0.241
1.19 [1.01–1.40]	0.035	RH % (nightly mean)#	1.12 [1.01–1.24]	0.030
1.36 [1.02–1.82]	0.038	Topographic wetness (TWI)#	1.48 [1.10–2.00]	0.010
1.73 [1.28–2.32]	<0.001	Human structures#	1.79 [1.33–2.40]	<0.001
–		Hour (after 18:00)	0.89 [0.85–0.93]	<0.001
–		Treatment::hour (Follow-up)	0.91 [0.85–0.97]	0.005
–		Treatment::hour (Baseline)	1.01 [0.96–1.07]	0.633
–		Follow-up period::hour	1.07 [1.02–1.12]	0.005
Random effects				
Collection date			Collection date/hour	
Location/cluster			Location/cluster/hour	
865 (875)		Degrees freedom	10,352 (10,367)	
3370 (3739)		AIC	10,980 (13,787)	

coefficients were scaled and centered around study means

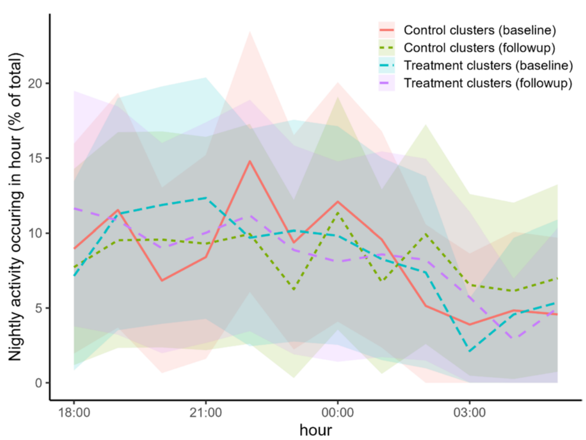

Fig. 3 Proportional hourly host seeking activity in control and treatment clusters. *Lines* refer to the mean proportion of host seeking activity which occurred in each hour across all study nights in each cluster and study period, with the 95% confidence interval ribbons displayed

significantly associated with topographic wetness index, land coverage by human structures, and relative humidity in nightly and hourly models. The SE interventions had a significant but lesser effect on non-*Anopheles* mosquitoes (RR: 0.74 [0.62–0.89], $p = 0.001$), with full model results reported in Table S2. Device age in weeks after opening, nightly temperature, and rainfall were not predictive in any model.

Discussion

The SE devices had a significant impact on localized mosquito host-seeking activity (i.e., personal protective effects for individuals residing within the protected structures) over the duration of the study period. The intervention was attributed to an approximately 70% reduction in nightly *Anopheles* host seeking activity compared to placebo devices, with hourly analysis indicating that the protective effect is more pronounced later in the night. In the hourly model, the interventions were predicted to reduce 63% of host seeking activity in the first hour (18:00–19:00), increasing to 88% reduction in the final hour of collection (05:00–06:00). It is possible that this represents an accumulation effect throughout the night, species-specific responses towards the SE device, or other variation in hourly host-seeking activity based on innate behaviour or random factors. Given the all-night nature of the malaria exposure in the region, it may be more appropriate to consider the all-night protective efficacy of ~70% rather than the hourly protection. Overall, the same trends were observed to a lesser

Table 3 Identified *Anopheles* mosquitoes ($n = 451$, 18.4% of total specimens) were selected from study baseline and follow-up periods. Table is organized by species group or complex in alphabetical order, with the total number of mosquitoes indicated in parenthesis next to each group name

Species	#	Species group	References
An. montanus	1	Albotaeniorhynchus (1)	
An. annularis	1	Annularis (1)	Sukkanon (2022) [46]
An. crawfordi	3	Hyrcanus (22)	
An. lesteri	1		Maquart (2021) [47]
An. nigerrimus	2		Sukkanon (2022) [46]
An. nitidus	13		
An. peditaeniatus	3		Sukkanon (2022) [46]
An. hyrcanus grp	1		Sukkanon (2022) [46]
An. kochi	9	Kochi (9)	Sukkanon (2022) [46]
An. latens	5	Leucosphyrus (15)	
An. leucosphyrus grp	10		Vythilingam (2018) [48]
An. maculatus	54	Maculatus (54)	Sukkanon (2022) [46]
An. minimus	1	Minimus (1)	Sukkanon (2022) [46]
An. tessellatus	1	Neomyzomyia (1)	
An. brevipalpis	8	Umbrosus (298)	
An. letifer	52		Permana (2023) [49]
An. roperi	1		
An. separatus	2		
An. umbrosus	191		
An. whartoni	16		
An. umbrosus grp	28		
An. vagus	21	Vagus (21)	Sukkanon (2022) [46]
An. spp.	27	Other (27)	
Total	451	(451)	

Table 4 Identified non-*Anopheles* mosquitoes ($n = 6518$, 26.0% of total non-*Anopheles* specimens) were selected from study baseline and follow-up periods. The number of specimens is presented alphabetically by genus, with percentage indicated in parentheses

Genus	#
Aedes spp.	385 (5.9%)
Armigeres spp.	638 (9.8%)
Coquillettidia spp.	25 (0.4%)
Culex spp.	3789 (58.1%)
Mansonia spp.	1636 (25.1%)
Other	45 (0.7%)
Total	6518

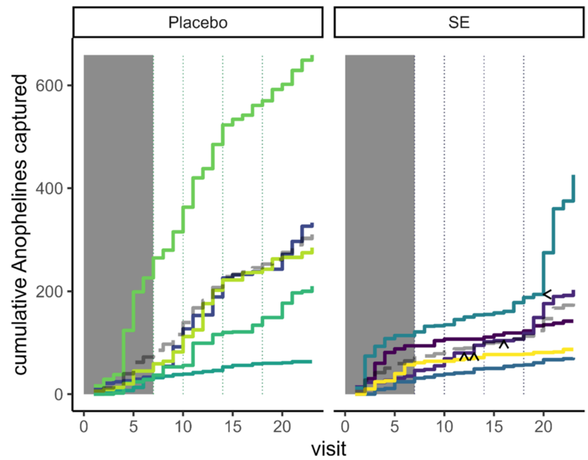

Fig. 4 Cumulative capture of *Anopheles* mosquitoes by study cluster over weekly entomological visits. Untreated clusters are displayed in panel A (*left*) with treated clusters in panel B (*right*). Each cluster is displayed as separate, *colored lines*, with mean values within the two treatment arms displayed in each panel as *gray dashed lines*. The baseline period is denoted by a *gray box* between weekly visits 1 and 7. Cluster movement occurred in two treatment clusters and is denoted by *arrows*. *Vertical dotted lines* represent device replacement

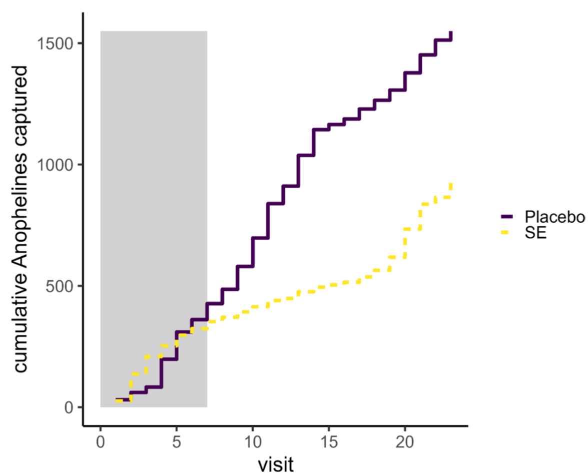

Fig. 5 Cumulative capture of *Anopheles* mosquitoes in treatment and placebo clusters. The number of mosquitoes is displayed cumulatively by visit on the x-axis by study arm. The baseline period is denoted by a *gray background* between visits 1 and 7

degree amongst non-*Anopheles* mosquitoes, with an overall nightly reduction of approximately 25% observed in these species. There was no discernable effect of device age on efficacy, indicating that mosquitoes continued to avoid SE-protected structures for their four-week deployment in rainy and humid conditions. Although these devices are not designed to get wet, these results suggest that hanging them underneath open-walled roof structures is a viable deployment strategy. A recent semi-field study of the SE devices in Zambia indicated a slight, but significant decline [24], and a Latin-square rotational comparison study conducted in one of the placebo clusters from this trial indicate possible age effects in one of the secondary analyses [46]. A further field study in Zambia did not indicate an age effect (manuscript in review). The longevity of the SE devices should continue to be investigated, with intent to understand the influential factors. Due to their portability and the presently demonstrated efficacy in challenging conditions, the SE devices may be applicable to a wide range of other cases where protection from mosquito biting is desired, ranging from military personnel deployed in the field to recreational activities or protecting public spaces.

A considerable degree of variation was observed throughout the study period. Clusters were nested within subgroups of the Orang Rimba population, with collection structures correspondingly nested within each cluster. When possible, this nested structure was preserved in the random effect term, with the complexity reduced to achieve successful model convergence when necessary. The models ascribed roughly equal variation to the random effects of collection date and site effect, with the inclusion of both improving the model fits considerably and resulting in similar modeled values of standard deviation for the random intercept terms. Adding additional fixed effects of relative humidity, human land use, and topographic wetness to the models appeared to reduce the total variability attributed to the random effects by roughly a third without changing the modelled estimate of SE efficacy. These effects are predictable based on mosquito ecology; mosquito host-seeking activity increases with higher relative humidity, higher human land use in an area, and higher predicted wetness based on topography. It is possible more detailed data, such as larval surveys, could help in further understanding these sources of variation across a localized study area. Such data would be useful in the case of two treatment clusters which moved locations to areas which had no corresponding baseline data. During these moves, the SE device in the cluster structures was not replaced nor were the individuals instructed on its proper transportation. It is unclear whether the increase in mosquitoes captured in the new location is due to elevated host seeking activity in the new location, SE damage/reduced efficacy, or some combination of both and possibly other factors.

The seasonal abundance of *Anopheles* mosquitoes was steady throughout the baseline and follow-up collection periods, highlighting the continuous possibility of malaria transmission in the area. *Anopheles* mosquitoes were encountered on most collection nights in most clusters, with the SE devices significantly reducing the abundance of *Anopheles* host-seeking activity in protected households during the follow-up period. Other, non-*Anopheles* mosquitoes significantly declined in abundance over the duration of the study period, but otherwise followed the same trends in modelled parameters as *Anopheles.* This decline may be indicative of species-specific behaviours among the non-*Anopheles* species or could be more easily detected in these mosquitoes compared to *Anopheles* due to the larger number of these species captured during the study.

Many of the *Anopheles* species morphologically identified during this study have been implicated in human and zoonotic malaria transmission. This includes *Anopheles letifer*, a member of the most frequently observed *Anopheles* group captured during the study (Umbrosus), alongside *An. maculatus, An. vagus,* members of Group Hyrcanus, and other individual species [47–50]. This study did not seek to assess the insecticide resistance status of captured *Anopheles*, but it should be noted that transfluthrin has shown efficacy towards mosquitoes resistant to other pyrethroids in previous studies [25–27]. Morphologically identified mosquitoes were randomly selected from the total collection without specific regard for treatment arm, cluster, or follow-up week. Therefore, individual species-specific effects were not assessed in this study. These are important to consider in the future, particularly in the species-rich environments of Southeast Asia, to ensure that effects are observed in species contributing to transmission. Understanding how the SE device operates in these cases will be vital in modeling predictions of efficacy and possible synergy between interventions. Presently, pyrethroid resistance is measured by WHO susceptibility assays of lab-reared local larvae, an approach which is time-consuming and particularly difficult among the species and larval habitat diversity of Indonesia [51]. When possible, the resistance status of local vectors should be prioritized in study design.

The results of this study support the findings of previous studies which have tested transfluthrin-based spatial repellent products, including other recently concluded and ongoing SE trials [28, 31, 36, 37, 52]. These studies have noted protective efficacy against multiple *Anopheles* species, typically African vectors of the *An. gambiae* complex. A limited number of studies have been conducted in Southeast Asia, with the majority in Indonesia itself during a large-scale field trial of a spatial repellent product applied indoors [36, 37, 39]. In transmission settings across Indonesia, the role of individual *Anopheles* species in transmission is complex and may vary across sampling locations. Furthermore, the effect of transfluthrin is not known against Indonesian or Southeast Asian *Anopheles* vectors in general [35, 37, 40]. This study evaluates the efficacy of the SE device on *Anopheles* mosquitoes in general without regard to species, while investigations into species-specific effects may be appropriate for larger scale field trials which capture a greater degree of variation in the behaviour of resident species. It is possible that species identification could aim in investigating sources of variation, such as species heterogeneity over the study area, and the specimens remain preserved with this as a possible future objective.

A previous randomized controlled trial conducted in Indonesia associated a 16% reduction in *Anopheles* landing with a 60% impact on malaria incidence [36]. The 70% outdoor reduction in *Anopheles* host seeking activity related to this SE represents considerable potential protection against not just malaria but also other vector borne diseases. These variable SE -based protections may be associated with geographic and vector specific factors with observed reductions in landing up to 95% in Cambodian trials [39]. In addition to general usage as an entomological intervention, the SE appeals to various use-cases, such as for military deployment, acute epidemic responses, or recreational purposes.

Conclusions

The SE device tested in this study reduced the host-seeking activity of *Anopheles* mosquitoes by 70% over the course of the follow-up period. No drop in efficacy was observed over the one-month replacement period for each device. Non-*Anopheles* mosquitoes were also significantly impacted by the intervention, with a 20% reduction in host-seeking activity. These results were observed in a setting with relatively consistent weather patterns and numbers of host-seeking *Anopheles* mosquitoes throughout the study period. This impact on host-seeking activity, particularly of *Anopheles* species, supports evidence that the SE device provides viable protection and should continue to be investigated as a malaria control tool. A significant interaction between hour of capture and device efficacy during the follow-up period indicates the possibility of species-specific or behaviour-specific effects which are worth investigating. SEs represent a potential effective outdoor protective intervention against *Anopheles* and non-*Anopheles* vectored diseases.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12936-025-05284-y.

Additional file 1.

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Author contributions

T.A.B. wrote the main manuscript text, prepared all manuscript materials, and performed statistical analysis. L.S., D.H.P., I.E.R., R.R., S.Z., S.Z., M.M.M., R.E., M.K. collected, validated, and analyzed data. P.B.S.A., D.S., N.F.L. conceived the study and secured funding. All authors were involved in study design and planning, and all authors reviewed the manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests

The authors declare no competing interests.

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