

Mosquito arboviral vectors at zoonotic interfaces in border provinces in Thailand

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Abstract

Background Thailand is a biodiversity hotspot with high risk for spillover of zoonotic mosquito-borne viruses. Mosquito vectors of endemic Flaviviruses, Japanese encephalitis virus (JEV) and dengue virus (DENV), are well characterised, yet rapid climate change, land-use shifts, and urbanisation necessitate current surveillance, especially where humans and animals overlap.

Methods Seven interfaces, houses ($n = 9$), temples ($n = 2$), dumpsites ($n = 6$), orchards ($n = 8$), forest edges ($n = 9$), forest interior ($n = 9$) and bat caves ($n = 2$), were sampled in dry and wet seasons of 2024 to determine mosquito species presence and abundance in four districts: Pong Nam Ron (PNR) and Soi Dao (SD) in Chanthaburi province, Mae Fah Luang (MFL) and Wiang Kaen (WK) in Chiang Rai province. Snapshot sampling deployed 2–3 daily replicates of multiple trap types (Light traps, Biogents Pro traps, Gravid Aedes traps, and Prokopack aspirators) over 48 h per interface. Mosquito presence/absence and abundance across interfaces and seasons were assessed with GLMMs, with Tukey post hoc tests. Rarefied diversity indices were calculated for host-seeking communities at interfaces.

Results Overall, dumpsites had the highest burden of arboviral vector species. *Culex gelidus* (45.6%, $n = 6,195$) and *Culex vishnui* (24.6%, $n = 3,348$) were the two most abundant vectors and were most prominent at dumpsites. *Aedes albopictus* (9.0%, $n = 1,014$) was the third top vector, found across all interfaces with high abundances in forests in all districts and at the MFL dumpsite. Host seeking communities at dumpsites were consistently low in diversity as were forest interiors in PNR and WK.

Conclusions This is the first investigation of arboviral vector ecology in Thailand to include dumpsites. These sites support high populations of mosquito vectors that feed day and night, and attract animal species presenting a zoonotic risk to workers and neighbouring farmland communities. Forest sites were also high-risk settings owing to *Ae. albopictus* predominance. Further investigation into its potential as a bridge vector and role in sylvatic transmission is required. Longitudinal monitoring of rural interfaces is recommended to inform vector control and One Health preparedness.

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Keywords Mosquito · Vector · Zoonosis · Arbovirus · Interface

Abbreviations

PNR	Pong Nam Ron district
SD	Soi Dao district
CTH	Pong Nam Ron and Soi Dao districts in Chanthaburi Province, Thailand
MFL	Mae Fah Luang district in Chiang Rai Province, Thailand
WK	Wiang Kaen district in Chiang Rai Province, Thailand
PRO	Biogents Pro Trap
LT	CDC Light Trap
GAT	Gravid Aedes Trap
PK-IN	Prokopack aspiration indoors
PK-OUT	Prokopack aspiration outdoors
BF	Blood-fed female mosquito
SG	Semi-gravid female mosquito
GLMM	Generalised linear mixed model
ANOVA	Analysis of variance
CHIKV	Chikungunya virus
DENV	Dengue virus
JEV	Japanese encephalitis virus
TMUV	Tembusu virus
WNV	West Nile virus

Background

Thailand is entirely within a recognised biodiversity hotspot and shares borders with several countries: Cambodia, Laos, Myanmar and Malaysia [1]. This makes it high priority for investigations into zoonotic viral pathogens with the potential to spillover from wildlife reservoirs to humans. Several transmission pathways exist that could result in spillover: direct contact (in excreta, aerosol, saliva, blood and through the environment) or via an intermediate host (a secondary animal or invertebrate vector) [2].

Known important mosquito-borne viruses exist in Thailand with moderate to significant human pathogenicity, namely dengue (DENV), chikungunya (CHIKV) and Zika (ZIKV), transmitted by *Aedes* spp. vectors; and Japanese encephalitis (JEV), Tembusu (TMUV) and West Nile (WNV) viruses, transmitted by *Culex* spp. [3]. Animal reservoirs for these known arboviruses can be multiple and diverse, for example, pigs and horses (JEV), birds (WNV and JEV), ducks, geese, pigeons and sparrows (TMUV) and monkeys (ZIKV) [3]. Chikungunya and dengue are human reservoir viruses although their origin is sylvatic; however, evidence of neutralising antibodies in wild macaques raises the question if zoonotic transmission could contribute to human infections [4]. The recognised DENV, JEV, TMUV, WNV and ZIKV viruses belong to the genus *Flavivirus*, a group with a history of epidemics and ability to spread with ease to new geographies [5]. The group has attention for its suitability for novel emergences and less well-known zoonotic flaviviruses posing a global threat [5, 6]. With climate change altering vector and reservoir host ecologies, the surveillance of established, new and emergent zoonotic arboviruses is a critical need for public health.

Although arboviruses are a significant public health challenge in Thailand, there are few up-to-date investigations describing vector compositions and ecologies to identify key areas of risk or surveillance for known and novel zoonotic arboviruses. As in many countries, surveillance is reactive, in response to human arboviral outbreaks [7]. Often these are directed at known focal vectors such as *Aedes aegypti* and *Aedes albopictus* (DENV) and overlook lesser known potentially important or emerging vector species [7]. In Thailand, investigations into

unique environments such as zoos [8], islands [9] or areas of land-use change [10] exist, and those on specific environments such as rubber plantations [11], forest and village settings [12]. The last comprehensive publication on mosquito composition in 2008 sampled populations in Central Thailand over a habitat degradation gradient from forest–agriculture–urban focussing on known vector-borne diseases [13]. As it stands, recent studies sampling habitats for arboviral risk are distinctly lacking, particularly those examined through a zoonotic lens.

Environments used daily by humans that overlap with animals or vectors, called interfaces, are crucial for identifying spillover events. This study aimed to fill the gap in arboviral research by examining mosquito composition at human–animal interfaces. The specific aim was to understand the risk of zoonotic arboviral transmission through characterisation of mosquito populations and the identification of vectors and their relative abundances at different interfaces.

Methods

Study site description

Two districts in each of two disparate provinces in Thailand (Fig. 1) were selected due to the presence of wildlife, neighbouring international borders, and diversity in social and cultural human behaviours. Pong Nam Ron (PNR) and Soi Dao (SD) districts in Chanthaburi province borders Cambodia to the east, and to the west, a mountainous forest national park and wildlife sanctuary areas. Chanthaburi is famous for fruit production, mainly longan, durian, rambutan and mangosteen. Water pools are often adjacent for irrigation. Here, human–elephant conflict is problematic with armed guards protecting routes to villages from the forest.

In Chiang Rai, Mae Fah Luang district (MFL) borders Myanmar to the west and Wiang Kaen (WK) borders Lao People's Democratic Republic to the east. Study sites in MFL are located along the eastern foothills of Doi Tung mountain where pine and mixed woodland make up the forest areas, interspersed with ornamental flower growing, macadamia and coffee plantations. The WK site is located at a mountain village, Ban Huai Kook near the Lao border. It is a small community of 100 families, where several generations live under one roof. There is mixed housing, as in MFL and Chanthaburi, concrete homes or wooden structured with thatched or corrugated iron roofing. There is a strong farming culture where families grow rubber, corn, spring onions, banana and lychee for sustenance. Both provinces experience dry-cool, dry-hot and wet seasons with the north suffering from flooding and landslides in monsoon season. For this reason, wet season collections in Chiang Rai could not be conducted subsequently to Chanthaburi collections in August (Table S1) and were postponed to November as the site was inaccessible.

Seven interfaces with human–animal overlap were selected: houses, temples, dumpsites, orchards, forest edges, forest interior and bat caves (Fig. 2). Interfaces were identified by Google maps and ground-truthed on arrival to determine suitability for mosquito trapping based on access. Interfaces from the two Chanthaburi districts (PNR and SD) were combined and reported as Chanthaburi (CTH), while Chiang Rai districts (MFL and WK) were analysed separately due to distinct geography and ecology. In each location (CTH, MFL, WK), 2–3 sampling points per interface were selected (Table S1). Sampling points were spaced ≥ 100 m apart to ensure independence, except at dumpsites (70–80 m) due to the lack of structures to hang traps. Sites within the same area were assigned a shared site code (e.g. C-HO1). Dry and wet season sites were generally consistent, except for forest edge and interior 2 and orchard 2 sites in MFL, which were relocated in the wet season due to high ant abundance.

Data collections

CDC Light traps (LT) and Biogents Pro traps (PRO) were used to collect host-seeking mosquitoes. Traps were baited with 2 kg dry ice in the evening; PRO traps received an additional 1 kg in the morning to attract diurnal species and were supplemented with BG lure (ammonia, lactic acid, caproic acid). Gravid Aedes traps (GAT)

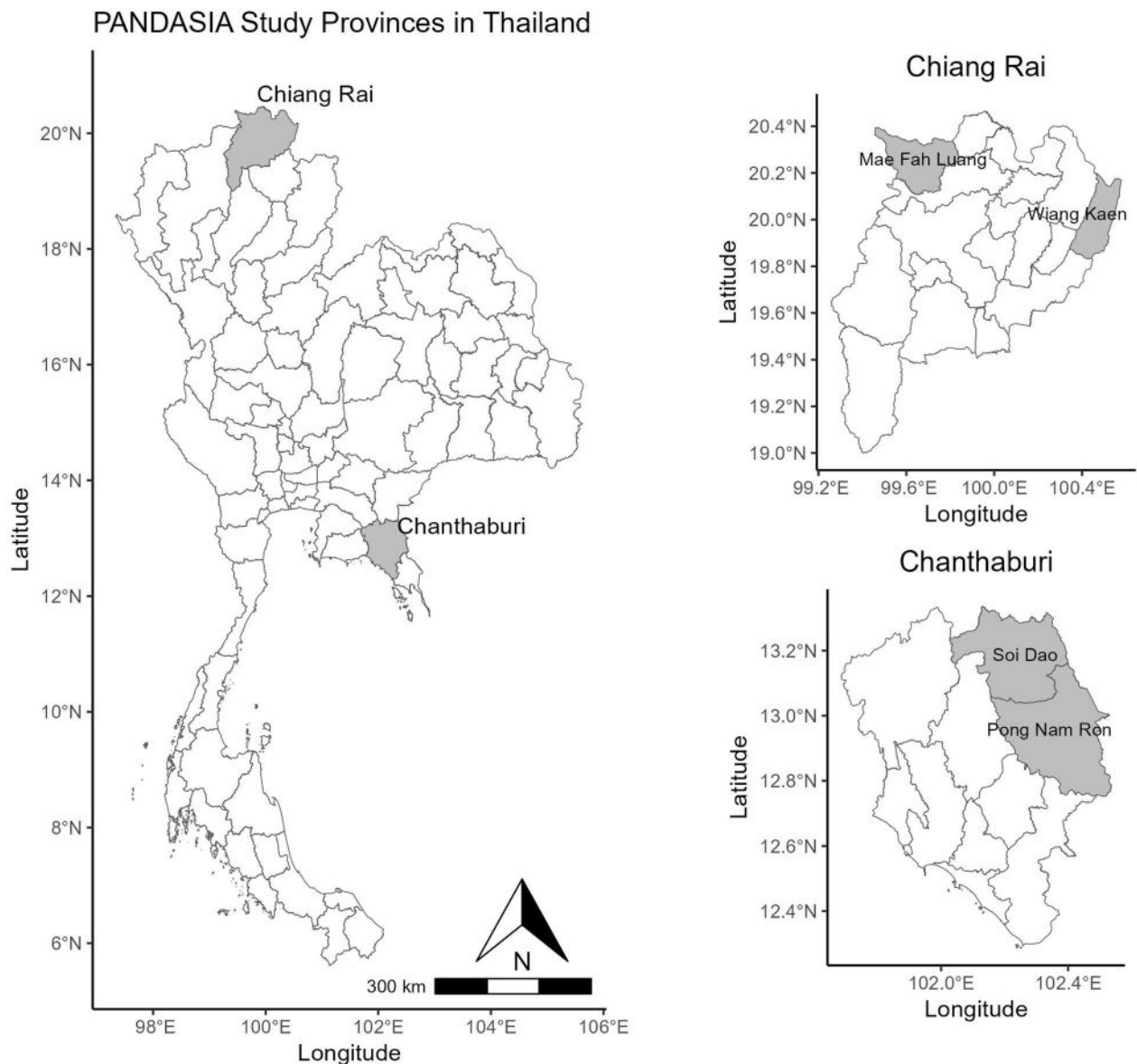


Fig. 1 Map of PANDASIA study provinces. Points represent districts: in Chanthaburi, these were Pong Nam Ron (East) and Soi Dao (West), and in Chiang Rai, Mae Fah Luang (East) and Wiang Kaen (West)

containing leaf litter-infused water were used for ovipositing mosquitoes, with sticky inserts to capture adults. Resting mosquitoes were collected using 10-min Prokopack aspirations (PK) outdoors (ground vegetation, tree holes and crevices up to chest height) and indoors (houses, temples, bat caves) where permitted.

At each sampling point (Table S1), a group of one PRO, one LT and one GAT was set up before dusk (16:00–18:00) on evening one. In the dry season, at one group per interface, the PRO trap was substituted with one LT due to a limited number of PROs. Traps were spaced ~20 m apart. The following mornings (06:00–08:00), LTs were collected, PROs rebaited, and PK performed. On the second evening, traps were reset, and GAT inserts replaced if required. Sampling continued for ~48 h before trap removal. Specimens were transported on dry ice to the field laboratory for processing.



Fig. 2 Example images of interfaces important for human–wildlife overlap selected for investigation into mosquito population composition. These were houses (A, E), dumpsites (B), forest edges (C), temples (D), orchards (F), forest interior (G) and bat caves (H)

Mosquito identification

Collections were processed individually. Specimens were sorted under a dissecting microscope, sexed, and identified to the lowest possible taxonomic level using morphological keys [14–19]. Female feeding status was recorded and each specimen was assigned a unique identification code.

Data analysis

Female mosquito abundance

Data were analysed in R (v4.4.2). Unsuccessful trapping events (e.g. battery failure or interference) were removed; the number of successful collections is shown in Table 1. Mean mosquito abundances per trapping event were analysed using generalised linear mixed models (GLMMs) fitted with the glmmTMB package. Data were subset by trap type to represent host-seeking (PRO, LT), resting (PK), and oviposition (GAT) behaviours. Catch count was modelled as the response variable, with district, interface, and season as fixed effects and site (Table S1) as a random effect. A negative binomial distribution was used to account for overdispersion. Effects of explanatory variables were tested by backward elimination using analysis of deviance. Significant predictors were used to estimate mean abundances across interfaces and seasons (ggeffects). Model fit was assessed using DHARMa. Group differences were evaluated using Tukey post hoc tests (multcomp), and figures were produced with ggplot2.

Presence and abundance of arboviral vectors

The presence/absence and count data for major female arboviral vectors captured in PRO and LT were analysed to estimate host-seeking probability and mean abundance as measures of exposure across interfaces. GLMMs with a binomial distribution were fitted for the presence/absence of *Ae. albopictus*, *Cx. gelidus*, *Cx. quinquefasciatus*, *Cx. tritaeniorhynchus*, and *Cx. vishnui*, with district, interface, and season as fixed effects. Abundance models used negative binomial GLMMs with site (Table S1) as a random effect. Model fit was assessed using DHARMa, and ggeffects was used to generate predicted probabilities and mean abundances. Group differences were assessed using Tukey post hoc tests.

Bat caves were excluded from analyses of *Cx. gelidus*, *Cx. tritaeniorhynchus*, and *Cx. vishnui* due to zero captures. Wiang Kaen was also excluded from *Cx. gelidus* and *Cx. tritaeniorhynchus* analyses because these species were absent.

Table 1 Mosquitoes caught by different trapping methods at interfaces in Pong Nam Ron/Soi Dao (Chanthaburi), Mae Fah Luang and Wiang Kaen (Chiang Rai) study districts

District	Interface	PRO trap			LT trap			GAT trap			Prokopack—indoors			Prokopack—outdoors			Total
		f	m	repl	f	m	repl	f	m	repl	f	m	repl	f	m	repl	
Pong Nam Ron and Soi Dao	House	224	45	12	73	1	12	34	1	13	32	53	12	15	9	6	487
Pong Nam Ron and Soi Dao	Temple	116	9	7	141	3	10	11	0	10	35	74	8	6	7	10	402
Pong Nam Ron and Soi Dao	Dumpsite	3977	3	10	1156	7	9	2	0	12				0	0	12	5145
Pong Nam Ron and Soi Dao	Orchard	420	7	10	188	4	10	16	0	11				3	19	13	657
Pong Nam Ron and Soi Dao	Forest edge	81	3	9	70	9	11	32	0	12				20	3	14	218
Pong Nam Ron and Soi Dao	Forest interior	69	32	8	269	17	11	13	0	12				62	35	15	497
Pong Nam Ron and Soi Dao	Bat cave	3	0	3	1	1	4	8	0	4	0	0	4	0	0	2	13
Mae Fah Luang	House	98	7	10	83	2	11	6	0	12	10	6	18	12	7	18	231
Mae Fah Luang	Dumpsite	3491	3	8	1190	16	14	4	0	12				40	17	18	4761
Mae Fah Luang	Orchard	78	1	8	320	0	8	0	0	8				1	2	12	402
Mae Fah Luang	Forest edge	300	4	8	166	3	8	8	0	8				13	2	12	496
Mae Fah Luang	Forest interior	161	12	7	45	6	6	2	0	8				12	7	12	245
Wiang Kaen	House	23	0	10	36	9	14	8	0	12	6	7	17	3	6	17	98
Wiang Kaen	Orchard	34	1	8	76	4	9	7	3	8				4	1	11	130
Wiang Kaen	Forest edge	29	0	10	32	3	13	6	0	12				18	7	18	95
Wiang Kaen	Forest interior	97	3	10	66	7	14	11	6	13				16	7	18	213
Wiang Kaen	Bat cave	4	0	4	5	0	4	0	0	4	2	0	4	1	1	2	13

Females (f) and males (m) are presented alongside the number of trap replicates (repl.)

Female species diversity at study interfaces

The iNEXT package in R was used to analyse the diversity of female mosquito species in different districts at the interface level. Only females that could be identified to species level were included to measure Shannon diversity index and Simpson's diversity index for each population found at different interfaces. The Shannon diversity index (H):

$$H = - \sum_{i=1}^R p_i \ln(p_i)$$

measures species diversity by considering richness (number of different species) and evenness (abundance of different species) with a focus on rare species where R is total richness and p_i is the proportion of R of the i th species. The Simpson's diversity index (D):

$$D = \sum_{i=1}^R \left(\frac{n_i(n_i - 1)}{N(N - 1)} \right)$$

measures the probability of two individuals being from the same species with a focus on evenness and dominant species where n_i is the number of individuals in species i and N is the total number of species in the sample. To account for differences in sample size between interfaces, iNEXT computes diversity estimates for rarefied and extrapolated samples. Estimates were extrapolated to a specified endpoint of 750 for the PNR, MFL and WK samples.

Results

Mosquito composition

A total of 14,108 (96.4%, $n = 13,601$ female and 3.6%, $n = 502$ male) mosquitoes were sampled and processed over the study period from seven interfaces in the study districts (Table 1). Only 0.04% ($n = 5$) individuals could not be assigned a sex. The full catch was generated from 124 PRO, 168 LT, 171 GAT, 63 PK-IN and 210 PK-OUT collections. In the dry season, 1,767 mosquito specimens were caught (83.2%, $n = 1,470$ females and 17.1%, $n = 296$ males ($n = 1$ unknown sex)) and in the wet season 12,341 (98.3%, $n = 12,131$ females and 1.7%, $n = 206$ males ($n = 4$ unknown sex)) were caught. The overall catch from CTH was 7,423 (dry 14.1%: wet 85.9%), from MFL 6,136 (dry 7.9%: wet 92.1%) and from WK 549 (dry 43.2%: wet 56.8%). The highest counts were from dumpsites in CTH and MFL, and lowest were found inside the CTH and WK bat caves (Table 1).

Mosquitoes representing nine genera, *Aedes*, *Anopheles*, *Armigeres*, *Coquillettia*, *Culex*, *Mansonia*, *Toxorhynchites*, *Tripteroides* and *Uranotaenia*, were collected (Table S2). *Culex* was the most common genus, comprising 77.4% ($n = 10,530$) of the catch, followed by *Aedes* 9.0% ($n = 1,229$) and *Mansonia* 8.8% ($n = 1,200$). The *Anopheles* genus, containing important malaria vectors, comprised only 1.2% ($n = 161$) of the total catch identifiable to genus. Overall, 51 different species and 6 subgroups (lowest possible taxonomic class using key) were detected. In both CTH and MFL, the interface where the highest number of different species or subgroups was caught was from orchard sites (22 and 18 respectively, Table S2). In WK, the highest was from the forest edge (16). The number of different species or subgroups in dumpsites was similar or more than those found in the forest interior. Very few species were caught in both CTH and WK bat caves (2 and 3 species, respectively).

The total catch of arboviral vectors was 72.5% ($n = 5379$) in CTH, 92.0% ($n = 5643$) in MFL and 46.0% ($n = 252$) in WK. Dumpsites had the highest burden of vector species (Table 2). *Culex gelidus* (45.6%, $n = 6,195$) and *Cx. vishnui* (24.6%, $n = 3,348$), vectors of JEV and TMUV, were the most abundant vectors. Both were most prominent in dumpsites. The vector of DENV and CHIKV, *Ae. albopictus*, was the third most common species, distributed across all interfaces with highest counts from the forest interior in all districts sampled, and at the dumpsite in MFL. Other notable known viral vectors were *Cx. quinquefasciatus*, vector of JEV and ZIKA (3.3%, $n = 451$), and *Cx. tritaeniorhynchus*, vector of JEV and TMUV, (1.2%, $n = 167$) detected in high counts at the dumpsite in MFL and CTH respectively. Few *Ae. aegypti*, vector of DENV, CHIKV, and ZIKA (0.6%, $n = 83$), were found overall with the largest catches from around homes in CTH.

Blood-fed status

Blood-fed females were more common in the dry season ($n = 43$) than wet season ($n = 21$). Most were *Cx. quinquefasciatus* from houses and temples (Table 3). The lowest catches of blood-feds were from orchards and the forest interior (both $n = 2$) and none were found in the bat cave.

Female mosquito abundances at study interfaces

Female host-seeking abundance was dependent on district ($\chi^2 = 10.5$, $df = 2$, $p < 0.01$), interface ($\chi^2 = 26.0$, $df = 6$, $p < 0.001$), and season ($\chi^2 = 44.5$, $df = 1$, $p < 0.001$) but not trap type (PRO or LT) ($\chi^2 = 2.1$, $Df = 1$, $p = 0.1481$). PRO and LT performed the same regardless of interface because no interaction effect between interface and trap was detected ($\chi^2 = 11.6$, $df = 6$, $p = 0.07233$). A higher abundance of host-seeking females was found at dumpsites than in houses, orchards, forest edges, forest interior, bat caves ($p < 0.001$) or temple sites ($p < 0.01$). More females were host seeking at orchards ($p < 0.01$) and forest interior ($p < 0.05$) than in bat caves (Fig. 3). Higher mean catches of host-seeking females were found at CTH ($p < 0.001$) and MFL ($p < 0.05$) than WK, and similar abundances were found in CTH vs MFL ($p = 0.6575$). Higher host-seeking abundances were detected in the wet vs the dry season ($p < 0.001$).

Table 2 Female arboviral vector species collected at study interfaces in Chanthaburi and Chiang Rai provinces during the dry and wet seasons of 2024

District, province	Interface	<i>Ae. aegypti</i>	<i>Ae. albopictus</i>	<i>Cx. fusco- cephala</i>	<i>Cx. gelidus</i>	<i>Cx. quinque- fasciatus</i>	<i>Cx. tritaenio- rhynchus</i>	<i>Cx. vishnui</i>	<i>Cx. pseudo- vishnui</i>	Total
Pong Nam Ron and Soi Dao districts, Chanthaburi	House	61	28	1	115	54	8	65	0	332
	Temple	4	41	1	31	53	3	95	0	228
	Dumpsite	1	70	2	2630	9	83	1132	0	3927
	Orchard	1	86	0	177	22	12	215	1	514
	Forest edge	2	75	1	0	5	0	39	0	122
	Forest interior	1	204	0	0	3	5	31	0	244
	Bat cave	0	9	0	0	3	0	0	0	12
Mae Fah Luang district, Chiang Rai	House	2	7	1	40	63	1	50	0	164
	Dumpsite	6	105	6	2711	115	11	1606	0	4560
	Orchard	0	5	0	214	58	42	4	2	325
	Forest edge	5	66	0	247	6	2	98	0	424
	Forest interior	0	103	1	30	25	0	11	0	170
	House	0	12	0	0	0	0	0	0	12
	Orchard	0	53	0	0	19	0	0	0	72
Wiang Kaen district, Chiang Rai	Forest edge	0	24	0	0	6	0	2	0	32
	Forest interior	0	117	0	0	10	0	0	0	127
	Bat cave	0	9	0	0	0	0	0	0	9
	Total	83	1014	13	6195	451	167	3348	3	11,274

Ae. Aedes (Stegomyia), *Cx.* Culex (Culex)

Table 3 Blood-fed (BF) and semi-gravid (SG) female mosquitoes collected at study interfaces in Chanthaburi and Chiang Rai provinces during the dry and wet seasons of 2024

Genus and species	Status	House	Temple	Dumpsite	Orchard	Forest edge	Forest interior	Total
<i>Culex (Cux.) quinquefasciatus</i>	BF	15	14	0	2	3	0	34
<i>Aedes (Ste.) aegypti</i>	BF	7	1	2	0	0	0	10
<i>Culex (Cux.) vishnui</i>	BF	2	0	4	0	1	0	7
<i>Aedes (Ste.) albopictus</i>	BF	1	1	1	0	1	1	5
<i>Armigeres (Arm.) subalbatus</i>	BF	1	0	0	0	0	1	2
<i>Culex (Cux.) gelidus</i>	BF	1	0	1	0	0	0	2
<i>Culex (Cux.) quinquefasciatus</i>	SG	2	0	0	0	0	0	2
<i>Aedes spp.</i>	BF	0	0	0	0	1	0	1
<i>Anopheles (Cel.) minimus</i>	BF	1	0	0	0	0	0	1
<i>Culex (Cux.) gelidus</i>	SG	0	0	1	0	0	0	1
<i>Culex (Cux.) hutchinsoni</i>	BF	0	0	1	0	0	0	1
<i>Mansonia (Mnd.) annulata</i>	BF	0	1	0	0	0	0	1
Total		30	17	10	2	6	2	67

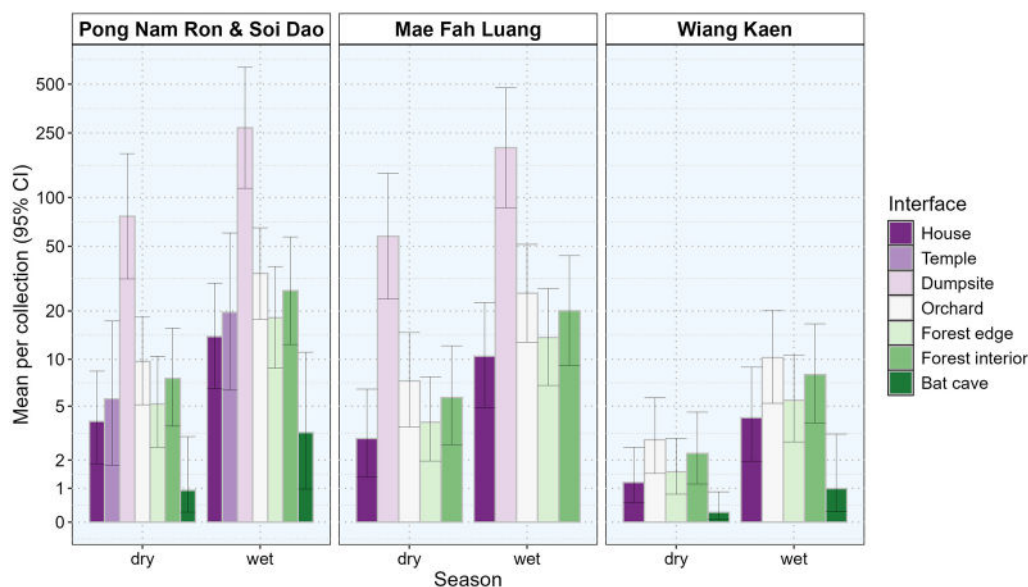


Fig. 3 Mean female mosquito host-seeking abundances from Light trap and Pro trap collections at study interfaces in Pong Nam Ron and Soi Dao districts combined (Chanthaburi province), Mae Fah Luang district (Chiang Rai province) and Wiang Kaen district (Chiang Rai province). Mean values derived from negative binomial GLMM predictions of female abundance ~ interface + district + season + (1 | site). Error bars are 95% confidence intervals

There was no difference in the abundance of resting females across district ($\chi^2 = 1.2$, $df = 2$, $p = 0.5527$), interface ($\chi^2 = 12.0$, $df = 6$, $p = 0.06233$) or season ($\chi^2 = 0.3$, $df = 1$, $p = 0.5896$) (Fig. S1 and S2). Females caught in oviposition traps varied with district ($\chi^2 = 8.7$, $df = 2$, $p < 0.05$), season ($\chi^2 = 6.5$, $df = 1$, $p < 0.05$) but not interface ($\chi^2 = 4.6$, $df = 6$, $p = 0.5972$). Higher abundances were detected in the wet vs the dry season ($p < 0.01$, Fig. S3). Gravid trap catches in WK were lower than CTH ($p < 0.05$), but similar abundances were found between CTH vs MFL ($p = 0.0513$) and MFL vs WK ($p = 0.998$).

Presence and abundance of arboviral vectors at study interfaces

The abundance of all arboviral vector species examined differed with interface and season (Table 4). For *Cx. quinquefasciatus*, *Cx. tritaeniorhynchus* and *Cx. Vishnui*, their abundances also differed with district sampled (Table 4). A higher abundance of *Ae. albopictus* was found host seeking in the dumpsite, orchard, forest edge and forest interior than around houses ($p < 0.001$) and in the forest interior than the temple, bat cave ($p < 0.01$), forest edge or orchard ($p < 0.05$, Fig. 4A). Higher abundances of *Cx. gelidus* were trapped in the dumpsite than around houses, temples, forest edge, forest interior ($p < 0.001$) and in orchards than the forest interior ($p < 0.01$, Fig. 4B). More *Cx. quinquefasciatus* were found in the orchard than forest edge ($p < 0.05$, Fig. 4C) and a higher abundance was detected in MFL than WK ($p < 0.001$) or PNR ($p < 0.01$). More *Cx. tritaeniorhynchus* were trapped in the dumpsite than the forest edge ($p < 0.01$) or around houses ($p < 0.05$), and in the orchard than the forest edge ($p < 0.05$, Fig. 4D). Higher abundances of *Cx. vishnui* were trapped at the dumpsite than in house, temple, orchard, forest edge and forest interior sites ($p < 0.001$, Fig. 4E). Significantly more were caught in CTH and MFL than in WK ($p < 0.001$). There was a higher abundance of *Ae. albopictus* ($p < 0.01$), *Cx. gelidus* ($p < 0.001$), *Cx. quinquefasciatus* ($p < 0.05$), *Cx. tritaeniorhynchus* ($p < 0.05$) and *Cx. vishnui* ($p < 0.001$) in the wet season compared to the dry season.

The presence of all arboviral vector species examined differed with interface and season, except for *Cx. quinquefasciatus* (Table 4). Further, the detection of *Cx. quinquefasciatus*, *Cx. tritaeniorhynchus* and *Cx. vishnui* differed between districts but this was not true for other vectors (Table 4). There was a higher chance of detecting *Ae. albopictus* at the dumpsite, forest interior ($p < 0.001$), orchard ($p < 0.01$) and forest edge ($p < 0.05$) than at houses (Fig. 5). *Culex vishnui* was more likely to be present at dumpsites than at houses or forest edges ($p < 0.001$), forest interior ($p < 0.01$) or in orchard and temple sites ($p < 0.05$, Fig. 5). The probability of detecting this vector was higher in CTH than in MFL ($p < 0.05$), and in both CTH and MFL than WK ($p < 0.001$). *Culex gelidus* was more likely to be host seeking at the dumpsite than the forest edge ($p < 0.001$) or forest interior ($p < 0.01$), and in orchards than the forest edge ($p < 0.01$) (Fig. 5). There was a higher chance of *Cx. quinquefasciatus* presence in MFL than WK ($p < 0.05$) but not between CTH or MFL ($p = 0.6280$) and WK ($p = 0.0891$). *Culex*

Table 4 Interface, district and seasonal influence on the presence and abundance of key arboviral vector species using analysis of deviance for GLMMs

Arboviral vector	Response variable	Interface	Season	District
<i>Ae. albopictus</i>	Presence/absence	Dev=36.1, Df=6, $p < 0.001$	Dev=5.7, Df=1, $p < 0.05$	Dev=2.7, Df=2, $p = 0.2517$
	Abundance	Dev=56.0, Df=6, $p < 0.001$	Dev ² =9.7, Df=1, $p < 0.01$	Dev=5.0, Df=2, $p = 0.0843$
<i>Cx. gelidus</i>	Presence/absence	Dev ² =37.4, Df=5, $p < 0.001$	Dev=89.2, Df=1, $p < 0.001$	Dev=0.4, Df=1, $p = 0.5209$
	Abundance	Dev=47.4, Df=5, $p < 0.001$	Dev=88.4, Df=1, $p < 0.001$	Dev=1.9, Df=1, $p = 0.1699$
<i>Cx. quinquefasciatus</i>	Presence/absence	Dev=8.7, Df=6, $p = 0.1888$	Dev=0.1, Df=1, $p = 0.7887$	Dev=8.5, Df=2, $p = 0.7887$
	Abundance	Dev=14.7, Df=6, $p < 0.05$	Dev=4.1, Df=1, $p < 0.05$	Dev=17.5, Df=2, $p < 0.001$
<i>Cx. tritaeniorhynchus</i>	Presence/absence	Dev=16.6, Df=5, $p < 0.01$	Dev=5.3, Df=1, $p < 0.05$	Dev=13.1, Df=1, $p < 0.001$
	Abundance	Dev=18.8, Df=5, $p < 0.01$	Dev=4.7, Df=1, $p < 0.05$	Dev=0.3, Df=1, $p = 0.5833$
<i>Cx. vishnui</i>	Presence/absence	Dev=27.3, Df=5, $p < 0.001$	Dev=10.6, Df=1, $p < 0.01$	Dev=64.5, Df=2, $p < 0.001$
	Abundance	Dev=71.0, Df=5, $p < 0.001$	Dev=45.1, Df=1, $p < 0.001$	Dev=72.6, Df=2, $p < 0.001$

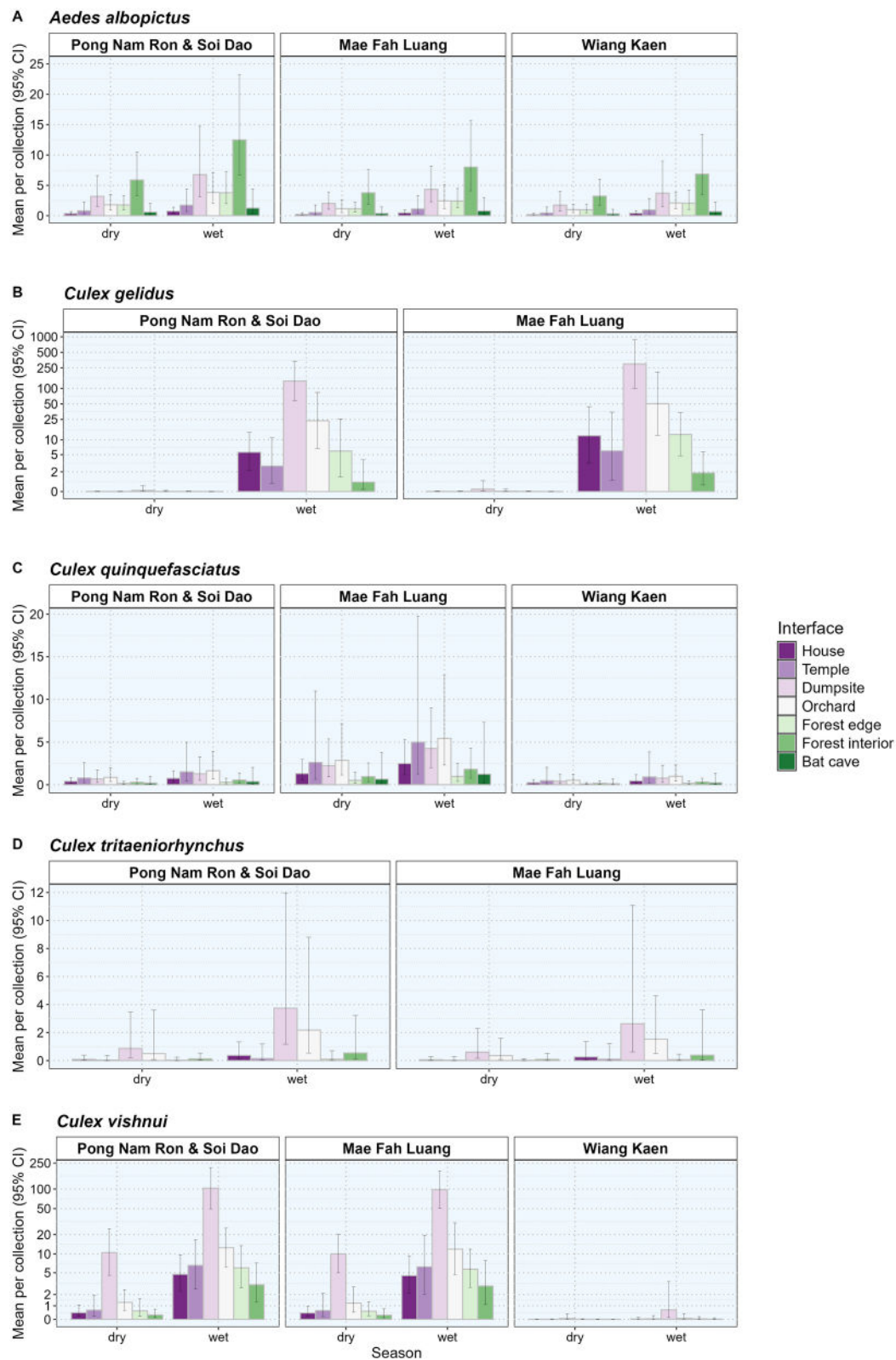


Fig. 4 Daily mean probability of detecting arboviral vectors at study interfaces in Pong Nam Ron and Soi Dao (Chanthaburi), Mae Fah Luang (Chiang Rai) and Wiang Kaen (Chiang Rai) districts. Mean values derived from binomial GLMM predictions of female presence/absence ~ interface + district + season. Error bars are 95% confidence intervals

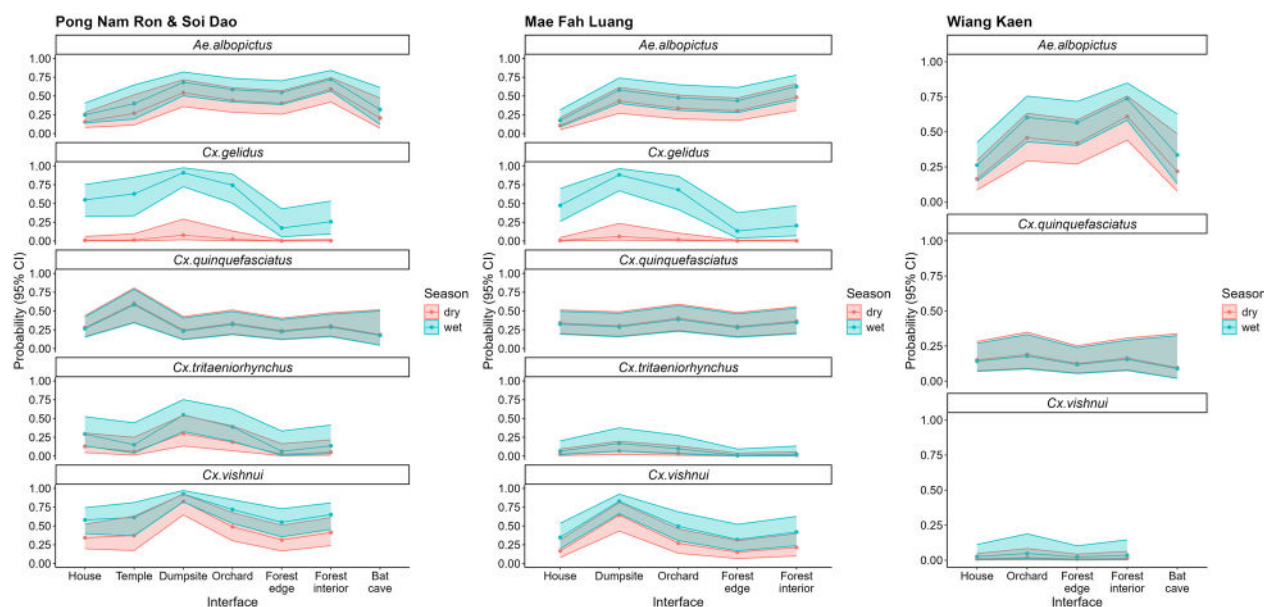


Fig. 5 Daily mean number of host-seeking female mosquitoes at study interfaces in Pong Nam Ron and Soi Dao (Chanthaburi), Mae Fah Luang (Chiang Rai) and Wiang Kaen (Chiang Rai) districts: **A** *Ae. albopictus*, **B** *Cx. gelidus*, **C** *Cx. quinquefasciatus*, **D** *Cx. tritaeniorhynchus* and **E** *Cx. vishnui*. Mean values derived from negative binomial GLMM predictions of female arboviral vector abundance ~ interface + district + season. Error bars are 95% confidence intervals

tritaeniorhynchus were more likely to be detected in CTH than MFL ($p < 0.001$) and in the dumpsite than at forest edge, but this was not significant ($p = 0.0818$) (Fig. 5). There was an increased probability of detecting *Ae. albopictus* ($p < 0.05$), *Cx. gelidus* ($p < 0.001$), *Cx. tritaeniorhynchus* ($p < 0.05$) and *Cx. vishnui* ($p < 0.01$) in the wet season compared to the dry season. This seasonal effect was not found for *Cx. quinquefasciatus* ($p = 0.789$).

Female species diversity at study interfaces

The most diverse mosquito communities were found in temple sites at CTH, house and forest interior sites in MFL and at the forest edge in WK (Fig. 6). The least diverse communities were found inside bat caves for CTH and WK and at the dumpsite (for MFL). The diversity measures derived for the dumpsite in CTH were also low, together with the forest interior sites. In WK, forest interior and house sites had similarly low diversity indices.

Discussion

This study provides updated evidence of arboviral vector exposure across key human–animal interfaces in Thailand. The outcome was to highlight important risk areas for arboviral transmission and generate an evidence base for guiding further surveillance or control measures.

To our knowledge, this is the first broad investigation into zoonotic arboviral vectors in rural interfaces to include dumpsites. Several studies, including in Thailand, have drawn inferences between the presence of solid waste around households and larval indices relating to dengue transmission [14–18]. This is due to the plastic containers in household waste that collect rainwater and provide suitable breeding habitats for *Ae. aegypti* and *Ae. albopictus*. As yet, however, there is a lack of reporting on mosquito composition at larger district-level dumpsites, including data on species other than *Aedes* spp. Despite that Chanthaburi and Chiang Rai are far from one another, commonalities were found. High abundances of host-seeking females were detected, in the dry as well as the wet season, which included high proportions of known arboviral vectors. Notable were the high abundances

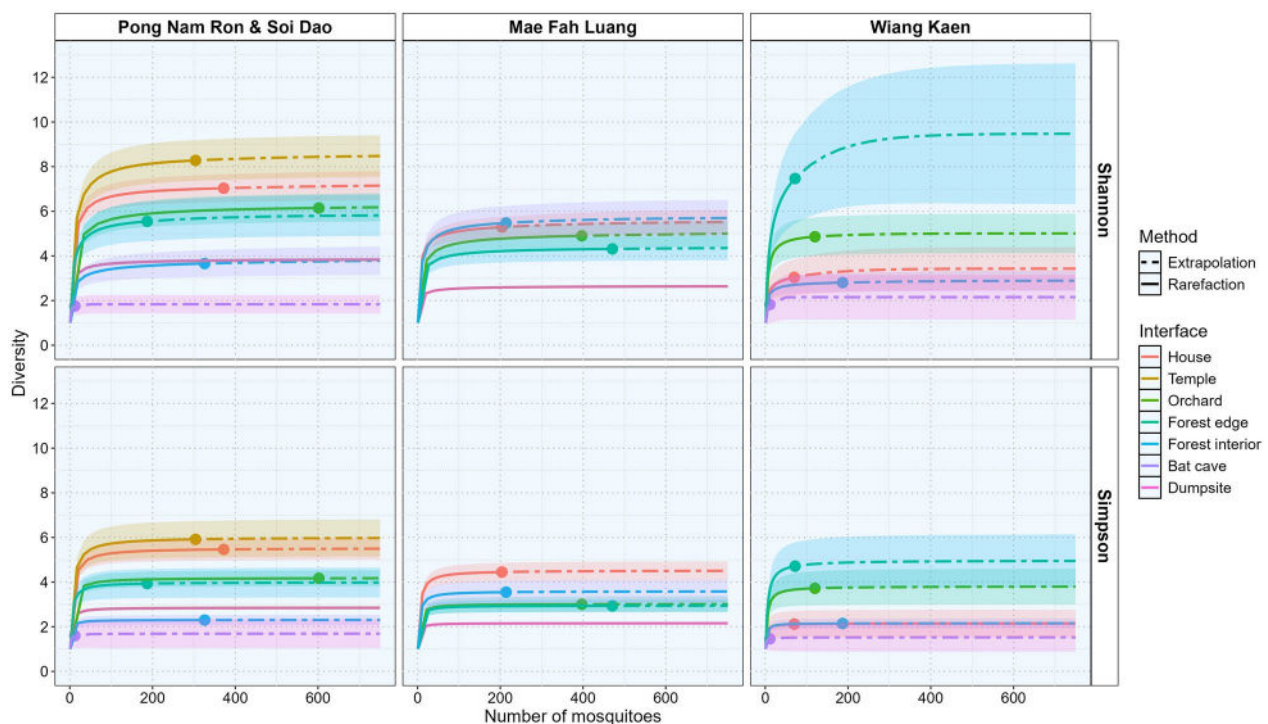


Fig. 6 Shannon and Simpson's diversity estimates for rarefied and extrapolated mosquito samples from study interfaces in Pong Nam Ron and Soi Dao (Chanthaburi), Mae Fah Luang (Chiang Rai) and Wiang Kaen (Chiang Rai) districts

of host seeking *Cx. gelidus*, *Cx. vishnui*, *Ae. albopictus* and *Cx. tritaeniorhynchus* at dumpsites vs. around homes and in other interfaces. Aside from the infinite opportunities for breeding in discarded containers at dumpsites, other factors contributing to high mosquito densities may be at play. First, containers tend to be small and collect little insect diversity of larval predators increasing mosquito survival rate and density [19]. Further, access to abundant sugars can support long adult life spans, as shown with banana, sweet potato and condensed milk residue to mimic sweet food waste in the lab [16]. Although assumptions cannot be made about the *Culex* spp. in our collections, it is important to note that Bisphenol A (BPA) in plastics has shown to decrease the development time of *Cx. quinquefasciatus* but not *Ae. aegypti* or *Ae. albopictus* [20]. Furthermore, the *Culex* spp. we detected in high numbers have similar breeding preferences, utilising groundwater habitats such as ditches, footprints and rice fields, that are readily available near the dumpsite areas, with *Cx. gelidus* and *Cx. quinquefasciatus* also able to breed in containers [21]. Therefore, suitable breeding sites, food sources and a lack of larval predation may have contributed to high mosquito abundances found at dumpsites.

Culex gelidus and *Cx. vishnui* are well known in Thailand for transmitting JEV (circulating in pigs and ardeid birds), with *Cx. tritaeniorhynchus* as the principal vector [21]. These species can also transmit the neurotrophic avian TMBU virus that causes significant losses in the poultry industry [22]. TMBU has been detected in avian farm workers, but it is unknown if mammals present severe neurological outcomes like infected birds. All are zoophilic, preferring to feed on livestock such as cows and pigs but in their absence will feed on humans and birds [3, 21]. In our study, an observation was that aside from places of high human activity and dwellings like houses and temples, a substantial number of blood fed females were collected from dumpsites. This denoted an availability of hosts to support the blood fed females found. Several potential hosts were observed in the daytime collections: cattle, dogs, wild birds, rodents, frogs and humans, and together with nocturnal hosts, present several pathways for viruses to circulate between species at these sites. Molecular analysis to identify the host species of these blood meals and explore the possibility of animal reservoirs involved in arboviral transmission is ongoing.

Aedes albopictus, considered a secondary vector of DENV and CHIKV in Thailand to *Ae. aegypti*, opportunistically feeds on mammals but prefers humans [23]. Its activity peaks at dawn and dusk which is when people are present at waste sites. Apart from government officials and garbage dump managers, people from nearby communities are attracted to sort through garbage to collect plastic bottles or other items to sell. Generally, people avoid these sites at night thus vectors like *Ae. albopictus* that are active in the day pose the highest risk for arboviral transmission to humans.

Dumpsites are areas that are in a constant ecological flux, with new material arriving daily, attracting people and animals from different locations. Solid waste sites attract high numbers of murine rodents due to the availability of abundant food sources and are indicated as places where different species (including humans) can interact, facilitating the spread of disease [24]. Known zoonotic diseases from rodents alone are hantavirus, leptospirosis and gastro helminth parasites [24], with little investigation of infections circulating in other animals at dumpsites. As well as rodents, garbage pickers come into contact with reptiles and flies, and eat in the working day without proper sanitation to wash hands [25]. Surveys note the use of basic PPE such as long-sleeved clothing, hats, gloves and shoes; however, none mention using topical insect repellents for protection against mosquito bites [25]. Waste at dumpsites is managed over time, compacted in set areas and vegetation is allowed to recover. This creates fringe environments where wildlife are bitten by mosquitoes which subsequently bite humans over a small scale, posing risk of zoonotic arboviral spillover. Further study of the effect of these changing environments on mosquito vectors and infections could guide waste or wildlife management of the site, tailored towards reducing risk of vector-borne transmission.

A limitation of the study was the snapshot sampling approach where up to seven interfaces in each location were studied in a short time frame. The advantage of this was a wide coverage of sites, allowing exploration of compositions across different interfaces at a single time point. As expected, the abundance of host-seeking females overall was higher in the wet vs. the dry season, a pattern that was repeated consistently across interfaces and districts sampled. *Aedes albopictus* was more abundant in the wet vs. dry season, likely due to its generalist oviposition behaviour, laying in domestic as well as natural water containers [26]. Therefore, with the increased availability of larval habitats in the rainy season, the adult *Aedes* abundance naturally increased [26]. Similarly, a higher abundance of *Cx. vishnui* was found in the wet than the dry season, due to a higher availability of breeding habitats. *Culex vishnui* and *Cx. gelidus* prefer ground pool habitats, including marshy depressions, ditches, pits, pools, rice fields, wheel tracks and footprints [21]. *Culex vishnui* immatures can also be found in natural containers and *Cx. gelidus* in artificial containers [21]. Very few *Cx. gelidus* were found in the dry season overall, followed by a vast increase in abundance in the wet season timepoint. This agrees with seasonal data generated for *Cx. gelidus* in Central Thailand where very low abundances were found from October to April, then a small primary peak in May (after our first sampling event), increasing fourfold to peak abundances in September [27]. Our findings differed in finding high abundances of *Cx. gelidus* even at the November sampling points. The crude approach to seasonal sampling here allowed for spatial breadth but limits the scope for interpreting seasonal dynamics. Thus, detail is lacking on how vector populations fluctuate in species presence and abundance throughout the year. To build on the findings here, we recommend longitudinal survey of interfaces, like dumpsites, where significant proportions of vector species were detected to understand population dynamics at these sites. The consistent low mosquito diversity found in dumpsites is characteristic of a degraded natural environment where select species can flourish. When the environment supports predominant species that are also vectors, these become risky systems if an infection is introduced. Thus, longitudinal surveys would review abundances and provide an opportunity to investigate arboviral infections to determine if and/or how risk varies throughout the year. This would allow for detailed recommendations to be made to accurately warn personnel and implement control measures at high-risk periods.

The distribution of *Ae. albopictus* across all interfaces in each district is a significant finding to bring to discussion. Its flexible feeding behaviour on humans, wild and domestic animals, in addition to its ability to breed in both natural, e.g. treeholes, bamboo stumps, and man-made containers make it a robust and prolific species in

all habitats. Its widespread presence and history of maintaining sylvatic and urban arboviral cycles [26] makes it a high-risk species as a bridge vector and mediator of zoonotic transmission between animals and humans. Several studies provide evidence of *Ae. albopictus* transmitting DENV and CHIKV in Thailand [26]; however, further investigation into its infection status in areas away from homes is required for a comprehensive picture of the disease ecology of this vector.

Our study points to the risk of arboviral transmission from high vector burden in areas away from home, including dumpsites, orchards, forest edges and forest interiors, that people visit for work or recreation and are not targets of government vector control interventions. Thongsripong et al. 2013 surveyed urban and rural settlements, rice fields, forest edges and interior in Thailand and found high abundances of *Ae. albopictus* in rural and fragmented forest habitats, *Cx. vishnui* in rice fields and forest fringes, and *Cx. gelidus* in suburban settlements and rice fields. In line with their findings, we found that *Ae. aegypti* and *Cx. quinquefasciatus* were more common in human settlement areas. Current control including breeding site reduction and insecticide spraying will contribute to reduction of these household vectors; however, those biting away from the home present an ongoing challenge for control. Personal protective measures such as topical repellents and insecticide treated clothing are appropriate for non-domestic vectors but rely on community education and individual compliance. There is opportunity for outdoor spatial repellents, outdoor residual spray, space spraying, mass trapping, attractive toxic sugar baits and zoophylaxis [28] in places such as dumpsites or agricultural areas where people work.

Conclusions

To conclude, this study is the most up-to-date assessment of mosquito communities at a range of human–animal interfaces in Thailand. We highlight previously undocumented, large, district-level dumpsites as important for zoonotic arbovirus transmission and emerging infectious diseases due to the high vector abundances (consistent throughout the dry and wet seasons), the range of wild and domestic animal hosts and the presence of humans working with little mosquito protection. Further directed study is required to investigate the role of *Ae. albopictus* as a bridge vector of zoonotic arboviruses between sylvatic forest cycles and in rural settlement areas. Longitudinal surveys are recommended to understand mosquito population and pathogen dynamics in human–animal interfaces throughout the year to guide interventions with evidence base.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1186/s13071-026-07667-9>.

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Author contributions RB: Design, fieldwork, analysis and writing. TP: Fieldwork and writing. PP: Fieldwork and writing. AJ: Fieldwork and writing. WR: Fieldwork and writing. SP: Design and writing. CP: Design and writing. HJO: Design, fieldwork and writing.

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Data availability Mosquito data presented and the Rmarkdown script used to perform the analysis in this publication has been uploaded as supplementary information 'Additional file 5: Dataset S1. Mosquito data 2024 for analysis', and 'Additional file 6: Rmd script S1. Data analysis'.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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