Journal of Medical Entomology, 2017, 1–16
doi: 10.1093/jme/tjx071
Research article

Diversity of Mosquitoes (Diptera: Culicidae) Attracted to Human Subjects in Rubber Plantations, Secondary Forests, and Villages in Luang Prabang Province, Northern Lao PDR

Julie-Anne A. Tangena,[1,2,3] Phoutmany Thammavong,[1] Naritsara Malaithong,[4] Thavone Inthavong,[5] Phuthasone Ouanesamon,[5] Paul T. Brey,[1,]* and Steve W. Lindsay[2]*

[1]Department of Medical Entomology, Institut Pasteur du Laos, Samsenthai Rd, Ban Kao-gnot, PO Box 3560, Vientiane, Lao PDR (jtangena@gmail.com; phoutmany_tick@hotmail.com; ptb1957@yahoo.com), [2]School of Biological and Biomedical Sciences, Durham University, South Road, Durham, DH1 3LE, United Kingdom (s.w.lindsay@durham.ac.uk), [3]Corresponding author, e-mail: jtangena@gmail.com, [4]Department of Entomology, Kasetsart University, 50 Ngam Wong Wan Rd., Ladyaow Chatuchak Bangkok 10900, Thailand (naritsara_malaithong@hotmail.com), and [5]Agriculture and Forestry Policy Research Center, National Agriculture and Forestry Research Institute, Nongviengkham Village, Vientiane, P.O Box 7170, Lao PDR (i_thavone@yahoo.com; phuthasone_vt@yahoo.com)

*Joint senior authors.

Subject Editor: Richard Wilkerson

Received 22 December 2016; Editorial decision 4 March 2017

Abstract

The impact of the rapid expansion of rubber plantations in South-East Asia on mosquito populations is uncertain. We compared the abundance and diversity of adult mosquitoes using human-baited traps in four typical rural habitats in northern Lao PDR: secondary forests, immature rubber plantations, mature rubber plantations, and villages. Generalized estimating equations were used to explore differences in mosquito abundance between habitats, and Simpson's diversity index was used to measure species diversity. Over nine months, 24,927 female mosquitoes were collected, including 51 species newly recorded in Lao PDR. A list of the 114 mosquito species identified is included. More mosquitoes, including vector species, were collected in the secondary forest than immature rubber plantations (rainy season, odds ratio [OR] 0.33, 95% confidence interval [CI] 0.31–0.36; dry season, 0.46, 95% CI 0.41–0.51), mature rubber plantations (rainy season, OR 0.25, 95% CI 0.23–0.27; dry season, OR 0.25, 95% CI 0.22–0.28), and villages (rainy season, OR 0.13, 95% CI 0.12–0.14; dry season, 0.20, 95% CI 0.18–0.23). All habitats showed high species diversity (Simpson's indexes between 0.82–0.86) with vectors of dengue, Japanese encephalitis (JE), lymphatic filariasis, and malaria. In the secondary forests and rubber plantations, *Aedes albopictus* (Skuse), a dengue vector, was the dominant mosquito species, while in the villages, *Culex vishnui* (Theobald), a JE vector, was most common. This study has increased the overall knowledge of mosquito fauna in Lao PDR. The high abundance of *Ae. albopictus* in natural and man-made forests warrants concern, with vector control measures currently only implemented in cities and villages.

Key words: mosquito population dynamics, mosquito fauna, rubber plantation, Lao People's Democratic Republic

South-East Asia (SEA) is a region where the population is at high risk of exposure to vector-borne diseases (Jones et al. 2008, Suwonkerd et al. 2013). This risk is exacerbated by changes in the environment, such as changes in land use, surface water availability, large-scale cross-border migration of people, and climate change (Githeko et al. 2000, Foley et al. 2005, Reisen 2010, Parham et al. 2015). One major land-use change in the region is the expansion of rubber plantations. The largest rubber plantations in the world are located in Indonesia, Thailand, and Malaysia. Together with other rubber-producing countries in SEA, they covered 9.2 million ha of land in 2010 (Food and Agriculture Organization 2010). These man-made forests provide environments for vector mosquitoes. Outbreaks of dengue (Ministry of Health Malaysia 2013, Palaniyandi 2014), malaria (Watson 1921, Singhasivanon et al. 1999, Garros et al. 2008, Wangroongsarb et al.

2012, Bhumiratana et al. 2013b), and chikungunya (Kumar et al. 2011, Palaniyandi 2014) have been recorded in rubber plantations of India, Myanmar, Thailand, and Viet Nam. However, data on the abundance and diversity of mosquitoes in rubber plantations remain limited (Tangena et al. 2016).

Lao PDR (People's Democratic Republic) has one of the fastest growing economies in SEA, with a 6.4% increase in gross domestic product (GDP) in 2015 (The World bank 2015). This growth has partly been achieved by the 160-fold expansion of mature rubber plantations from 2010 to 2015. Rubber tree cultivation is a new kind of mass farming, and the impact of these changes on local vectors remains poorly understood (Rueda et al. 2015). Since rubber plantations are likely to expand in the country for at least the next decade (National Agriculture and Forestry Research Institute 2016), there is a need to understand the risk of vector-borne diseases in these habitats.

The objective of this study was to identify the mosquito dynamics, specifically for vectors of human diseases in rural habitats common in Lao PDR. A longitudinal study was carried out in northern Lao PDR to determine the abundance and diversity of adult mosquitoes in four typical rural habitats: secondary forests, immature rubber plantations, mature rubber plantations, and villages. We hypothesized that mature rubber plantations in Lao PDR, with similarly high canopy cover, high humidity, and stable temperatures as forest habitats, would provide a good alternative habitat for forest mosquitoes, including important dengue and malaria vectors.

Materials and Methods

Study Design

Entomological surveys were conducted monthly from July to November 2013 and in February, March, May, and July of 2014. Collections were not conducted in December 2013, January 2014, April 2014, and June 2014 due to national holidays, local festivals, peak in rice planting activities, and a large temporary migration of villagers. Temperature and humidity data were collected in all habitats throughout the study period, with the additional environmental measurements collected once between June and July 2014.

Study Sites

The study was conducted in three sites: Thinkeo (19° 41′02.13″ N, 102° 07′05.49″ E), Silalek (19° 37′02.80″ N, 102° 03′05.70″ E), and Houayhoy (19° 33′03.22″ N, 101° 59′42.42″ E) in Xieng-Ngeun and Nane district, Luang Prabang province, northern Lao PDR. The study area is a hilly region at an altitude of 570–650 m, with patches of secondary forest and rubber plantations. The area has a tropical monsoon climate, with a single rainy season from May to October, when vector-borne disease transmission is highest (Ministry of Health Lao PDR 2010). Dengue and Japanese encephalitis (JE) are endemic in the area, with an unknown number of lymphatic filariasis cases. No malaria transmission has been recorded in the study area. According to the Lao climatology center, the average temperature in the area during the rainy season of 2013/2014 was 25.4°C (range 15.3–39.9°C) with 84.2% relative humidity (RH; range 19.0–100%) and in the dry season of 2013/2014, 23.2°C (range 8.8–41.9°C) with 75.8% RH (range 20.3–100%). The average daily rainfall was 8.0 mm (range 0.2–141.2 mm) in the rainy season and 0.4 mm (range 0.0–55.9 mm) in the dry season.

In each of the three study sites four habitats were surveyed ($n_{\text{total}} = 12$ sampling sites): a secondary forest, an immature rubber plantation, a mature rubber plantation, and a local village. The secondary forests are forests that had re-grown after the primary forests had been cut for timber. There are few mature trees with bamboo shrubs and small trees dominating. Immature plantations are those with rubber trees less than five years old, which had not been tapped for latex. These immature plantations have little canopy cover and a high density of undergrowth. Mature rubber plantations are where the trees were more than five years old and over 70% of the trees had been tapped for latex for at least one year. As the trees are accessed regularly, the plantations had little undergrowth. The villages were linear rural settlements with 700–1,000 inhabitants. Generally, villagers lived either in one-storey bamboo houses with thatched roofs or brick houses with metal roofs. Aquatic habitats near the trapping sites were investigated for the immature rubber plantations, mature rubber plantations, and villages (Tangena et al. 2016). The greatest number of waterbodies positive for immature mosquito stages were found in the mature rubber plantations. The most important waterbodies were latex collection cups, tyres, and water container > 10 liters. In the villages, the second greatest number of waterbodies positive for mosquitoes were found. Mainly water containers > 10 liters and cut bamboo were positive for immature mosquitoes. In the immature rubber plantations, the lowest number of waterbodies were found. Larvae and pupae were found in leaf axils and cut bamboo.

Longitudinal Mosquito Collections

Adult blood-questing female mosquitoes were sampled using the human-baited double net (HDN) trap (Tangena et al. 2015). Briefly, the HDN trap consisted of one participant resting on a bamboo bed covered by two untreated bed nets: the smaller one completely surrounded the human subject, while the larger one was positioned over the smaller net and raised off the ground to let mosquitoes be caught between the two nets. Every hour for 10 min the participant raised the bottom of the inner net and aspirated all mosquitoes caught between the two bed nets into labelled paper-cups. Specimens were frozen at −20°C and morphologically identified to species or species complex using Thai identification keys (Rattanarithikul et al. 2005–2010). Members of the malaria vector groups *Anopheles funestus* and *An. maculatus* were identified using Allele-specific polymerase chain reaction (AS PCR) assays and species-specific primers (Linton et al. 2001, Garros et al. 2004, Walton et al. 2007). Unfortunately, there were problems with the PCR system, preventing us from identifying the *An. dirus* complex to species.

Three HDNs, placed 5 m apart, were used to collect mosquitoes in each of the four different habitats. This resulted in 36 HDN collection sites (i.e. 3 study sites × 4 habitats × 3 HDNs). Every month adult mosquitoes were collected for 3 wk. In the first week, 12 collectors (4 habitats × 3 HDNs) collected mosquitoes for 6 h in one study site. After 6 h, the collectors were replaced by a second group of 12 collectors, who continued collecting mosquitoes for another 6 h. This 12-h collection period lasted from 6.00–18.00 h or 18.00–6.00 h. This was repeated four times over several days until a total of 48-h collections were conducted in each habitat. The participants were distributed randomly between collection sites using the research randomizer program (Urbaniak and Plous 2013). Two supervisors checked on the collecting participants periodically during the collecting period. Similar collections were done in the other two study sites over the other two weeks' time-period. A total of 78 healthy male and female villagers, between 18 and 55 years old (i.e. 2 time periods × 12 collectors and two supervisors per study site), were recruited and paid for their participation.

Environmental Measurements

During the period when mosquitoes were collected temperature and relative humidity were measured in each habitat using data-loggers (HOBO Pro Onset Computer Corporation model H08-031-08). The data logger was attached to a tree or pole nearest to the HDN traps at 1.80 m above the ground before mosquito collections commenced. Precipitation data of the districts were collected from the governmental climatology center in Luang Prabang province.

Measurements of physical structure were made in the three forest habitat types—secondary forests, immature rubber plantations, and mature rubber plantations. Using Google Earth, a 10- by 10-m grid was fitted to each of the nine habitats. Each square in a grid was numbered sequentially and in each habitat 10 squares were chosen randomly using the research randomizer program (Urbaniak and Plous 2013). In each of the 90 squares undergrowth density, canopy cover, tree density, tree height, and tree circumference were recorded. Undergrowth density was measured at the four corners of each square, by placing the center of a 2- by 2-m white sheet vertically on one of the four corners. The sheet was set with one side facing north and the other side facing south. Pictures of the sheet were taken using a camera (Stylus TG-830 Tough, Olympus) on a tripod from 4 m away and 1 m above the ground (Palmer et al. 2004). Forty colour photographs were taken of the undergrowth in each habitat (four corners in 10 squares). The canopy cover proportion was also measured on the corners of each square by taking pictures of the sky 1 m above the ground (Palmer et al. 2004). All pictures were analyzed, using the threshold function of image J software (version 1.47, National Institutes of health, USA), to measure proportion of vegetation and canopy cover (Rasband 2014). Tree density was measured by counting the number of trees (defined as a perennial woody plant with the main trunk > 20 cm circumference) in each square. Tree height was measured for all trees using a clinometer (FIN-01510, Valimotie 7, Suunto, Finland), and tree circumference was measured at standard breast height, 1.37 m from the ground (Gregoire et al. 1995).

Data Analysis

For both the rainy and dry season, generalized estimating equations (GEE) were used to estimate the difference in mosquito density between habitats, study sites, and months. The hour factor was included in the GEE model. GEE analysis was done using a negative binomial model with log-link function (IBM SPSS statistics, version 20). Species diversity was compared using Simpson's index of diversity with 95% confidence interval (CI; Simpson 1949, Zhang and Zhou 2010). The positively skewed daily mean temperature and the daily mean humidity were square rooted and analyzed with GEE using a linear distribution with odds ratio (OR) and 95% CI. Undergrowth, canopy cover, tree height, and tree circumference were averaged for each square before analysis. Undergrowth and height were positively skewed and transformed with $\log_{10}(x+1)$ for undergrowth and with $\log_{10}(x)$ for height. Undergrowth and height data were analyzed with generalized linear modeling (GLM) using a linear distribution with OR and 95% CI. Canopy cover and circumference were negatively skewed and were analyzed with GLM using a gamma with log-link distribution with OR and 95% CI. Tree density data were analyzed with GLM using a Poisson log-linear distribution with OR and 95% CI.

Ethical Considerations

Verbal informed consent was provided by village leaders and written informed consent was collected from the HDN participants. Ethical consent for this study was provided by the ethics committee of the Ministry of Health in Lao PDR (approval number 017/NECHR issued 21-04-2013) and the School of Biological and Biomedical Sciences Ethics Committee, Durham University (issued 25-06-2013).

Results

Longitudinal Mosquito Collections

During 15,552 hours of collection, 24,927 adult female mosquitoes were collected. One hundred and fourteen mosquito species were identified, including 51 species not been recorded in Lao PDR before (Table 1; Apiwathnasorn 1986). Thirteen female mosquitoes could not be identified to species. Most mosquitoes were collected in secondary forests (55.3%, 13,789/24,927), followed by immature rubber plantations (21.4%, 5,323/24,927), mature rubber plantations (14.6%, 3,651/24,927), and villages (8.7%, 2,164/24,927). More than 60% (9,395/15,552) of the sampling hours yielded no mosquitoes (37.8%, 1,470/3,888 in secondary forests; 64.7%, 2,300/3,888 in immature rubber plantations; 59.2%, 2,514/3,888 in mature rubber plantations; 80%, 3,111/3,888 in villages). The average number of adult female mosquitoes collected during the night from 18.00 h to 6.00 h was 13.3 (95% CI 11.7–14.8). The average number of mosquitoes collected during the day from 6.00 h to 18.00 h was 25.2 (95% CI 21.9–28.5).

Mosquito Density

The number of female mosquitoes collected varied per habitat (GEE, $P < 0.001$), study site ($P < 0.001$), and month ($P < 0.001$; Table 2). In both the rainy season and dry season more female mosquitoes were collected in the secondary forests than the other three habitats (all $P < 0.0001$). Most mosquitoes were collected in Thinkeo study site and the fewest in Silalek. The variability between collection months within one season was high, with collection numbers varying between 1.5 times higher and 1.5 times lower for different months within both in the rainy season and dry season.

In the secondary forests, more female mosquitoes were collected in August and September 2013, when rainfall was highest, than in the other months combined (Fig. 1). There was a similar monthly trend in the rubber plantations. During the August 2013 peak in rainfall, between four and five times more mosquitoes were collected than in February 2014 when there was no rain (Fig. 1). Generally, a lower number of mosquitoes was collected in mature plantations than in immature rubber plantations. In the villages, the numbers of female mosquito collected were low throughout the year, with generally less than one female mosquito collected per person per hour (Fig. 1).

Mosquito Diversity

In the secondary forests 89 species were collected with a Simpson's index of 0.853 (95% CI 0.850–0.856). This was slightly higher than for immature rubber plantations where 79 species were collected (0.843 with 95% CI 0.838–0.848, t-test $P < 0.001$) and mature rubber plantations where 72 species were collected (0.816 with 95% CI 0.806–0.825, $P < 0.001$). The diversity index in the secondary forests was similar to the diversity index found in the villages, where 62 mosquito species were collected with an index of 0.864 (95% CI 0.855–0.873, $P = 0.0182$). The species distribution in the natural and man-made forest habitats showed similar trends, with *Aedes* species dominating in the rainy season and *Culex* species dominating in the dry season (Fig. 2). In villages *Culex* species were most common in the rainy season and *Anopheles* mosquitoes most common in

Table 1. List of all female adult mosquito species identified in the different habitats during the 9 mo of collection using the illustrated keys of the mosquitoes of Thailand (Rattanarithikul et al. 2005–2010).

	Village	Immature rubber plantation	Mature rubber plantation	Secondary forest	Total	References of previous identification in Lao PDR of previous identification in Lao PDR
Ayurakitia sp*	0	1	1	4	6	
Aedes (*Aedimorphus*) *caecus**	0	2	0	3	5	
Aedes (*Aedimorphus*) *orbitae**	0	0	1	0	1	
Aedes (*Borichinda*) *cavernicola**	0	0	0	1	1	
Aedes (*Bothaella*) *eldridgei*	2	8	11	13	34	(Rueda et al. 2015)
Aedes (*Bothaella*) *helenae**	0	6	4	5	15	
Aedes (*Bruceharrisonius*) *greenii**	1	6	3	17	27	
Aedes (*Danielsia*) *albotaeniata**	0	10	7	46	63	
Aedes (*Downsiomyia*) *inermis**	0	0	1	0	1	
Aedes (*Downsiomyia*) *novonivea* and *Aedes* (*Downsiomyia*) *litorea**	103	344	183	274	904	
Aedes (*Fredwardsius*) *vittatus*	4	15	14	2	35	(Apiwathnasorn 1986, Tsuda et al. 2002, Vythilingam et al. 2006, Rueda et al. 2015)
Aedes (*HuIecoeteomyia*) *chrysolineatus*	0	1	1	0	2	(Apiwathnasorn 1986, Vythilingam et al. 2006, Rueda et al. 2015, Walter Reed Biosystematics Unit 2016)
Aedes (*HuIecoeteomyia*) *formosensis* and *Aedes* (*HuIecoeteomyia*) *pallirostris*	0	10	5	36	51	(Rueda et al. 2015)
Aedes (*HuIecoeteomyia*) *reinerti*	1	18	5	47	71	(Rueda et al. 2015)
Aedes (*HuIecoeteomyia*) *saxicola**	0	0	1	0	1	
Aedes (*Kenknightia*) *dissimilis*	0	0	2	16	18	(Rueda et al. 2015)
Aedes (*Kenknightia*) *harbachi**	0	0	1	5	6	
Aedes (*Lorrainea*) *fumida**	0	0	0	2	2	
Aedes (*Mucidus*) *quasiferinus**	0	0	1	0	1	
Aedes (*Phagomyia*) *khazani*	0	8	5	23	36	(Apiwathnasorn 1986)
Aedes (*Phagomyia*) *prominens*	0	0	0	1	1	(Apiwathnasorn 1986, Rueda et al. 2015)
Aedes (*Stegomyia*) *aegypti*	1	0	0	0	1	(Apiwathnasorn 1986, Tsuda et al. 2002, Hiscox et al. 2013b, Rueda et al. 2015, Walter Reed Biosystematics Unit 2016)
Aedes (*Stegomyia*) *albopictus*	83	1248	1331	3640	6302	(Apiwathnasorn 1986, Vythilingam et al. 2006, Chen-Hussey 2012, Hiscox et al. 2013a, b; Rueda et al. 2015)
Aedes (*Stegomyia*) *gardnerii imitator*	2	48	33	104	187	(Vythilingam et al. 2006)
Aedes (*Stegomyia*) *seatoi**	0	0	2	1	3	
Aedes (*Stegomyia Heteraspidion*) *annandalei**	4	37	40	367	448	
Aedes (*Stegomyia Heteraspidion*) *craggi**	0	1	0	0	1	
Aedes (*Stegomyia Huangmyia*) *malikuli* and *Aedes* (*Stegomyia Huangmyia*) *perplexa**	0	36	25	211	272	
Aedes (*Stegomyia Xyele*) *desmotes**	0	4	12	18	34	
Aedes (*Verrallina Harbachius*) *yusafi**	0	0	0	6	6	
Aedes (*Verrallina Verrallina*) *lugubris**	0	1	0	0	1	
Anopheles (*Anopheles*) sp (*Aitkenii* group)*	0	3	1	22	26	
Anopheles (*Anopheles*) *baezai*	15	0	0	3	18	(Chen-Hussey 2012)
Anopheles (*Anopheles*) *baileyi*	1	0	0	0	1	(Apiwathnasorn 1986, Walter Reed Biosystematics Unit 2016)
Anopheles (*Anopheles*) *barbirostris* s.l.	45	12	11	102	170	(Lefebvre 1938, Apiwathnasorn 1986, Kobayashi et al. 1997, Kobayashi et al. 2000, Vythilingam et al. 2001, Toma et al. 2002, Vythilingam et al. 2003, Vythilingam et al. 2006, Chen-Hussey 2012, Hiscox et al. 2013a, Walter Reed Biosystematics Unit 2016)
Anopheles (*Anopheles*) *barbumbrosus*	388	49	35	48	520	(Kobayashi et al. 1997, Toma et al. 2002)
An. sp. near *Anopheles* (*Anopheles*) *gigas**	2	0	0	5	7	
Anopheles (*Anopheles*) *hodgkini*	3	0	0	4	7	(Toma et al. 2002, Chen-Hussey 2012, Suwonkerd et al. 2013)
Anopheles (*Anopheles*) *insulaeflorum**	1	0	0	0	1	
Anopheles (*Anopheles*) *separatus**	6	4	2	0	12	

(continued)

Table 1. Continued

	Village	Immature rubber plantation	Mature rubber plantation	Secondary forest	Total	References of previous identification in Lao PDR of previous identification in Lao PDR
Anopheles (*Anopheles*) *umbrosus*	16	1	1	0	18	(Apiwathnasorn 1986, Toma et al. 2002, Vythilingam et al. 2003, Chen-Hussey 2012)
Anopheles (*Anopheles*) *whartoni**	1	0	0	0	1	
Anopheles (*Cellia*) *aconitus*	36	12	8	7	63	(Gaschen 1934, Apiwathnasorn 1986, Vythilingam et al. 2001, Toma et al. 2002, Vythilingam et al. 2003, Vythilingam et al. 2006, Hiscox et al. 2013a, Walter Reed Biosystematics Unit 2016)
Anopheles (*Cellia*) *culcifacies*	2	1	0	0	3	(Gaschen 1934, Kobayashi et al. 1997, Toma et al. 2002, Chen-Hussey 2012, Walter Reed Biosystematics Unit 2016)
Anopheles (*Cellia*) *dirus* s.l.	1	31	9	5	46	(Kobayashi et al. 1997, Kobayashi et al. 2000, Vythilingam et al. 2001, Toma et al. 2002, Tsuda et al. 2002, Vythilingam et al. 2003, Vythilingam et al. 2005, Vythilingam et al. 2006, Chen-Hussey 2012, Suwonkerd et al. 2013, Walter Reed Biosystematics Unit 2016)
Anopheles (*Cellia*) *epiroticus**	1	1	0	1	3	
Anopheles (*Cellia*) *jamesii*	3	2	1	0	6	(Apiwathnasorn 1986, Kobayashi et al. 2000, Vythilingam et al. 2001, Toma et al. 2002, Chen-Hussey 2012, Walter Reed Biosystematics Unit 2016)
Anopheles (*Cellia*) *jeyporiensis*	0	1	1	0	2	(Lefebvre 1938, Apiwathnasorn 1986, Kobayashi et al. 1997, Vythilingam et al. 2003, Chen-Hussey 2012, Suwonkerd et al. 2013, Walter Reed Biosystematics Unit 2016)
Anopheles (*Cellia*) *kochi*	14	7	4	6	31	(Lefebvre 1938, Apiwathnasorn 1986, Kobayashi et al. 1997, Kobayashi et al. 2000, Vythilingam et al. 2001, Toma et al. 2002, Vythilingam et al. 2003, Vythilingam et al. 2006, Chen-Hussey 2012, Walter Reed Biosystematics Unit 2016)
Anopheles (*Cellia*) *maculatus* s.l.	53	137	49	55	294	(Lefebvre 1938, Apiwathnasorn 1986, Kobayashi et al. 1997, Vythilingam et al. 2001, Toma et al. 2002, Vythilingam et al. 2003, Vythilingam et al. 2005, Vythilingam et al. 2006, Chen-Hussey 2012, Suwonkerd et al. 2013, Walter Reed Biosystematics Unit 2016)
Anopheles (*Cellia*) *minimus* s.l.	47	16	16	9	88	(Lefebvre 1938, Apiwathnasorn 1986, Kobayashi et al. 1997, Kobayashi et al. 2000, Vythilingam et al. 2001, Toma et al. 2002, Vythilingam et al. 2003, Vythilingam et al. 2005, Vythilingam et al. 2006, Chen-Hussey 2012, Suwonkerd et al. 2013, Walter Reed Biosystematics Unit 2016)
Anopheles (*Cellia*) *pampanai*	6	1	1	3	11	(Vythilingam et al. 2001, Toma et al. 2002, Vythilingam et al. 2006, Chen-Hussey 2012, Walter Reed Biosystematics Unit 2016)
Anopheles (*Cellia*) *phillipinensis*	0	0	0	1	1	(Lefebvre 1938, Apiwathnasorn 1986, Kobayashi et al. 1997, Kobayashi et al. 2000, Vythilingam et al. 2001, Toma et al. 2002, Vythilingam et al. 2003, Vythilingam et al. 2006, Chen-Hussey 2012, Hiscox et al. 2013a, Suwonkerd et al. 2013, Walter Reed Biosystematics Unit 2016)
Anopheles (*Cellia*) *tessellatus*	7	2	0	2	11	(Apiwathnasorn 1986, Kobayashi et al. 1997, Vythilingam et al. 2001, Toma et al. 2002, Vythilingam et al. 2006, Chen-Hussey 2012, Hiscox et al. 2013a, Walter Reed Biosystematics Unit 2016)

(continued)

Table 1. Continued

	Village	Immature rubber plantation	Mature rubber plantation	Secondary forest	Total	References of previous identification in Lao PDR of previous identification in Lao PDR
Anopheles (*Cellia*) *varuna*	0	1	0	0	1	(Vythilingam et al. 2001, Toma et al. 2002, Vythilingam et al. 2003, Vythilingam et al. 2006, Walter Reed Biosystematics Unit 2016)
Armigeres (*Armigeres*) *confusus**	0	0	0	1	1	
Armigeres (*Armigeres*) *foliatus**	5	4	9	95	113	
Armigeres (*Armigeres*) *jugraensis**	4	0	2	4	10	
Armigeres (*Armigeres*) *kesseli**	204	76	129	2212	2621	
Armigeres (*Armigeres*) *kuchingensis*	1	0	0	2	3	(Apiwathnasorn 1986, Vythilingam et al. 2006, Walter Reed Biosystematics Unit 2016)
Armigeres (*Armigeres*) *malayi**	0	2	2	12	16	
Armigeres (*Armigeres*) *moultoni*	7	21	16	42	86	(Apiwathnasorn 1986, Walter Reed Biosystematics Unit 2016)
Armigeres (*Armigeres*) *subalbatus*	8	23	51	186	268	(Apiwathnasorn 1986, Vythilingam et al. 2006, Hiscox 2011, Walter Reed Biosystematics Unit 2016)
Armigeres (*Armigeres*) *theobaldi*	7	4	7	33	51	(Vythilingam et al. 2006, Walter Reed Biosystematics Unit 2016)
Armigeres (*Leicesteria*) *annulipalpis**	0	1	0	1	2	
Armigeres (*Leicesteria*) *annulitarsis*	0	2	1	5	8	(Apiwathnasorn 1986, Walter Reed Biosystematics Unit 2016)
Armigeres (*Leicesteria*) *balteatus**	0	0	1	3	4	
Armigeres (*Leicesteria*) *digitatus**	0	3	2	5	10	
Armigeres (*Leicesteria*) *dolichocephalus*	1	57	36	101	195	(Apiwathnasorn 1986, Walter Reed Biosystematics Unit 2016)
Armigeres (*Leicesteria*) *flavus*	11	109	81	480	681	(Apiwathnasorn 1986, Rueda et al. 2015, Walter Reed Biosystematics Unit 2016)
Armigeres (*Leicesteria*) *inchoatus**	0	1	0	2	3	
Armigeres (*Leicesteria*) *longipalpis*	0	10	7	15	32	(Apiwathnasorn 1986, Rueda et al. 2015, Walter Reed Biosystematics Unit 2016)
Armigeres (*Leicesteria*) *magnus*	1	1	5	7	14	(Apiwathnasorn 1986, Walter Reed Biosystematics Unit 2016)
Armigeres (*Leicesteria*) *omissus**	0	1	0	0	1	
Armigeres (*Leicesteria*) *pectinatus* and *Armigeres* (*Leicesteria*) *vimoli*	0	0	0	4	4	(Apiwathnasorn 1986, Walter Reed Biosystematics Unit 2016)
Armigeres (*Leicesteria*) *traubi**	0	1	0	0	1	
Coquillettidia sp	0	25	27	47	99	(Apiwathnasorn 1986, Vythilingam et al. 2006, Walter Reed Biosystematics Unit 2016)
Culex (*Culex*) *alis*	85	55	52	133	325	(Chen-Hussey 2012)
Culex (*Culex*) *edwardsi**	0	0	0	1	1	
Culex (*Culex*) *fuscocephala*	41	17	2	10	70	(Apiwathnasorn 1986, Vythilingam et al. 2006, Chen-Hussey 2012, Hiscox et al. 2013a, Walter Reed Biosystematics Unit 2016)
Culex (*Culex*) *gelidus*	2	3	0	5	10	(Apiwathnasorn 1986, Toma et al. 2002, Vythilingam et al. 2006, Hiscox 2011, Chen-Hussey 2012, Walter Reed Biosystematics Unit 2016)
Culex (*Culex*) *hutchinsoni*	7	9	2	1	19	(Vythilingam et al. 2006, Chen-Hussey 2012, Walter Reed Biosystematics Unit 2016)
Culex (*Culex*) *mimulus* and *Culex* (*Culex*) *murrelli*	1	0	1	0	2	(Apiwathnasorn 1986)
Culex (*Culex*) *perplexus*	0	1	1	0	2	(Chen-Hussey 2012)
Culex (*Culex*) *quinquefasciatus*	7	3	3	8	21	(Apiwathnasorn 1986, Vythilingam et al. 2006, Chen-Hussey 2012, Hiscox et al. 2013a, Rueda et al. 2015, Walter Reed Biosystematics Unit 2016)
Culex (*Culex*) *sitiens*	56	31	34	91	212	(Apiwathnasorn 1986, Chen-Hussey 2012)
Culex (*Culex*) *vishnui*	604	1041	440	1477	3562	(Toma et al. 2002, Vythilingam et al. 2006, Chen-Hussey 2012, Hiscox et al. 2013a, Walter Reed Biosystematics Unit 2016)
Culex (*Culex*) *whitei*	194	125	67	244	630	(Chen-Hussey 2012)

(continued)

Table 1. Continued

	Village	Immature rubber plantation	Mature rubber plantation	Secondary forest	Total	References of previous identification in Lao PDR of previous identification in Lao PDR
Culex (*Culex*) *whitmorei*	2	0	0	2	4	(Apiwathnasorn 1986, Toma et al. 2002, Vythilingam et al. 2006, Chen-Hussey 2012, Hiscox et al. 2013a, Walter Reed Biosystematics Unit 2016)
Culex (*Culiciomyia*) *dispectus**	0	0	0	1	1	
Culex (*Culiciomyia*) *fragilis* and *Culex* (*Culex*) *spathifurca**	1	2	1	3	7	
Culex (*Culiciomyia*) *nigropunctatus*	12	11	12	5	40	(Vythilingam et al. 2006, Chen-Hussey 2012, Rueda et al. 2015, Walter Reed Biosystematics Unit 2016)
Culex (*Culiciomyia*) *papuensis**	5	1	1	2	9	
Culex (*Culiciomyia*) *termi**	1	0	0	0	1	
Culex (*Eumelanomyia*) *brevipalpis* and *Culex* (*Eumelanomyia*) *phangngae*	0	4	11	8	23	(Apiwathnasorn 1986)
Culex (*Eumelanomyia*) *foliatus*	0	1	0	0	1	(Chen-Hussey 2012)
Culex (*Lophoceraomyia*) *infantulus* and *Culex* (*Lophoceraomyia*) *minutissimus**	0	0	0	2	2	
Culex (*Lophoceraomyia*) sp (*Mamilifer* subgroup and *Wilfredi* group)*	0	0	0	2	2	
Culex (*Oculeomyia*) *bitaeniorhynchus*	7	26	11	31	75	(Apiwathnasorn 1986, Vythilingam et al. 2006, Chen-Hussey 2012, Hiscox et al. 2013a, Walter Reed Biosystematics Unit 2016)
Culex (*Oculeomyia*) *longicornis**	0	1	0	1	2	
Culex (*Oculeomyia*) *pseudosinensis*	0	0	0	1	1	(Vythilingam et al. 2006, Walter Reed Biosystematics Unit 2016)
Heizmania (*Heizmania*) *chengi**	5	255	99	793	1152	
Heizmania (*Heizmania*) *complex*	0	1	0	0	1	(Apiwathnasorn 1986, Walter Reed Biosystematics Unit 2016)
Heizmania (*Heizmania*) *demeilloni**	0	0	0	2	2	
Heizmania (*Heizmania*) *mattinglyi**	22	1244	635	2497	4398	
Lutzia (*Metalutzia*) *vorax**	2	0	0	1	3	
Malaya sp*	3	4	3	9	19	
Mansonia sp	2	19	25	10	56	(Apiwathnasorn 1986, Vythilingam et al. 2006, Chen-Hussey 2012, Walter Reed Biosystematics Unit 2016)
Mimomyia sp	1	1	0	1	3	(Apiwathnasorn 1986, Chen-Hussey 2012, Walter Reed Biosystematics Unit 2016)
Topomyia sp	0	3	2	4	9	(Apiwathnasorn 1986, Miyagi and Toma 2001, Chen-Hussey 2012, Walter Reed Biosystematics Unit 2016)
Toxorhynchites splendens and Toxorhynchites amboinensis	0	0	1	2	3	(Apiwathnasorn 1986)
Tripteroides sp	5	29	44	62	140	(Apiwathnasorn 1986, Chen-Hussey 2012, Walter Reed Biosystematics Unit 2016)
*Udaya argyrurus**	0	1	0	1	2	
Uranotaenia sp	1	3	0	0	4	(Apiwathnasorn 1986, Chen-Hussey 2012, Walter Reed Biosystematics Unit 2016)

*Species not recorded in Lao PDR before.

the dry season. Overall, *Aedes albopictus* (Skuse) was the dominant mosquito species in the secondary forests and rubber plantations habitats with *Culex vishnui* (Theobald) most common in the villages.

The dengue vectors *Ae. albopictus* ($n = 6{,}302$) and *Ae. aegypti* (L.) ($n = 1$), and the JE vectors *Cx. vishnui* ($n = 3{,}562$), *Cx. bitaeniorhynchus* (Giles) ($n = 75$), *Cx. fuscocephalus* (Theobald) ($n = 70$), *Cx. quinquefasciatus* (Say) ($n = 21$), and *Cx. gelidus* (Theobald) ($n = 10$) were collected during our study (Table 1). Many lymphatic filariasis vectors were also collected, including *Armigeres kesseli* (Ramalingam) ($n = 2{,}621$), *Ar. subalbatus* (Coquillett) ($n = 268$), and *Cx. quinquefasciatus*. Furthermore the malaria vectors *An. maculatus* complex ($n = 294$), *An. barbirostris* complex ($n = 170$), *An. funestus* group ($n = 151$), *An. dirus* complex ($n = 46$), *An. culcifacies* (Giles) ($n = 3$), *An. epiroticus* (Linton and Harbach) ($n = 3$), and *An. philippinensis* (Ludlow) ($n = 1$) were collected. Members of the *An. maculatus* complex were molecularly identified to *An. maculatus* (Theobald) ($n = 180$), *An. pseudowillmori* (Theobald) ($n = 36$), *An. dravidicus* (Christophers) ($n = 10$), and *An. sawadwongporni* (Rattanarithikul and Green) ($n = 9$; Table 3). The remaining 59 mosquitoes could not be identified to species. For the *An. funestus* group, *An. minimus* s.s. (Theobald) ($n = 85$) and *An.*

Table 2. Multivariate analysis of variables associated with female adult mosquitoes collected using human-baited double net traps

Season	Explanatory variable	n	Mean no. collected per person/h (95% CI)	OR (95% CI)	P
Rainy (May–Oct.)	*Habitat*				
	Immature rubber plantation	4,118	1.59 (1.49–1.68)	0.33 (0.31–0.36)	<0.0001*
	Mature rubber plantation	3,007	1.16 (1.08–1.24)	0.25 (0.23–0.27)	<0.0001*
	Village	1,652	0.64 (0.55–0.72)	0.13 (0.12–0.14)	<0.0001*
	Secondary forest	11,427	4.41 (4.19–4.62)	1	
	Study site				
	Thinkeo	8,158	2.36 (2.22–2.50)	1.48 (1.39–1.57)	<0.0001*
	Silalek	5,811	1.68 (1.57–1.80)	0.88 (0.83–0.94)	<0.0001*
	Houayhoy	6,235	1.80 (1.69–1.92)	1	
	Month				
	July 2013	3,442	1.99 (1.82–2.16)	1.05 (0.96–1.14)	0.311
	Aug. 2013	4,852	2.81 (2.59–3.02)	1.50 (1.38–1.64)	<0.0001*
	Sept. 2013	3,348	1.94 (1.75–2.12)	0.94 (0.86–1.02)	0.139
	Oct. 2013	2,350	1.36 (1.23–1.49)	0.68 (0.62–0.74)	<0.0001*
	May 2014	2,883	1.67 (1.51–1.83)	0.84 (0.77–0.92)	<0.0001*
	June 2014	3,329	1.93 (1.76–2.10)	1	
Dry (Nov.–April)	*Habitat*				
	Immature rubber plantation	1,205	0.93 (0.77–1.09)	0.46 (0.41–0.51)	<0.0001*
	Mature rubber plantation	644	0.50 (0.42–0.57)	0.25 (0.22–0.28)	<0.0001*
	Village	512	0.40 (0.32–0.47)	0.20 (0.18–0.23)	<0.0001*
	Secondary forest	2,362	1.82 (1.64–2.01)	1	
	Study site				
	Thinkeo	2,492	1.44 (1.30–1.59)	2.07 (1.87–2.29)	<0.0001*
	Silalek	889	0.78 (0.67–0.89)	0.65 (0.58–0.73)	<0.0001*
	Houayhoy	1,342	0.51 (0.43–0.60)	1	
	Month				
	Nov. 2013	1,832	1.06 (0.94–1.18)	1.12 (1.01–1.25)	0.026*
	Feb. 2014	1,205	0.70 (0.59–0.80)	0.74 (0.66–0.82)	<0.0001*
	Mar. 2014	1,686	0.98 (0.84–1.11)	1	

Results are shown for generalized estimating equations of factors affecting the collection of adult female mosquitoes with odds ratio (OR) and 95% confidence interval (CI).

*Significantly different, $P < 0.05$.

aconitus (Dönitz) ($n = 63$) were molecularly identified. Three samples from the *An. funestus* group could not be identified to species.

About 73% of the collected *Aedes* mosquitoes were *Ae. albopictus* (6,305/8,585). Most *Aedes* and *Ae. albopictus* were collected in the secondary forests during both the rainy season and dry season (all $P < 0.001$; Tables 4 and 5). A similar pattern was found for *Culex* mosquitoes. The most abundant species was *Cx. vishnui* (71%, 3,562/5,022), with largest numbers collected in the secondary forests during both seasons (all $P \leq 0.001$; Tables 4 and 5). Few

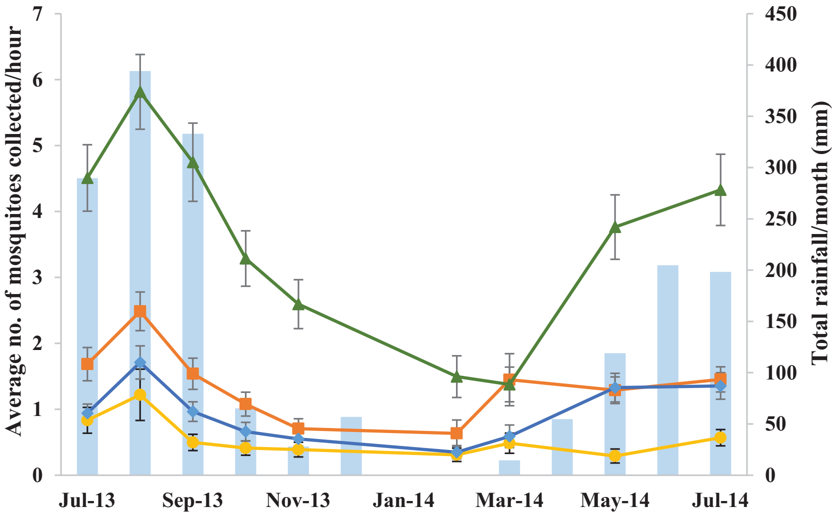

Fig. 1. Seasonal variation of female mosquito numbers in the different habitats. The average number of female mosquitoes collected per person per hour for each collection month in the four habitats is shown with 95% confidence intervals (—▲— secondary forest, —■— immature plantation, —◆— mature plantation, —●— village). Total rainfall per month is indicated with light blue bars (▨).

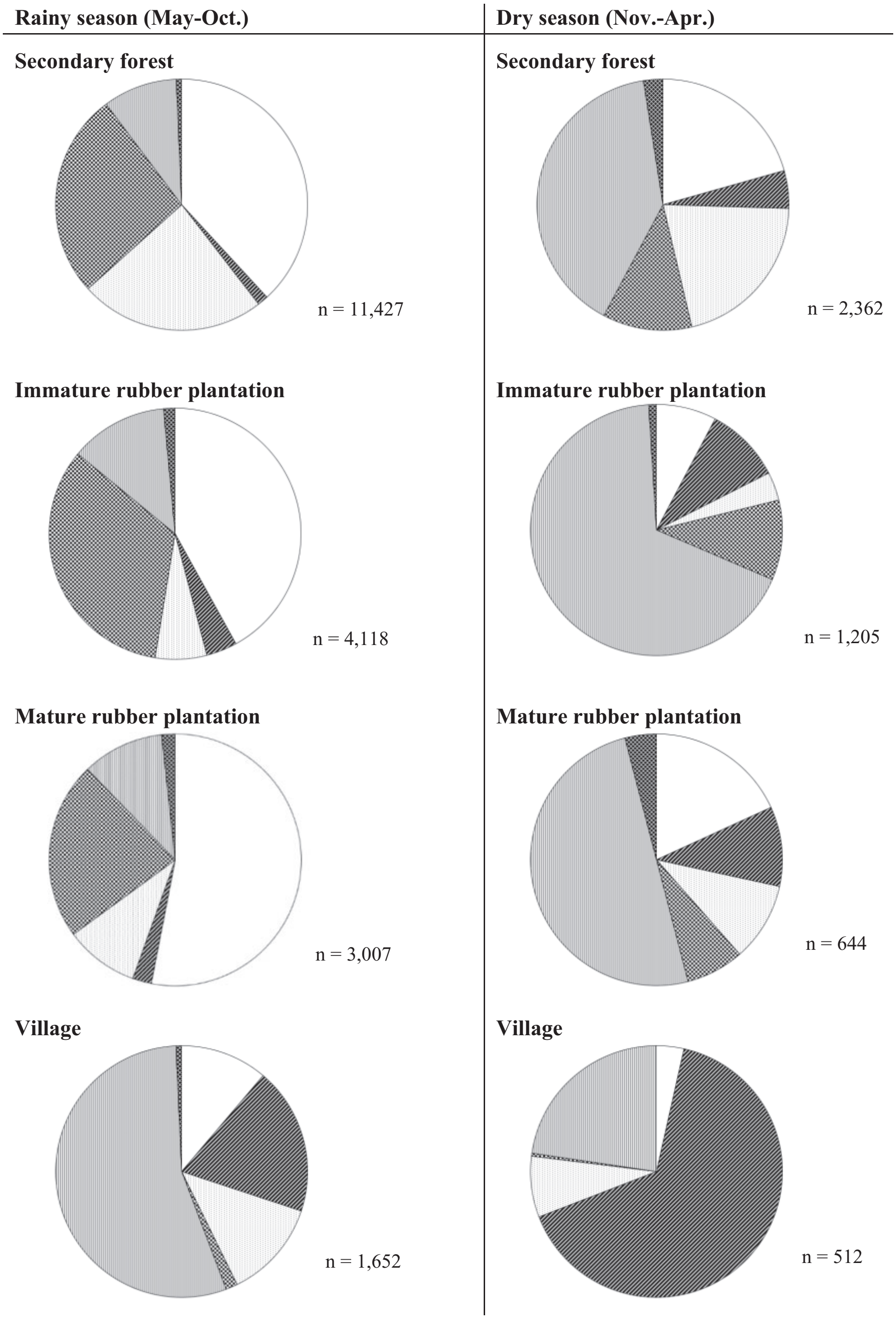

Fig. 2. Distribution of mosquito species collected during the rainy season and dry season in the secondary forest, immature rubber plantation, mature rubber plantation and village with □ *Aedes* species, ▨ *Anopheles* species, □ *Armigeres* species, ▩ *Heizmania* species, ▥ *Culex* species, and ▩ other species.

Anopheles mosquitoes were caught during the survey ($n = 1{,}341$), with 48% of samples collected in the village (648/1,341; Tables 4 and 5). The putative malaria vectors *An. maculatus* s.l. and *An. dirus* s.l. were most common in immature rubber plantations, *An. minimus* s.l. in villages, and *An. barbirostris* s.l. in secondary forests.

In the secondary forests the highest number of dengue vectors (57.8%, 3,640/6,303), JE vectors (41.0%, 1,523/3,717), and lymphatic filariasis vectors (82.7%, 2,406/2,910) were collected. Malaria vector numbers were similarly high in the secondary forests (27.0%, 177/655), immature rubber plantations (31.8%, 208/655), and villages (27.0%, 177/655). In the secondary forests, more than half of the mosquitoes collected were putative vector species (56.2%, 7,746/13,786), including the dengue vector (26.4%, 3,640/13,789; Fig. 3). In the immature rubber plantations, almost half of the collected mosquitoes were vector species (49.7%, 2,678/5,323; Fig. 3). Both dengue and JE vectors were most frequently collected there (dengue 23.4%, 1,248/5,323 and JE 20.4%, 1,087/5,323). In the mature plantations, 56.4% of the collected mosquitoes were putative vector species (2,060/3,651; Fig. 3). Similar to the secondary

Table 3. Molecular identification of members of the *An. funestus* group and the *An. maculatus* complex collected in the different habitats

		Secondary forest	Immature rubber plantation	Mature rubber plantation	Village
An. maculatus complex	*An. maculatus* s.s.	28	96	27	29
	An. pseudowillmori	3	9	10	14
	An. dravidicus	8	2	0	0
	An. sawadwongporni	3	2	1	3
An. funestus group	*An. minimus* s.s.	8	17	16	44
	An. aconitus	7	12	8	36

Table 4. Multivariate analysis of habitat variabilities associated with adult female mosquito species collected using human-baited double net traps during the rainy season

Rainy season (May–Oct.)	Habitat	*n*	Mean no. collected per person/h (95% CI)	OR (95% CI)	*P*
Aedes mosquitoes	Immature rubber plantation	1,729	0.67 (0.61–0.72)	0.41 (0.38–0.45)	<0.001*
	Mature rubber plantation	1,595	0.62 (0.56–0.67)	0.37 (0.35–0.41)	<0.001*
	Village	185	0.07 (0.06–0.08)	0.04 (0.04–0.05)	<0.001*
	Secondary forest	4,361	1.68 (1.58–1.79)	1	
Ae. albopictus	Immature rubber plantation	1,185	0.46 (0.42–0.50)	0.38 (0.35–0.41)	<0.001*
	Mature rubber plantation	1,233	0.48 (0.43–0.52)	0.38 (0.35–0.42)	<0.001*
	Village	77	0.03 (0.02–0.04)	0.02 (0.02–0.03)	<0.001*
	Secondary forest	3,281	1.27 (1.18–1.36)	1	
Culex mosquitoes	Immature rubber plantation	517	0.20 (0.17–0.23)	0.47 (0.41–0.53)	<0.001*
	Mature rubber plantation	316	0.12 (0.10–0.14)	0.29 (0.25–0.33)	<0.001*
	Village	909	0.35 (0.28–0.42)	0.75 (0.67–0.84)	<0.001*
	Secondary forest	1,090	0.42 (0.35–0.49)	1	
Cx. vishnui	Immature rubber plantation	273	0.11 (0.09–0.12)	0.47 (0.40–0.55)	<0.001*
	Mature rubber plantation	142	0.05 (0.04–0.07)	0.24 (0.20–0.30)	<0.001*
	Village	518	0.20 (0.14–0.26)	0.79 (0.68–0.91)	0.001*
	Secondary forest	584	0.23 (0.18–0.27)	1	
Anopheles mosquitoes	Immature rubber plantation	163	0.06 (0.05–0.08)	1.03 (0.82–1.30)	0.790
	Mature rubber plantation	73	0.03 (0.02–0.04)	0.46 (0.35–0.61)	<0.001*
	Village	312	0.12 (0.10–0.14)	1.95 (1.60–2.39)	<0.001*
	Secondary forest	158	0.06 (0.05–0.07)	1	
An. maculatus s.l.	Immature rubber plantation	100	0.04 (0.03–0.05)	2.20 (1.54–3.14)	<0.001*
	Mature rubber plantation	29	0.01 (0.01–0.02)	0.65 (0.40–1.03)	0.068
	Village	42	0.02 (0.01–0.02)	0.93 (0.61–1.42)	0.722
	Secondary forest	46	0.02 (0.01–0.02)	1	
An. minimus s.l.	Immature rubber plantation	11	0.00 (0.00–0.01)	1.22 (0.50–2.95)	0.662
	Mature rubber plantation	16	0.01 (0.00–0.01)	1.66 (0.72–3.80)	0.234
	Village	50	0.02 (0.01–0.02)	4.99 (2.43–10.25)	<0.001*
	Secondary forest	9	0.00 0.00–0.01)	1	
An. barbirostris s.l.	Immature rubber plantation	9	0.00 (0.00–0.01)	0.18 (0.09–0.36)	<0.001*
	Mature rubber plantation	8	0.00 (0.00–0.01)	0.16 (0.07–0.33)	<0.001*
	Village	28	0.01 (0.01–0.02)	0.55 (0.35–0.88)	0.013*
	Secondary forest	51	0.02 (0.01–0.03)	1	
An. dirus s.l.	Immature rubber plantation	20	0.01 (0.00–0.01)	3.99 (1.49–10.67)	0.006*
	Mature rubber plantation	5	0.00 (0.00–0.01)	0.99 (0.29–3.45)	0.994
	Village	0			
	Secondary forest	5	0.00 (0.00–0.00)	1	

Results are shown using generalized estimating equations with odds ratio (OR) and 95% confidence interval (CI).

*Significantly different, $P < 0.05$.

forests, a majority of these were dengue vectors (36.5%, 1,331/3,651). In the villages 52.4% of the collected mosquitoes were putative vector mosquitoes (1,134/2,164), with JE the most abundant vector species (30.2%, 654/2,164; Fig. 3).

Environmental Measurements

The temperature and humidity was similar between the four habitats investigated, with temperature only slightly lower in the secondary forests than the other habitats during the rainy season (Table 6). The physical structure differed between the natural and man-made forests. Undergrowth density and canopy cover were higher in the secondary forests than in the immature rubber plantations and mature rubber plantations (Table 7). Furthermore, the tree height and tree circumference was lower in the secondary forest than in both the immature rubber plantations and the mature rubber plantations. In addition, the tree density in the secondary forests was 1.24 times lower than in the

Table 5. Multivariate analysis of habitat variability associated with female adult mosquito species collected using human-baited double net traps during the dry season

Dry season (Nov.–April)	Habitat	*n*	Mean no. collected per person/h (95% CI)	OR (95% CI)	*P*
Aedes mosquitoes	Immature rubber plantation	93	0.07 (0.05–0.09)	0.19 (0.15–0.24)	<0.001*
	Mature rubber plantation	117	0.09 (0.07–0.11)	0.24 (0.19–0.30)	<0.001*
	Village	18	0.01(0.00–0.02)	0.04 (0.02–0.06)	<0.001*
	Secondary forest	487	0.38 (0.32–0.44)	1	
Ae. albopictus	Immature rubber plantation	63	0.05 (0.03–0.07)	0.17 (0.13–0.23)	<0.001*
	Mature rubber plantation	98	0.08 (0.06–0.10)	0.27 (0.21–0.35)	<0.001*
	Village	6	0.00 (0.00–0.01)	0.02 (0.01–0.04)	<0.001*
	Secondary forest	359	0.28 (0.23–0.32)	1	
Culex mosquitoes	Immature rubber plantation	814	0.63 (0.48–0.77)	0.80 (0.70–0.91)	0.001*
	Mature rubber plantation	322	0.25 (0.19–0.31)	0.32 (0.28–0.38)	<0.001*
	Village	116	0.09 (0.07–0.11)	0.13 (0.10–0.16)	<0.001*
	Secondary forest	938	0.72 (0.60–0.84)	1	
Cx. vishnui	Immature rubber plantation	768	0.59 (0.45–0.74)	0.78 (0.68–0.90)	0.001*
	Mature rubber plantation	298	0.23 (0.17–0.29)	0.31 (0.26–0.37)	<0.001*
	Village	86	0.70 (0.05–0.09)	0.10 (0.08–0.12)	<0.001*
	Secondary forest	893	0.69 (0.57–0.81)	1	
Anopheles mosquitoes	Immature rubber plantation	118	0.09 (0.06–0.12)	1.00 (0.76–1.31)	0.971
	Mature rubber plantation	66	0.05 (0.04–0.07)	0.55 (0.40–0.76)	<0.001*
	Village	336	0.26 (0.20–0.32)	2.76 (2.20–3.48)	<0.001*
	Secondary forest	115	0.09 (0.07–0.11)	1	
An. maculatus s.l.	Immature rubber plantation	37	0.03 (0.02–0.04)	4.13 (1.98–8.60)	<0.001*
	Mature rubber plantation	20	0.02 (0.01–0.02)	2.20 (1.00–4.86)	0.051
	Village	11	0.01 (0.00–0.01)	1.22 (0.50–2.96)	0.658
	Secondary forest	9	0.01 (0.03–0.05)	1	
An. minimus s.l.	Immature rubber plantation	17	0.01 (0.01–0.02)	2.43 (1.00–5.89)	0.050
	Mature rubber plantation	8	0.01 (0.00–0.01)	1.14 (0.41–3.16)	0.803
	Village	33	0.03 (0.02–0.04)	4.66 (2.05–10.60)	<0.001*
	Secondary forest	7	0.01 (0.00–0.01)	1	
An. barbirostris s.l.	Immature rubber plantation	3	0.00 (0.00–0.00)	0.06 (0.02–0.19)	<0.001*
	Mature rubber plantation	3	0.00 (0.00–0.00)	0.06 (0.02–0.19)	<0.001*
	Village	17	0.01 (0.01–0.02)	0.33 (0.19–0.57)	<0.001*
	Secondary forest	51	0.04 (0.32–0.44)	1	
An. dirus s.l.	Immature rubber plantation	11	0.01 (0.00–0.01)	N.A.	
	Mature rubber plantation	4	0.00 (0.00–0.01)		
	Village	1	0.00 (0.00–0.00)		
	Secondary forest	0			

Results are shown using generalized estimating equations with odds ratio (OR) and 95% confidence interval (CI).
*Significantly different, $P < 0.05$.

mature rubber plantations. However, the tree density was similar for the secondary forests and immature rubber plantation.

Discussion

This study described the abundance and diversity of adult mosquitoes, including vector species, in four rural habitats typical in northern Lao PDR. Species diversity was extremely high in each habitat type, with 114 mosquito species identified during the study. Three of the four most common mosquitoes found in our study were vectors: the dengue vector *Ae. albopictus* (Paupy et al. 2009), the JE vector *Cx. vishnui* (Sirivanakarn 1975), and the lymphatic filariasis vector *Ar. kesseli* (Izzati Mohd et al. 2010).

There have been few studies that describe the mosquito fauna in Lao PDR, and none that have studied mosquitoes in Lao rubber plantations. To date 101 mosquito species have been recorded in the country, including 41 *Anopheles* species (Gaschen 1934, Lefebvre 1938, Apiwathnasorn 1986, Pholsena 1992; Kobayashi et al. 1997; Kobayashi et al. 2000; Miyagi and Toma 2001; Vythilingam et al. 2001, 2003, 2005, 2006; Toma et al. 2002; Tsuda et al. 2002; Hiscox 2011; Chen-Hussey 2012; Chen-Hussey et al. 2013; Hiscox et al. 2013a, b; Suwonkerd et al. 2013; Rueda et al. 2015; Walter Reed Biosystematics Unit 2016). This is in marked contrast with neighboring Thailand where >300 different mosquito species have been recorded, including at least 73 *Anopheles* species (Rattanarithikul et al. 2005–2010, Thongsripong et al. 2013). The present study adds a further 51 species to the mosquito species list in Lao PDR. Although this study provides a substantial addition to the species distribution literature, the true mosquito diversity is certainly much greater than our study suggests. Not least because our trapping method used human baits, which underestimates zoophilic mosquitoes (Tangena et al. 2015). A more systematic surveillance of vectors in Lao PDR would provide valuable information on the risk from mosquito-borne diseases across the country.

In this study only the Thai morphological identification keys were used to identify the mosquito species (Rattanarithikul et al. 2005–2010). No cross-references were done with the Vietnamese

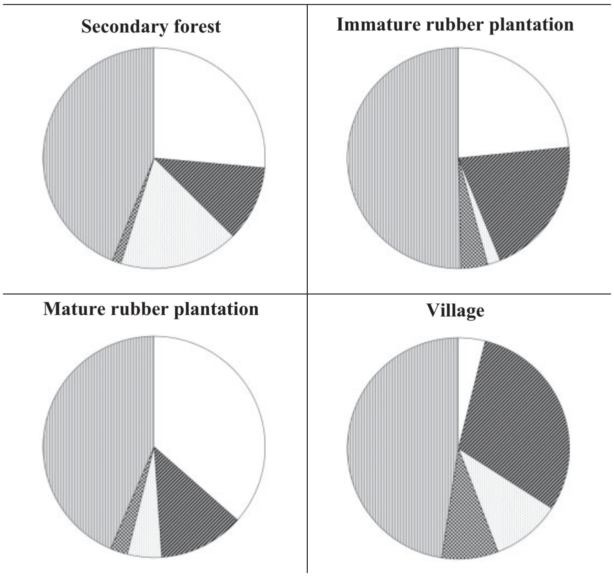

Fig. 3. Proportion of putative vector species collected in the different habitats, with □ Dengue vectors (*Ae. albopictus* and *Ae. aegypti*), ▨ Japanese encephalitis vectors (*Cx. vishnui*, *Cx. bitaeniorhynchus*, *Cx. fuscocephalus*, and *Cx. gelidus*), ▫ Lymphatic filariasis vectors (*Ar. kesseli*, *Ar. subalbatus*, and *Cx. quinquefasciatus*), ▩ Malaria vectors (*An. maculatus* complex, *An. barbirostris* complex, *An. funestus* group, *An. dirus* complex, *An. culcifacies*, *An. epiroticus*, and *An. philippinensis*), and ▥ non vectors.

keys nor with keys from other neighboring countries. A total overlap between the Thai and Lao mosquito population is highly unlikely, with the mosquito populations differing between the eastern neighbors (Viet Nam) and western neighbors (Thailand) of Lao PDR (Stojanovich and Scott 1966, Rattanarithikul et al. 2005–2010, IMPE 2008). For future entomological studies in Lao PDR, it will be of importance to use the identification keys from China, Myanmar, and Viet Nam for confirmation and cross-reference (Chow 1949, Stojanovich and Scott 1966, Baolin and Houyong 2003, Oo et al. 2006, IMPE 2008). Additionally, as has already been done for *Anopheles* mosquitoes (Manguin et al. 2008, Morgan et al. 2013, Obsomer et al. 2013), it would be interesting to make a list of all the mosquito species that have been identified in different parts of Cambodia, Lao PDR, Myanmar, Thailand, Southern China, and Viet Nam. Such a list would be important for identifying shifts in species' distributions associated with environmental change and the changing threat from vector-borne diseases.

The highest numbers of mosquitoes were collected from the secondary forests. In the secondary forests and rubber plantations *Ae. albopictus* was the dominant species in the rainy season, while *Cx. vishnui* dominated in the dry season. In contrast, in the villages *Cx. vishnui* was abundant in the rainy season and *Anopheles* species in the dry season. It is important to note that mosquito data in the rainy season consisted of 6 mo of collection, while mosquito data in the dry season consisted of only 3 mo. As the number of mosquitoes collected in the dry season is generally lower than in the rainy season, more frequent monthly surveys are advised for future studies to increase the reliability of the data. Additionally, local daily rainfall data should be collected in future studies for detailed analysis of relation between rainfall and mosquito abundance. The current study included precipitation data from the climatology centre in Luang Prabang province, which does not include detailed local data. As rainfall can differ significantly between small areas, especially in hilly regions such as our study area, the data from the province is not sufficient for detailed analysis of relation. Overall, there was a high risk of exposure to dengue vectors in natural and man-made forests, with a moderate risk of exposure to vectors of JE, lymphatic filariasis and malaria in all habitats.

The important dengue, chikungunya, and possibly zika vector *Ae. albopictus* (Hawley 1988, Gratz 2004, Paupy et al. 2009, Rianthavorn et al. 2010, Rezza 2012, Wong et al. 2013, Grard et al. 2014, Gardner et al. 2016) was the dominant species in the natural and man-made forests. It is not surprising to find high numbers of *Ae. albopictus* in the forests of northern Lao PDR, since it is a forest mosquito that originated from tropical forest areas in SEA (Paupy et al. 2009, Higa 2011) and prefers shaded areas (Horsfall 1955, Hawley 1988, Vanwambeke et al. 2007). Similar studies in other parts of SEA also found *Ae. albopictus* to be the dominant species in forests and rubber plantations (Sulaiman and Jeffery 1986, Sumodan 2003, Charlwood et al. 2014, Sumodan et al. 2015). The dominance of *Ae. albopictus* in secondary forest and rubber plantations is worrying. Many people work and live in these environments, with the incidence of arbo-viral diseases such as dengue becoming more frequent. Protecting people from this mosquito remains a high priority and requires a combination of protection methods, including larval source reduction and personal protection methods (Tangena et al. 2016). Detailed studies are needed in dengue endemic areas to identify the main breeding sites of *Ae. albopictus* in

Table 6. Mean temperature and relative humidity during the rainy season and dry season in the immature rubber plantations, mature rubber plantations, and villages compared to the secondary forests

Season	Habitat	Temp (°C)			Relative humidity (%)		
		Mean (95% CI)	OR (95% CI)	*P*	Mean (95% CI)	OR (95% CI)	*P*
Rainy (May–Oct.)	Immature rubber plantation	25.0 (24.4–25.7)	1.04 (1.02–1.06)	<0.001*	83.7 (80.0–87.4)	1.26 (0.92–1.72)	0.151
	Mature rubber plantation	25.0 (24.2–25.7)	1.03 (1.00–1.06)	<0.001*	81.5 (74.2–88.8)	1.07 (0.70–1.64)	0.742
	Village	26.1 (25.3–26.9)	1.15 (1.12–1.18)	<0.001*	82.7 (80.1–85.3)	1.20 (0.87–1.65)	0.264
	Secondary forest	24.7 (24.0–25.4)	1		79.9 (73.4–86.5)	1	
Dry (Nov.–April)	Immature rubber plantation	23.3 (21.5–25.1)	1.03 (0.98–1.07)	0.260	75.8 (68.0–83.5)	1.01 (0.92–1.12)	0.819
	Mature rubber plantation	22.9 (21.3–24.5)	0.99 (0.95–1.03)	0.628	77.0 (69.3–84.7)	1.09 (0.97–1.21)	0.139
	Village	23.5 (21.2–25.8)	1.04 (0.98–1.11)	0.208	74.0 (65.6–80.4)	0.92 (0.81–1.04)	0.166
	Secondary forest	23.1 (20.9–25.2)	1		75.7 (66.3–85.1)	1	

Results are shown using generalized estimating equations with odds ratio (OR) and 95% confidence interval (CI).

*Significantly different, $P < 0.05$.

Table 7. Difference in physical forest structure of the secondary forest compared to the immature rubber plantations and mature rubber plantations

Environmental factors	Habitat	Mean (95% CI)	OR (95% CI)	*P*
Undergrowth (% covered by undergrowth)	Immature rubber plantation	12.1 (9.0–15.2)	0.63 (0.54–0.74)	<0.001*
	Mature rubber plantation	4.5 (2.4–6.7)	0.40 (0.34–0.47)	<0.001*
	Secondary forest	30.7 (25.5–35.9)	1	
Canopy (% covered by canopy)	Immature rubber plantation	81.9 (76.3–87.5)	0.88 (0.83–0.94)	<0.001*
	Mature rubber plantation	87.4 (85.2–89.5)	0.94 (0.88–1.00)	<0.001*
	Secondary forest	93.0 (92.0–94.0)	1	
Tree density[a] (no. of trees/grid of 10 by 10 m)	Immature rubber plantation	6.1 (5.3–6.9)	1.17 (0.94–1.45)	0.158
	Mature rubber plantation	6.4 (5.8–7.1)	1.24 (1.00–1.53)	0.048*
	Secondary forest	5.2 (4.3–6.1)	1	
Height (m)	Immature rubber plantation	11.6 (10.7–12.5)	1.05 (1.00–1.11)	0.033*
	Mature rubber plantation	13.9 (13.3–14.6)	1.15 (1.10–1.21)	<0.001*
	Secondary forest	10.8 (8.7–12.8)	1	
Circumference (cm)	Immature rubber plantation	40.6 (37.3–44.0)	1.59 (1.33–1.89)	<0.001*
	Mature rubber plantation	47.8 (45.7–49.9)	1.89 (1.58–2.26)	<0.001*
	Secondary forest	25.8 (18.3–33.4)	1	

Results are shown using generalized linear modeling with odds ratio (OR) and 95% confidence interval (CI). [a] All perennial trees, including rubber trees, were counted for tree density.

*Significantly different, $P < 0.05$.

rubber plantations before larval source reduction can be successful (World Health Organization 2013), with an indication that latex collection cups may be important aquatic habitats for this species (Sumodan 2003). Further research is also needed to identify the best outdoor personal protection method, with currently limited field studies on the use of permethrin-treated clothing, transfluthrin-emitting devices and mosquito coils in a clip-on holder. In the future, to protect both local villagers and rubber workers from exposure to arboviruses, further research is needed to 1) identify the main larval breeding sites to determine whether targeted larval source management could be protective, and 2) develop improved methods of personal protection.

Few malaria vectors were collected in our study, although this resulted in near daily exposure. In our study area, no malaria transmission was recorded. The absence of local malaria transmission could be related to a number of factors such as 1) few, if any, malaria-infected individuals in the area, 2) the low efficiency of vectors to transmit malaria parasites, as well as 3) underreporting of the disease and 4) sleeping under an insecticide-treated net (ITN), using repellents and wearing long-sleeved clothing.

Overall, the most commonly caught *Anopheles* species were *An. maculatus* s.s and *An. minimus* s.s., which is similar to other parts of the country (Centre of Malariology, Parasitology and Entomology (CMPE) of Lao PDR). The species composition of malaria vectors differed between habitats, with *An. minimus* s.s. dominant in the villages, *An. maculatus* s.s. dominant in the rubber plantations and *An. barbirostris* s.l. dominant in the secondary forests. In the rubber plantations, the malaria vectors *An. maculatus* s.s., *An. minimus* s.s, *An. dirus* s.l., *An. barbirostris* s.l., *An. umbrosus* s.l. and *An. jeyporiensis* were found. All species, except *An. jeyporiensis*, have been recorded in rubber plantations before (Singh and Tham 1988, Rosenberg et al. 1990, Sallum et al. 2005, Sinka et al. 2011, Bhumiratana et al. 2013a). Interestingly, hardly any specimens of *An. dirus* s.l. were collected in the secondary forests, even though these primary malaria vectors are often found in SEA forests (Obsomer et al. 2007, Tananchai et al. 2012). Although we could not confirm the species of *An. dirus* s.l. using PCR, it seems likely that the samples we collected are *An. dirus* s.s. (Manguin et al. 2008, Morgan et al. 2013).

This study highlights the rich and heterogeneous mosquito dynamics in SEA (Trung et al. 2005, Gryseels et al. 2015). The highest species heterogeneity was found in the secondary forests and villages, which was slightly lower in the rubber plantation monocultures. The rural village and fragmented forests have been described as ecotones (Thongsripong et al. 2013), a transition area between two habitats where multiple habitat communities integrate. This generally entails a higher number of species compared to other habitats, as they include species from bordering ecological systems (Despommier et al. 2006). The higher species heterogeneity in the secondary forests and villages could be related to the higher habitat diversity providing a higher diversity of aquatic habitats and consequently a higher heterogeneity of mosquito species (Shililu et al. 2003, Thongsripong et al. 2013, Overgaard et al. 2015).

It is important to emphasize that in this study, we did not test for the presence of pathogens. Therefore, it is difficult to identify the actual risk of exposure to vector-borne diseases. The risk analysis is further complicated by human behavior, which often results in a heterogeneous pathogen-exposure pattern within the population (Schwartz and Goldstein 1990, Reuben 1993, Vlassoff and Manderson 1998, National Institute for Occupational Safety and Health [NIOSH] 2005, Finch et al. 2014, Herdiana et al. 2016, Sang et al. 2016). It is therefore important to relate the entomological data with the pathogen dynamics and the social dynamics in a more interdisciplinary fashion. Then control measures can be focussed on the groups of people most at risk, making control efforts more effective. This focussed approach is especially important for countries such as Thailand, where hotspots of disease transmission remain (Lyttleton 2016).

Evidence of the impact of land use change on mosquito dynamics, and thus the risk of vector-borne diseases, is growing (McMichael et al. 1998, Norris 2004, Foley et al. 2005, Patz et al. 2008, Reisen 2010, Parham et al. 2015). Yet a deeper understanding on the functional relationships between the abundance and diversity of mosquito species, and the different habitats is often limited, since one habitat type may contain numerous functional resources for mosquitoes (Overgaard et al. 2003, Manguin et al. 2008, Van Dyck 2012, Obsomer et al. 2013, Overgaard et al. 2015, Fornace et al. 2016). In this study, we attempted to provide a richer description of the physical characteristics of each habitat type. The secondary forests in our study were relatively young forests with high undergrowth and canopy cover, yet with a lower density and smaller trees than the rubber plantations. Compared to the secondary forests the immature rubber plantations had less undergrowth with lower canopy cover and slightly bigger trees. The mature rubber plantations consisted of a high density of big trees with similar canopy cover as the immature rubber plantations, but little undergrowth. The low undergrowth in the mature rubber plantations results from regular weeding and pruning by rubber workers, so they can tap latex. The high mosquito numbers in the secondary forests and immature rubber plantations were associated with high undergrowth, lower temperatures, and higher humidity. These conditions may increase mosquito survival by providing shelter from predators, shade and flowers for sugar. Furthermore, the leaves of the undergrowth might provide more suitable larval habitats for *Aedes* and *Culex* mosquitoes than areas with less vegetation. If this association is correct, cutting undergrowth might be a potential vector control method. Secondary forest and rubber plantations provide ideal habitats for *Ae. albopictus*, an efficient vector of many human arboviruses, to flourish. Protecting people entering forest habitats from this vector should be a priority.

Acknowledgments

We would like to thank the staff of Institut Pasteur du Laos and the National Agriculture and Forestry Research Institute, especially the ECOMORE team, for providing support throughout this study. We would also like to thank the entomology department of Kasetsart University in Bangkok, Thailand, for their work on the molecular identification of the *Anopheles* mosquitoes. This project would not have been possible without the hospitality of the residents of Thinkeo, Silalek, and Houayhoy village, the assistance from the district heads of Nane and Xieng Ngeun, and support from the provincial head of Luang Prabang. This study was supported by the ECOMORE project, funded by the Agence Française de Développement (AFD).

References Cited

Apiwathnasorn, C. 1986. A list of mosquito species in Southeast Asia, SEAMEO-TROPMED, National Centre of Thailand, Fac. Trop. Med. Mahidol University, Bangkok.

Baolin, L., and W. Houyong. 2003. Classification and identification of important medical insects of China, vol. 10, Henan Science and Technology Publishing House.

Bhumiratana, A., P. Sorosjinda-Nunthawarasilp, W. Kaewwaen, P. Maneekan, and S. Pimnon. 2013a. Malaria-associated rubber plantations in Thailand. Travel Med Infect Dis. 11: 37–50.

Bhumiratana, A., A. Intarapuk, P. Sorosjinda-Nunthawarasilp, P. Maneekan, and S. Koyadun. 2013b. Border malaria associated with multidrug resistance on Thailand-Myanmar and Thailand-Cambodia borders: Transmission dynamic, vulnerability, and surveillance. BioMed Res. Int. 2013: 363417.

Charlwood, J., E. Tomas, L. Kelly-Hope, and O. Briet. 2014. Evidence of an 'invitation' effect in feeding sylvatic *Stegomyia albopicta* from Cambodia. Parasit Vectors 7: 324.

Chen-Hussey, V. 2012. A cluster-randomised trial to assess whether the insect repellent N,N-diethyl-m-toluamide (DEET) can provide additional protection against clinical malaria over current best practice in Lao PDR. Ph.D. dissertation. London School of Hygiene & Tropical Medicine, London.

Chen-Hussey, V., I. Carneiro, H. Keomanila, R. Gray, S. Bannavong, S. Phanalasy, and S. W. Lindsay. 2013. Can topical insect repellents reduce malaria? A cluster-randomised controlled trial of the insect repellent *N*, *N*-diethyl-m-toluamide (deet) in Lao PDR. PLoS ONE 8: e70664.

Chow, C. Y. 1949. The identification and distribution of Chinese anopheline mosquitoes. J. Natl. Malar. Soc. 8: 121–131.

Despommier, D., B. R. Ellis, and B. A. Wilcox. 2006. The role of ecotones in emerging infectious diseases. EcoHealth 3: 281–289.

Finch, C., M. S. Al-Damluji, P. J. Krause, L. Niccolai, T. Steeves, C. F. O'Keefe, and M. A. Diuk-Wasser. 2014. Integrated assessment of behavioral and environmental risk factors for Lyme disease infection on Block Island, Rhode Island. PLoS ONE 9: e84758.

Foley, J. A., R. DeFries, G. P. Asner, C. Barford, G. Bonan, S. R. Carpenter, F. S. Chapin, M. T. Coe, G. C. Daily, H. K. Gibbs, et al. 2005. Global consequences of land use. Science 309: 570–574.

Food and Agriculture Organization 2010. Global forest resources assessment 2010. FAO, Rome.

Fornace, K. M., T. R. Abidin, N. Alexander, P. Brock, M. J. Grigg, A. Murphy, T. William, J. Menon, C. J. Drakeley, and J. Cox. 2016. Association between Landscape Factors and Spatial Patterns of *Plasmodium knowlesi* Infections in Sabah, Malaysia. Emerg. Infect. Dis. 22: 201–209.

Gardner, L. M., N. Chen, and S. Sarkar. 2016. Global risk of Zika virus depends critically on vector status of *Aedes albopictus*. Lancet Infect. Dis. 16: 522–523.

Garros, C., L. L. Koekemoer, M. Coetzee, M. Coosemans, and S. Manguin. 2004. A single multiplex assay to identify major malaria vectors within the African *Anopheles funestus* and the Oriental *An. minimus* groups. Am. J. Trop. Med. Hyg. 70: 583–590.

Garros, C., C. Van Nguyen, H. Trung, W. Van Bortel, M. Coosemans, and S. Manguin. 2008. Distribution of *Anopheles* in Vietnam, with particular attention to malaria vectors of the *Anopheles minimus* complex. Malar. J. 7: 11.

Gaschen, H. 1934. Prospection entomologique au Laos. Bull Soc. Medico-Chirurgicale Indochine 12: 533–540.

Githeko, A. K., S. W. Lindsay, U. E. Confalonieri, and J. A. Patz. 2000. Climate change and vector-borne diseases: A regional analysis. Bull. World Health Organ. 78: 1136–1147.

Grard, G., M. Caron, I. M. Mombo, D. Nkoghe, S. Mboui Ondo, D. Jiolle, D. Fontenille, C. Paupy, and E. M. Leroy. 2014. Zika virus in Gabon (Central Africa) – 2007: A new threat from *Aedes albopictus*? PLoS Negl. Trop. Dis. 8: e2681.

Gratz, N. G. 2004. Critical review of the vector status of *Aedes albopictus*. Med. Vet. Entomol. 18: 215–227.

Gregoire, T. G., H. T. Valentine, and G. M. Furnival. 1995. Sampling method to estimate foliate and other characteristics of individual trees. Ecology 76: 1181–1194.

Gryseels, C., L. Durnez, R. Gerrets, S. Uk, S. Suon, S. Set, P. Phoeuk, V. Sluydts, S. Heng, T. Sochantha, et al. 2015. Re-imagining malaria: heterogeneity of human and mosquito behaviour in relation to residual malaria transmission in Cambodia. Malar. J. 14: 165.

Hawley, W. A. 1988. The biology of *Aedes albopictus*. J. Am. Mosq. Control Assoc. 1: 1–39.

Herdiana, H., C. Cotter, F. N. Coutrier, I. Zarlinda, B. W. Zelman, Y. K. Tirta, B. Greenhouse, R. D. Gosling, P. Baker, M. Whittaker, et al. 2016. Malaria risk factor assessment using active and passive surveillance data from Aceh Besar, Indonesia, a low endemic, malaria elimination setting with *Plasmodium knowlesi*, *Plasmodium vivax*, and *Plasmodium falciparum*. Malar. J. 15: 468.

Higa, Y. 2011. Dengue vectors and their spatial distribution. Trop. Med. Health 39: 17–27.

Hiscox, A. 2011. Studies on mosquito vectors in areas affected by the nam theun 2 hydroelectric project, Lao PDR. Ph.D. dissertation. London School of Hygiene and Tropical Medicine, London.

Hiscox, A., P. Khammanithong, S. Kaul, P. Sananikhom, R. Luthi, N. Hill, P. T. Brey, and S. W. Lindsay. 2013a. Risk factors for mosquito house entry in the Lao PDR. PLoS ONE 8: e62769.

Hiscox, A., A. Kaye, K. Vongphayloth, I. Banks, M. Khammanithong, P. Sananikhom, S. Kaul, N. Hill, S. Lindsay, and P. Brey. 2013b. Risk factors for the presence of *Stegomyia aegypti* and *Stegomyia albopicta* in domestic water-holding containers in areas impacted by the Nam Theun 2 hydroelectric project, Laos. Am. J. Trop. Med. Hyg. 88: 1070–1078.

Horsfall, W. R. 1955. Their bionomics and relation to disease, pp. 245, 249–255, 257–264 and 595, Mosquitoes. The Ronald press company, New York.

IMPE 2008. Keys to identify the Anopheles in Vietnam (Adult - Pupae - Larvae). Institute of Malariology Parastology and Entomology Quy Nhon, Hanoi.

Izzati Mohd, N.-Z., C. Chee Dhang, L. Han Lim, F. Kalid, and S.-A. Mohd. 2010. Parity status and filarial infection in field-collected *Armigeres kesseli* ramalingam and *Armigeres salbabatus* coquillett from forested and urban areas in Malaysia, pp. 259–262, Environmental Engineering and Applications (ICEEA), International Conference from 10–12 September 2010. IEEE.

Jones, K. E., N. G. Patel, M. A. Levy, A. Storeygard, D. Balk, J. L. Gittleman, and P. Daszak. 2008. Global trends in emerging infectious diseases. Nature 451: 990–993.

Kobayashi, J., S. Nambanya, I. Miyagi, B. Vanachone, K. Manivong, T. Koubouchan, H. Amano, H. Nozaki, S. Inthakone, and Y. Sato. 1997. Collection of anopheline mosquitos in three villages endemic for malaria in Khammouane, Lao PDR. Southeast Asian J. Trop. Med. Public Health 28: 20–21.

Kobayashi, J., P. Somboon, H. Keomanila, S. Inthavongsa, S. Nambanya, S. Inthakone, Y. Sato, and I. Miyagi. 2000. Malaria prevalence and a brief entomological survey in a village surrounded by rice fields in Khammouan province, Lao PDR. Trop. Med. Int. Health 5: 17–21.

Kumar, N. P., A. Suresh, P. Vanamail, S. Sabesan, K. G. Krishnamoorthy, J. Mathew, V. T. Jose, and P. Jambulingam. 2011. Chikungunya virus outbreak in Kerala, India, 2007: A seroprevalence study. Mem. Inst Oswaldo Cruz 106: 912–916.

Lefebvre, M. 1938. *A. minimus*, vecteur principal du paludisme au Laos. Bull Soc. Pathol. Exot. 3: 387–390.

Linton, Y. M., R. E. Harbach, S. Chang Moh, T. G. Anthony, and A. Matusop. 2001. Morphological and molecular identity of *Anopheles* (Cellia) *sundaicus* (Diptera: Culicidae), the nominotypical member of a malaria vector species complex in Southeast Asia. Syst. Entomol. 36: 357–366.

Lyttleton, C. 2016. Deviance and resistance: malaria elimination in the Greater Mekong subregion. Soc. Sci. Med. 150: 144–152.

Manguin, S., C. Garros, I. Dusfour, R. E. Harbach, and M. Coosemans. 2008. Bionomics, taxonomy, and distribution of the major malaria vector taxa of *Anopheles* subgenus *Cellia* in Southeast Asia: An updated review. Infect. Genet. Evol. 8: 489–503.

McMichael, A. J., J. Patz, and R. S. Kovats. 1998. Impacts of global environmental change on future health and health care in tropical countries. Br. Med. Bull. 54: 475–488.

Ministry of Health Malaysia 2013. Dengue health facts 2010. *In* **Ministry of Health Malaysia. Health facts. Ministry of Health Malaysia, Putrajaya.**

Miyagi, I., and T. Toma. 2001. *Tripteroides* (Rachionotomyia) *ponmeki* (Diptera: Culicidae): A new species from Khammouane Province, Lao PDR. Med. Entomol. Zool. 52: 269–277.

Morgan, K., P. Somboon, and C. Walton. 2013. Understanding *Anopheles* diversity in Southeast Asia and its applications for malaria control, pp. 327–355. *In* S. Manguin (ed.), Anopheles mosquitoes - New insights into malaria vectors. InTech.

National Agriculture and Forestry Research Institute 2016. Review of rubber plantations. *In* **National Agriculture and Forestry Research Institute and Ministry of agriculture and forestry [collab.], Rubber plantation information book. Ministry of Agriculture and Forestry, NAFRI, Vientiane.**

(NIOSH) National Institute for Occupational Safety and Health 2005. Recommendations for protecting outdoor workers from west nile virus exposure. Department of Health and Human Services, Centers for Disease Control and Prevention.

Norris, D. 2004. Mosquito-borne diseases as a consequence of land use change. EcoHealth 1: 19–24.

Obsomer, V., P. Defourny, and M. Coosemans. 2007. The *Anopheles dirus* complex: spatial distribution and environmental drivers. Malar. J. 6: 26.

Obsomer, V., M. Dufrene, P. Defourny, and M. Coosemans. 2013. Anopheles species associations in Southeast Asia: Indicator species and environmental influences. Parasites Vectors 6: 136.

Oo, T. T., A. Kaiser, and N. Becker. 2006. Illustrated keys to the anopheline mosquitoes of Myanmar. J. Vector Ecol. 31: 9–16.

Overgaard, H., B. Ekbom, W. Suwonkerd, and M. Takagi. 2003. Effect of landscape structure on anopheline mosquito density and diversity in northern Thailand: Implications for malaria transmission and control. Landsc. Ecol. 18:

Overgaard, H. J., W. Suwonkerd, and J. Hii. 2015. The malaria landscape: mosquitoes, transmission, landscape, insecticide resistance, and integrated control in Thailand, pp. 123–159. *In* S. Morand (ed.), Socio-ecological dimensions of infectious diseases in Southeast Asia, 1 ed. Springer Science, Business Media Singapore.

Palaniyandi, M. 2014. The environmental aspects of dengue and chikungunya outbreaks in India: GIS for epidemic control. Int. J. Mosq. Res. 1: 35–40.

Palmer, T., U. Andersen, P. Cantelaube, F. Doblas-Reyes, H. Feddersen, R. Graham, S. Gualdi, J.-F. Gueremy, R. Hagedorn, M. Hoshen, et al. 2004. Development of a European Ensemble System for Season to Inter-annual Prediction (DEMETER). BAMS 85: 853–872.

Parham, P. E., J. Waldock, G. K. Christophides, D. Hemming, F. Agusto, K. J. Evans, N. Fefferman, H. Gaff, A. Gumel, S. LaDeau, et al. 2015. Climate, environmental and socio-economic change: Weighing up the balance in vector-borne disease transmission. Phil. Trans. R. Soc. B. 370: 1665.

Patz, J. A., S. H. Olson, C. K. Uejio, and H. K. Gibbs. 2008. Disease emergence from global climate and land use change. Med. Clin. North Am. 92: 1473–1491.

Paupy, C., H. Delatte, L. Bagny, V. Corbel, and D. Fontenille. 2009. *Aedes albopictus*, an arbovirus vector: from the darkness to the light. Microb. Infect. 11: 1177–1185.

Pholsena, K. 1992. The malaria situation and antimalaria program in Laos. Southeast Asian J. Trop. Med. Public Health 23: 39–42.

Rasband, W. S. 2014. ImageJ computer program, version By Rasband, W. S., Maryland. (http://imagej.nih.gov/ij/)

Rattanarithikul, R., R. E. Harbach, B. A. Harrison, P. Panthusiri, R. E. Coleman, and J. H. Richardson. 2005–2010. Illustrated keys to the mosquitoes of Thailand II-VI. Southeast. Asian J. Trop. Med. Public Health 36, 37 and 41.

Reisen, W. K. 2010. Landscape epidemiology of vector-borne diseases. Annu. Rev. Entomol. 55: 461–483.

Reuben, R. 1993. Women and malaria - special risks and appropriate control strategy. Soc. Sci. Med. 37: 473–480.

Rezza, G. 2012. *Aedes albopictus* and the reemergence of dengue. BMC Public Health 12: 72.

Rianthavorn, P., K. Prianantathavorn, N. Wuttirattanakowit, A. Theamboonlers, and Y. Poovorawan. 2010. An outbreak of chikungunya in southern Thailand from 2008 to 2009 caused by African strains with A226V mutation. Int. J. Infect. Dis. 3.

Rosenberg, R., R. G. Andre, and L. Somchit. 1990. Highly efficient dry season transmission of malaria in Thailand. Trans. R. Soc. Trop. Med. Hyg. 84: 22–28.

Rueda, L. M., K. Vongphayloth, J. E. Pecor, I. W. Sutherland, J. Hii, M. Debboun, and P. T. Brey. 2015. Mosquito fauna of Lao People's Democratic Republic, with special emphasis on the adult and larval surveillance at Nakai district, Khammuane province. US Army Med. Dept. J. July-September: 25–32.

Sallum, M.A.M., E. L. Peyton, and R. C. Wilkerson. 2005. Six new species of the *Anopheles leucosphyrus* group, reinterpretation of *An. elegans* and vector implications. Med. Vet. Entomol. 19: 158–199.

Sang, S., S. Wang, L. Lu, P. Bi, M. Lv, and Q. Liu. 2016. The epidemiological characteristics and dynamic transmission of dengue in China, 2013. PLoS Negl. Trop. Dis. 10: e0005095.

Schwartz, B. S., and M. D. Goldstein. 1990. Lyme disease in outdoor workers: Risk factors, preventive measures, and tick removal methods. Am. J. Epidemiol. 131: 877–885.

Shililu, J., T. Ghebremeskel, F. Seulu, S. Mengistu, H. Fekadu, M. Zerom, A. Ghebregziabiher, D. Sintasath, G. Bretas, C. Mbogo, et al. 2003. Larval habitat diversity and ecology of anopheline larvae in Eritrea. J. Med. Entomol. 40: 921–929.

Simpson, E. H. 1949. Measurement of diversity. Nature 163: 688.

Singh, J., and A. S. Tham. 1988. Case history on malaria vector control through the application of environmental management in Malaysia. WHO, Geneva.

Singhasivanon, P., K. Thimasarn, S. Yimsamran, K. Linthicum, K. Nualchawee, D. Dawreang, S. Kongrod, N. Premmanisakul, W. Maneeboonyang, and N. Salazar. 1999. Malaria in tree crop plantations in south-eastern and western provinces of Thailand. Southeast Asian J. Trop. Med. Public Health 30: 399–404.

Sinka, M. E., M. J. Bangs, S. Manguin, T. Chareonviriyaphap, A. P. Patil, W. H. Temperley, P. W. Gething, I. R. F. Elyazar, C. W. Kabaria, R. E. Harbach, et al. 2011. The dominant *Anopheles* vectors of human malaria in the Asia-Pacific region: occurrence data, distribution maps and bionomic précis. Parasit Vectors 4: 89.

Sirivanakarn, S. 1975. The systematics of *Culex vishnui* complex in Southeast Asia with the diagnosis of three common species (Diptera: Culicidae). Mosq. Syst. 7: 69–86.

Stojanovich, C. J., and H. G. Scott. 1966. Illustrated key of mosquitoes of Vietnam, US Dept of Health, Education and Welfare, Public Health Service, Atlanta, GA.

Sulaiman, S., and J. Jeffery. 1986. The ecology of *Aedes albopictus* (Skuse) (Diptera: Culicidae) in a rubber estate in Malaysia. Bull. Entomol. Res. 76: 553–557.

Sumodan, P. K. 2003. Potential of rubber plantations as breeding source for *Aedes albopictus* in Kerala, India. Dengue Bull. 27: 197–198.

Sumodan, P. K., R. M. Vargas, J. Pothikasikorn, A. Sumanrote, R. Lefait-Robin, and J.-P. Dujardin. 2015. Rubber plantations as a mosquito box amplification in South and Southeast Asia, pp. 160–165. In S. Morand (ed.), Socio-ecological dimensions of infectious diseases in Southeast Asia. Springer Science Business Media, Singapore.

Suwonkerd, W., W. Ritthison, C. T. Ngo, K. Tainchum, M. J. Bangs, and T. Chareonviriyaphap. 2013. Vector biology and malaria transmission in Southeast Asia. *In* S. Manguin (ed.), Anopheles mosquitoes - New insights into malaria vectors. InTech, Rijeka, Croatia.

Tananchai, C., R. Tisgratog, W. Juntarajumnong, J. Grieco, S. Manguin, A. Prabaripai, and T. Chareonviriyaphap. 2012. Species diversity and biting activity of *Anopheles dirus* and *Anopheles baimaii* (Diptera: Culicidae) in a malaria prone area of western Thailand. Parasit Vectors 5: 211.

Tangena, J.-A. A., P. Thammavong, A. Hiscox, S. W. Lindsay, and P. T. Brey. 2015. The human-baited double net trap: An alternative to human landing catches for collecting outdoor biting mosquitoes in Lao PDR. PLoS ONE 10: e0138735.

Tangena, J.-A. A., P. Thammavong, A. L. Wilson, P. T. Brey, and S. W. Lindsay. 2016. Risk and control of mosquito-borne diseases in Southeast Asian rubber plantations. Trends Parasitol. 32: 402–415.

The World bank 2015. Countries: Lao PDR. (http://www.worldbank.org/en/country/lao) (accessed 12 November 2016).

Thongsripong, P., A. Green, P. Kittayapong, D. Kapan, B. Wilcox, and S. Bennett. 2013. Mosquito vector diversity across habitats in central Thailand endemic for dengue and other arthropod-borne diseases. PLoS Negl. Trop. Dis. 7: e2507.

Toma, T., I. Miyagi, T. Okazawa, J. Kobayashi, S. Saita, A. Tuzuki, H. Keomanila, S. Nambanya, S. Phompida, M. Uza, et al. 2002. Entomological surveys of malaria in Khammouane Province, Lao PDR, in 1999 and 2000. Southeast Asian J. Trop. Med. Public Health 33: 532–546.

Trung, H. D., W. V. Bortel, T. Sochantha, K. Keokenchanh, O.J.T. Briët, and M. Coosemans. 2005. Behavioural heterogeneity of *Anopheles* species in ecologically different localities in Southeast Asia: A challenge for vector control. Trop. Med. Int. Health 10: 251–262.

Tsuda, Y., J. Kobayashi, S. Nambanya, I. Miyagi, T. Toma, S. Phompida, and K. Manivang. 2002. An ecological survey of dengue vector mosquitos in central Lao PDR. Southeast Asian J. Trop. Med. Public Health 33: 63–67.

Urbaniak, G. C., and S. Plous. 2013. Research Randomizer computer program, version 4.0. By Urbaniak, G. C., and S. Plous.

Van Dyck, H. 2012. Changing organisms in rapidly changing anthropogenic landscapes: The significance of the ‘Umwelt’-concept and functional habitat for animal conservation. Evol. Appl. 5: 144–153.

Vanwambeke, S. O., P. Somboon, R. E. Harbach, M. Isenstadt, E. F. Lambin, C. Walton, and R. K. Butlin. 2007. Landscape and land cover factors influence the presence of *Aedes* and *Anopheles* larvae. J. Med. Entomol. 44: 133–144.

Vlassoff, C., and L. Manderson. 1998. Incorporating gender in the anthropology of infectious diseases. Trop. Med. Int. Health 3: 1011–1019.

Vythilingam, I., K. Keokenchan, S. Phommakot, S. Nambanya, and S. Inthakone. 2001. Preliminary studies of *Anopheles* mosquitos in eight provinces in Lao PDR. Southeast Asian J. Trop. Med. Public Health 32: 83–87.

Vythilingam, I., R. Phetsouvanh, K. Keokenchanh, V. Yengmala, V. Vanisaveth, S. Phompida, and S. L. Hakim. 2003. The prevalence of *Anopheles* (Diptera: Culicidae) mosquitoes in Sekong Province, Lao PDR in relation to malaria transmission. Trop. Med. Int. Health 8: 525–535.

Vythilingam, I., B. Sidavong, S. T. Chan, T. Phonemixay, V. Vanisaveth, P. Sisoulad, R. Phetsouvanh, S. L. Hakim, and S. Phompida. 2005. Epidemiology of malaria in Attapeu Province, Lao PDR in relation to entomological parameters. Trans. R. Soc. Trop. Med. Hyg. 99: 833–839.

Vythilingam, I., B. Sidavong, C. S. Thim, T. Phonemixay, S. Phompida, and J. Jeffery. 2006. Species composition of mosquitoes of Attapeu province, Lao People’s Democratic Republic. J. Am. Mosq. Control Assoc. 22: 140–143.

Walter Reed Biosystematics Unit 2016. Keys to the medically important mosquito species. *In* M. D. Suitland (ed.), Walter Reed Biosystematics Unit, Smithsonian Institution. [online database].

Walton, C., P. Somboon, S. M. O’Loughlin, S. Zhang, R. E. Harbach, Y. M. Linton, B. Chen, K. Nolan, S. Duong, M. Y. Fong, et al. 2007. Genetic diversity and molecular identification of mosquito species in the *Anopheles maculatus* group using the ITS2 region of rDNA. Infect. Genet Evol. 7: 93–102.

Wangroongsarb, P., P. Sudathip, and W. Satimai. 2012. Characteristics and malaria prevalence of migrant populations in malaria-endemic areas along the Thai-Cambodian border. Southeast Asian J. Trop. Med. Public Health 43: 261–269.

Watson, M. 1921. The prevention of malaria in the Federated Malay States, a record of 20 years progress, E.P. Dutton and company, New York, NY.

Wong, P.S.J., M.Z.I. Li, C. S. Chong, L. C. Ng, and C. H. Tan. 2013. *Aedes (Stegomyia) albopictus* (Skuse): A potential vector of Zika virus in Singapore. PLoS Negl. Trop. Dis. 7: e2348.

World Health Organization 2013. Larval source management: A supplementary measure for malaria vector control: an operational manual. WHO, Geneva.

Zhang, Z., and J. Zhou. 2010. Re-parameterization of multinomial distributions and diversity indices. J. Stat. Plan Inference. 140: 1731–1738.