

Seasons and socio-cultural practices affecting *Aedes* mosquito larvae in southern Thailand

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Abstract. The aims of this study are to examine the effects of (1) socio-cultural practices of using different types of water storage containers, and (2) seasons, on the numbers of *Aedes aegypti* and *Aedes albopictus* larvae in the five sub-districts of Lansaka district. We randomly selected 20 houses per sub-district per month, with a total of 600 households, and collected *Aedes* mosquito larvae from different types of containers (indoor/outdoor, natural/artificial, dark/light coloured, and with/without lids) during both the dry (March-May 2015) and wet (October-December 2015) seasons. *Ae. aegypti* and *Ae. albopictus* larvae found in each container were identified and counted. The larval indices (i.e., house index (HI), container index (CI), and Breteau index (BI)) were calculated for each sub-district, and for each season. Our results showed that socio-cultural practices of using different types of containers differed between sub-districts and seasons. *Ae. albopictus* larval indices were higher than *Ae. aegypti* larval indices in both seasons. *Ae. albopictus* larval indices were higher in the wet season than in the dry season; the opposite results were observed for *Ae. aegypti*. *Ae. albopictus* larvae were more commonly found in outdoor containers in both seasons, but were found to be more in light coloured and without lid containers during the dry season, whereas *Ae. aegypti* larvae were more often found in indoor containers in both seasons, but are found more in light coloured containers in the dry season. There were positive correlations between container numbers and mosquito larvae numbers in all sub-districts. These results indicate that seasons and water container types have strong effects on the breeding of *Aedes* mosquito larvae. This knowledge could be used to obtain a better understanding on how to take initiatives that could promote the prevention and control of dengue vectors.

INTRODUCTION

Dengue is the most important mosquito-borne virus disease in the world, with nearly 2500 million people at risk worldwide (WHO, 2017). Dengue fever is caused by dengue viruses of the family *Flaviviridae*, transmitted principally by *Aedes aegypti* (Linnaeus) and *Ae. albopictus* (Skuse) in the tropical and subtropical regions of the world (Brown *et al.*, 2014; Caraballo & King, 2014). These mosquitoes also transmit chikungunya, yellow fever, and Zika infection. Currently, there is no effective antiviral treatment or preventive vaccine for dengue (Xu *et al.*, 2016); *Aedes* larval

control is the most effective method for controlling dengue disease (Singh & Taylor-Robinson, 2017). For the prevention of dengue fever, the topographical factors that influence the key breeding sites of *Ae. aegypti* and *Ae. albopictus* larvae must be more closely investigated (Preechaporn *et al.*, 2007).

There are several factors influencing *Aedes* mosquitoes, including water container types, seasons, and topographic, climatic, and vectorial factors. Previous studies showed that seasonal effect on numbers of *Aedes* larvae depends on topographical location. For example, in Northern Thailand, *Ae. aegypti* was higher in number during the

dry season, and *Ae. albopictus* was higher in the wet season (Mogi *et al.*, 1988). The opposite results were observed in Eastern and Southern Thailand (Strickman & Kittiyapong, 2002; Preechaporn *et al.*, 2007). *Aedes* density, as well as the number of dengue cases, increased in the wet season in Malaysia, India, Sri Lanka, Myanmar, Indonesia and the Philippines, as well as in Thailand (Rozilawati *et al.*, 2007; Bar & Andrew, 2012; Wai *et al.*, 2012). However, according to Preechaporn *et al.* (2007), Codeco *et al.* (2009), and Lambdin *et al.* (2009), *Ae. aegypti* larvae numbers can actually be high in the dry season, and dengue transmission may occur in both the wet and dry seasons.

Container type is probably the most important factor determining the key breeding sites of mosquito species (Rajesh *et al.*, 2013). Mosquitoes breed in different kind of containers, such as water storage containers, water plant pots, animal feeding pans, ant-guards, trash containers, and coconut shells (Phuanukoonnon *et al.*, 2005; Wongkoon *et al.*, 2007a; Promprou *et al.*, 2011; Bartlett-Healy *et al.*, 2012). Previous studies have divided these containers into four types (i.e., indoor/outdoor, natural/artificial, dark/light coloured, and with/without lid containers) and investigated the effects of container types on the key breeding sites of *Aedes* larvae (e.g., Chen *et al.*, 2005; Vanwambeke *et al.*, 2007; Wongkoon *et al.*, 2007b; Wong *et al.*, 2011; Ferdousi *et al.*, 2015; Getachew *et al.*, 2015; Rozilawati *et al.*, 2015; Boonklong & Bhumiratana, 2016; Vannavong *et al.*, 2017). These studies observed that *Ae. aegypti* preferred to breed in indoor containers, and *Ae. albopictus* preferred to breed in outdoor containers, but both species preferred to breed in artificial, dark coloured, or without lid containers than in natural, light coloured, or with lid containers, respectively.

In Thailand, no research has been conducted to investigate how socio-cultural differences in using different types of containers in sub-districts, or seasons, affect the key breeding sites of *Aedes* spp. Nakhon Si Thammarat (NST) province in Southern Thailand is a suitable province for addressing this question, as several Dengue Hemorrhagic

Fever (DHF) outbreaks were reported in 2002, 2010, 2013, and 2016, with 6603, 5943, 4496, and 2284 dengue cases, respectively (NST Provincial Office). We predict that (1) if socio-culture practices affect the water storage usages, when we compare among sub-districts, there should be differences in container types based on socio-cultural practices among sub-districts, (2) if container types affect the numbers of *Aedes* larvae, then *Aedes* larvae should differ between indoor/outdoor, natural/artificial, dark/light coloured containers, and containers with/without lids, and (3) if seasons affect the numbers of *Aedes* larvae, then the numbers of *Ae. albopictus* larvae should be higher in the wet season than in the dry season due to higher numbers of outdoor containers available and *Ae. aegypti* larvae should be higher in the dry season than in the wet season.

MATERIALS AND METHODS

Study site and experiment duration

A mosquito larval survey was conducted in Lansaka district, Nakhon Si Thammarat province, Southern Thailand (8.40700 °N and 99.76891 °E) (Figure 1) from March-April, 2015, in the dry season, and from October-December, 2015, in the wet season. Lansaka district is one of 23 districts in this province, and has an area of 342.90 km². This district has five sub-districts (Lansaka, Khaokaew, Thadi, Kamlon, and Khunthale), 44 villages, 11,427 households, and 43,056 people (Lansaka District Register Office, 2014). Lansaka district was selected as our study site due to two main reasons: (1) Lansaka district is a dengue high risk area, with two major dengue outbreaks reported in 2010 and 2013, and (2) Lansaka district has five sub-districts with different socio-cultural practices that may affect key *Aedes* breeding sites.

Mosquito larvae collection, preservation and identification

We used a stratified random sampling technique using the proportional allocation method (also known as proportional stratified random sampling) (Hirzel & Guisan, 2002)

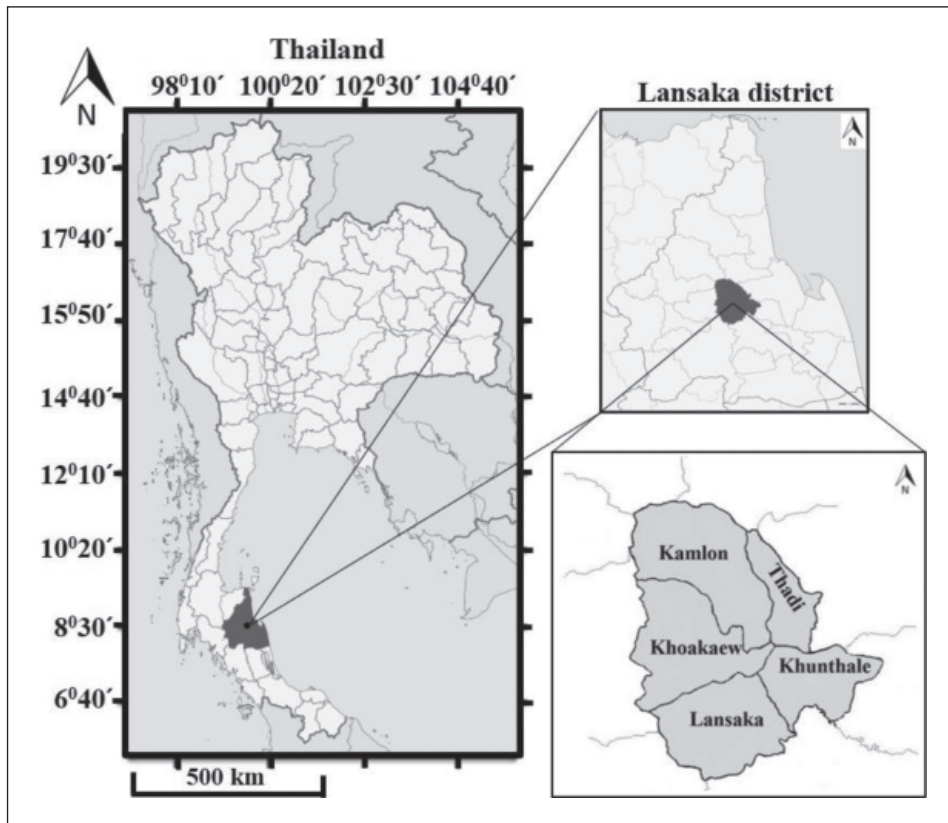


Figure 1. Location (black dot) of Lansaka district, Nakhon Si Thammarat, southern Thailand (left hand side). Study areas were the five sub-districts (Lansaka, Khoakaew, Thadi, Kamlon and Khunthale) in Lansaka district (right hand side).

to make sub-groups, or strata (i.e., one stratum in each sub-district) for data collection. Afterwards, we randomly selected 20 households/sub-district/month and collected mosquito larvae from them in the dry and wet seasons, a total of 600 households. We collected *Ae. aegypti* and *Ae. albopictus* mosquito larvae from different kinds of water containers, such as water storage containers (earthen jars, cement tanks, plastic buckets, and plastic tanks), areca nut containers, water plant pots, animal feeding pans, container lids, trash containers, ant-guards, refrigerator drainage containers, coconut shells, cement mixing containers, and rubber tapping knife sharpening containers. We identified the containers based on the researches of Phuanukoonnon *et al.* (2005), Wongkoon *et al.* (2007a), Promprou *et al.* (2011), and Bartlett-Healy *et al.* (2012).

We divided the containers into four types: (1) indoor (i.e., those water containers found inside houses, such as ant guards, cement tanks, and refrigerator drainage containers) and outdoor (i.e., those water containers found outside houses, such as cement mixing containers and areca nut containers); (2) natural (i.e., not man-made containers, such as coconut shells) and artificial (i.e., man-made containers, such as earthen jars, and cement tanks); (3) dark (i.e., dark coloured containers, such as black plastic buckets) and light (i.e., light coloured containers such as white plastic buckets), and (4) with lid (i.e., containers with a lid cover, such as plastic lids, wooden lids, and metal lids) and without lid containers (i.e. containers with no lid cover).

Mosquito larvae were collected using fishnets of 0.55 mm mesh size. All live mosquito larvae were collected in plastic

bags, brought to the Vector Borne Disease Control Centre, Nakhon Si Thammarat province laboratory and preserved in 70% ethanol (Adebote *et al.*, 2008; Webb *et al.*, 2016). Each mosquito larva was identified up to species level under a microscope by using Rattarithikul and Panthusiri's keys (Rattarithikul & Panthusiri, 1994).

Three larval indices (i.e., house index (HI), container index (CI), and Breteau index (BI)) were calculated, as per standard WHO guidelines (WHO, 2009). House index (HI) was calculated as the number of houses infested divided by the number of houses surveyed $\times 100$. Container index (CI) was calculated as the number of positive containers divided by the number of containers surveyed $\times 100$. Breteau Index (BI) was calculated as the number of positive containers divided by the number of houses surveyed $\times 100$. HI is widely used to calculate the presence and distribution of *Aedes* spp. populations in a given locality. BI and HI are commonly used for the determination of risk areas for control measures to be implemented in. Generally, either $HI > 5\%$ or $BI > 20\%$ for any locality indicates that the locality is dengue sensitive (WHO, 2009).

Statistical analysis

Before starting data analysis, normality was assessed. Parametric statistics were used when normality or other assumptions of parametric tests were met. An independent sample *t*-test was used to investigate the differences in mosquito larvae numbers (1) between the wet and dry seasons, and (2) between mosquito species (*Ae. aegypti* and *Ae. albopictus*), for each sub-district. We used two-way ANOVA tests to observe the effects of seasons and container types (indoor/outdoor, natural/artificial, dark/light coloured, and with/without lids) and their interactions on mosquito larval density. Spearman correlations were made between container numbers and mosquito larvae numbers in all sub-districts. We used SPSS 22, and all significant tests were two tailed. We considered a statistically significance level to be at $P < 0.05$.

RESULTS

Mosquito species, season, and sub-districts

Comparing the numbers of larvae seen among the between mosquito species (*Ae. aegypti* and *Ae. albopictus*) in the wet season, *Ae. albopictus* showed a higher number of larvae than *Ae. aegypti* larvae numbers were higher than *Ae. aegypti* in all sub-districts (Table 1). In the dry season, the number of *Ae. albopictus* larvae were higher than *Ae. aegypti* larvae in Thadi and Khunthale sub-districts, but *Ae. aegypti* and *Ae. albopictus* larvae did not differ in other sub-districts (Table 1). HI, BI, and CI of *Ae. albopictus* were higher than *Ae. aegypti* in all sub-districts in both seasons (Table 1).

Comparing the number of larvae between seasons, showed that *Ae. albopictus* larvae were higher in the wet season than in the dry season in most sub-districts (Khaokaew, Lansaka, and Kamlon), and larval indices were higher in the wet season than in the dry season in all sub-districts (Table 1). The numbers of *Ae. aegypti* larvae were higher in the dry season than in the wet season in two sub-districts (Lansaka and Kamlon), and larval indices were higher in the dry season than in the wet season in most sub-districts (Khaokaew, Thadi, and Kamlon sub-districts) (Table 1).

Mosquito species, season, and container group

In the case of indoor/outdoor containers, the numbers of *Ae. aegypti* larvae were higher in indoor containers than in outdoor containers in both seasons (two-way ANOVA: seasons: $F_{1,745} = 7.79$, $P < 0.01$, containers: $F_{1,745} = 112.37$, $P < 0.001$, interaction: $F_{1,745} = 0.03$, *ns*; Figure 2a). The numbers of *Ae. albopictus* larvae were higher in outdoor containers than in indoor containers in both seasons (two-way ANOVA: seasons: $F_{1,745} = 19.64$, $P < 0.001$, containers: $F_{1,745} = 23.27$, $P < 0.001$, interaction: $F_{1,745} = 0.20$, *ns*; Figure 2b).

Table 1. *Aedes* larvae numbers and larval indices in the wet and dry seasons in five sub-districts in Lansaka district, Nakhon Si Thammarat, southern Thailand

Sub-districts	Mosquito species	Wet season (Mean ± SE)	Dry season (Mean ± SE)	Statistical tests	Larval Indices					
					HI		CI		BI	
					Wet	Dry	Wet	Dry	Wet	Dry
Khaokaew	<i>Ae. aegypti</i>	4.21 ± 1.14	5.34 ± 1.07	$t_{135} = -0.71$	25.00	31.67	2.65	5.75	25.00	43.33
	<i>Ae. albopictus</i>	14.81 ± 1.48	8.33 ± 1.38	$t_{135} = 3.18^{**}$	51.67	33.33	10.09	8.89	95.00	66.67
	Statistical tests	$t_{138} = -5.64^{***}$	$t_{132} = -1.70$							
Lansaka	<i>Ae. aegypti</i>	1.74 ± 0.66	4.70 ± 1.22	$t_{57.11} = -2.13^*$	11.67	23.33	1.35	4.08	11.67	21.67
	<i>Ae. albopictus</i>	16.24 ± 1.87	8.62 ± 2.34	$t_{97} = 2.51^*$	50.00	25.00	10.19	6.27	88.33	33.33
	Statistical tests	$t_{122} = -7.28^{***}$	$t_{72} = -1.98$							
Thadi	<i>Ae. aegypti</i>	2.64 ± 0.57	4.67 ± 1.30	$t_{212} = -1.61$	38.33	30.00	2.28	4.87	46.67	33.33
	<i>Ae. albopictus</i>	14.93 ± 0.95	14.37 ± 2.04	$t_{212} = 0.27$	85.00	41.67	10.98	9.49	225.00	65.00
	Statistical tests	$t_{322} = -11.05^{***}$	$t_{102} = -3.99^{***}$							
Kamlon	<i>Ae. aegypti</i>	1.17 ± 0.38	5.41 ± 1.40	$t_{49.33} = -2.91^{**}$	18.33	23.33	1.43	3.55	20.00	23.33
	<i>Ae. albopictus</i>	14.02 ± 1.01	6.70 ± 1.37	$t_{154} = 3.98^{***}$	68.33	26.67	10.58	6.35	148.33	41.67
	Statistical tests	$t_{222} = -11.84^{***}$	$t_{86} = -0.66$							
Khunthale	<i>Ae. aegypti</i>	3.32 ± 1.56	6.89 ± 1.58	$t_{141} = -1.25$	23.33	18.33	1.88	3.04	25.00	20.00
	<i>Ae. albopictus</i>	17.01 ± 1.28	12.92 ± 2.54	$t_{141} = 1.56$	70.00	30.00	11.03	4.81	146.67	31.67
	Statistical tests	$t_{212} = -6.76^{***}$	$t_{70} = -2.01^*$							

*** $P < 0.005$, ** $P < 0.01$, and * $P < 0.05$.

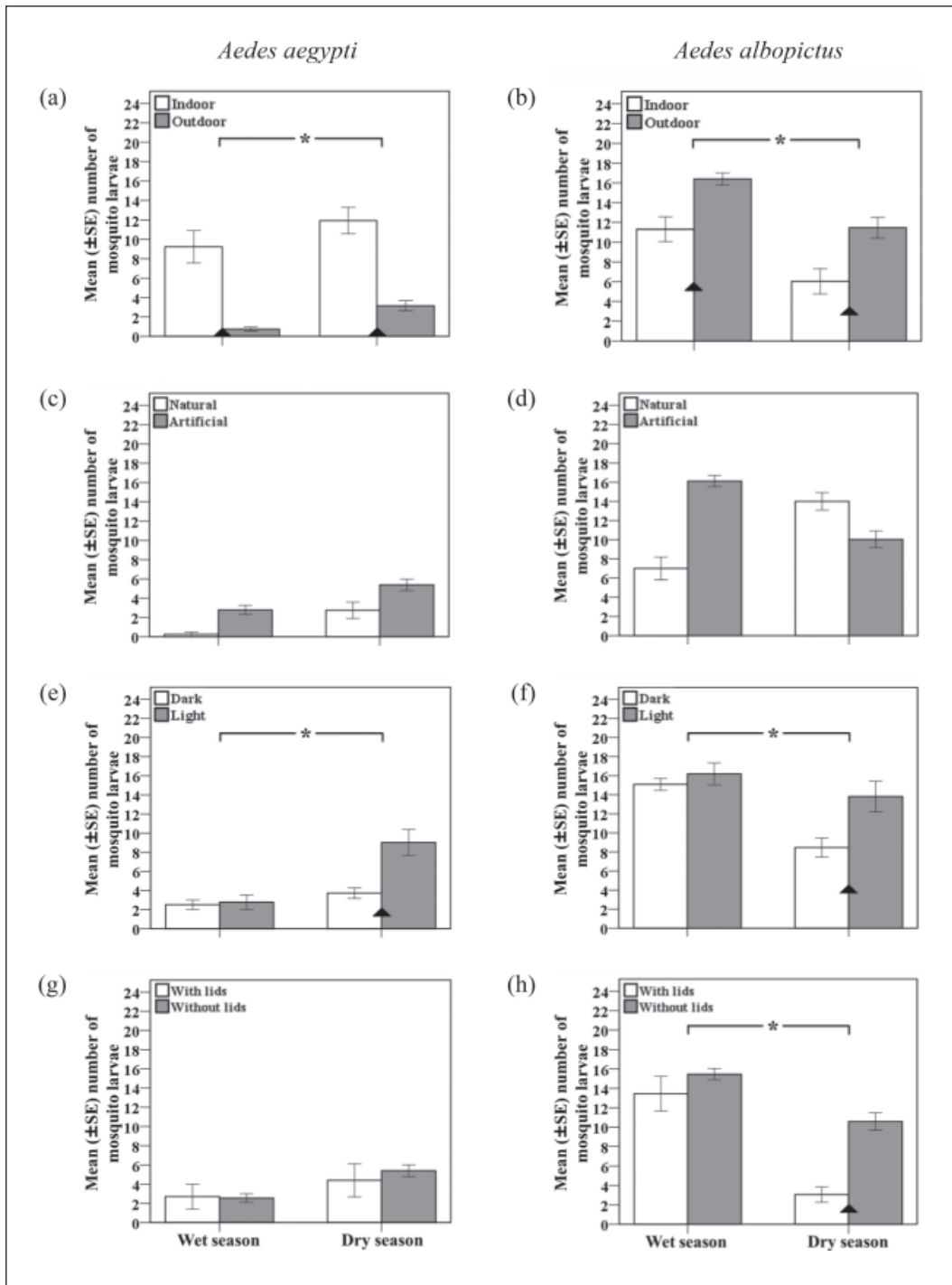


Figure 2. *Ae. aegypti* and *Ae. albopictus* larvae in the wet and dry seasons. (a, b) indoor/outdoor containers, (c, d) natural/artificial containers, (e, f) dark/light coloured containers and (g, h) containers with/without lids. ‘*’ indicates difference ($P < 0.05$) between seasons, and ‘▲’ indicates difference ($P < 0.05$) between container types.

Comparing between natural and artificial containers in both seasons, the numbers of *Ae. aegypti* and *Ae. albopictus* larvae did not differ between natural or artificial containers in either season, but the interaction between season and container type had an effect on the numbers of *Ae. albopictus* larvae (*Ae. aegypti*: seasons: $F_{1,745} = 0.99$, *ns*, containers: $F_{1,745} = 1.04$, *ns*, interaction: $F_{1,745} = 0.00$, *ns*; *Ae. albopictus*: seasons: $F_{1,745} = 0.02$, *ns*, containers: $F_{1,745} = 0.61$, *ns*, interaction: $F_{1,745} = 3.92$, $P < 0.05$; Figure 2c, d).

Comparing between dark and light coloured containers, both *Ae. aegypti* and *Ae. albopictus* larvae numbers were higher in light coloured containers than in dark coloured containers in the dry season, but no difference was observed in the wet season (*Ae. aegypti*: seasons: $F_{1,745} = 16.01$, $P < 0.001$, containers: $F_{1,745} = 7.93$, $P < 0.01$, interaction: $F_{1,745} = 2.10$, *ns*, *Ae. albopictus*: seasons: $F_{1,745} = 14.24$, $P < 0.001$, containers: $F_{1,745} = 6.74$, $P < 0.05$, interaction: $F_{1,745} = 3.76$, *ns*; Figure 2e, f).

Comparing between containers with lids and without lids, *Ae. aegypti* larvae numbers did not differ between containers with or without lids in either season (seasons: $F_{1,745} = 2.20$, *ns*, containers: $F_{1,745} = 0.05$, *ns*, interaction: $F_{1,745} = 0.09$, *ns*; Figure 2g), but *Ae. albopictus* larvae numbers were higher in containers without lids than in those with lids in the dry season (seasons: $F_{1,745} = 14.44$, $P < 0.001$, containers: $F_{1,745} = 5.83$, $P < 0.05$, interaction: $F_{1,745} = 1.96$, *ns*; Figure 2h).

Containers and seasons

Seven types of positive containers found inside houses were earthen jars, cement tanks, plastic buckets, plastic tanks, animal feeding pans, ant-guards, and refrigerator drainage containers (Table 2). Thirteen types of positive containers found outside houses were earthen jars, cement tanks, plastic buckets, plastic tanks, animal feeding pans, areca nut containers, water plant pots, container lids, trash containers, ant-guards, coconut shells, knife containers, and cement mixing containers (Table 2).

In all sub-districts, the numbers of outdoor, artificial, dark coloured and without lid containers seen were more than

indoor, natural, light coloured, and with lid containers, respectively (Table 3).

Container numbers and mosquito larvae

In all sub-districts, mosquito larvae numbers increased with increasing of container numbers (Khaokaew: $r^2 = 0.23$, $N = 120$, $P < 0.05$; Lansaka: $r^2 = 0.30$, $N = 120$, $P < 0.005$; Thadi: $r^2 = 0.32$, $N = 120$, $P < 0.001$; Kamlon: $r^2 = 0.35$, $N = 120$, $P < 0.001$; Khunthale: $r^2 = 0.29$, $N = 120$, $P < 0.005$).

DISCUSSION

In the wet season, the number of *Ae. albopictus* larvae was higher than *Ae. aegypti* in all sub-districts. We found higher numbers of *Ae. albopictus* larvae in outdoor, light coloured and without lid containers than indoor, dark coloured and with lid containers, respectively. In the dry season, *Ae. albopictus* numbers were higher in Thadi and Khunthale, whereas in Khaokaew, Lansaka, and Kamlon, *Ae. albopictus* and *Ae. aegypti* larvae numbers did not differ. On the other hand, *Ae. albopictus* numbers were not higher in Kamlon, Lansaka, and Khaokaew sub-districts. This could be because fewer numbers (<10%) of natural containers were found, compared to the other two sub-districts, which probably prohibited the numbers of *Ae. albopictus* larvae from being higher than the number of *Ae. aegypti* larvae. In addition, *Ae. albopictus* prefer to breed more in outdoor and natural containers, compared to indoor and artificial containers (Chen *et al.*, 2005; Wongkoon *et al.*, 2007b; Ferdousi *et al.*, 2015; Rozilawati *et al.*, 2015). However, few studies (Preechaporn *et al.*, 2007; Wongkoon *et al.*, 2007b) showed that *Ae. albopictus* larvae numbers were higher than *Ae. aegypti* larvae numbers in both the dry and wet seasons.

Ae. albopictus larvae numbers and larval indices were higher in the wet season than in the dry season in most sub-districts. Similar results were observed in Northern and Southern Thailand, South India, China, and Bangladesh (Mogi *et al.*, 1988; Thavara *et al.*, 2001; Tewari *et al.*, 2004; Almeida *et al.*, 2005; Ahmed *et al.*, 2007; Vanwambeke *et*

Table 2. Different kinds of containers in the wet and dry seasons in the five sub-districts

In/outdoor Sub-districts	Containers	Wet season										Dry season										
		Khaokaew		Lansaka		Thadi		Kamlon		Khunthale		Khaokaew		Lansaka		Thadi		Kamlon		Khunthale		
		T	P	T	P	T	P	T	P	T	P	T	P	T	P	T	P	T	P	T	P	
Indoor	EJ	10	2	6	0	13	6	14	4	7	0	17	4	33	6	35	6	30	0	48	6	
	CT	41	8	41	10	36	26	27	10	25	16	37	20	24	4	20	4	30	12	27	6	
	PB	11	2	14	0	23	6	14	2	19	6	7	6	5	0	2	0	2	0	6	0	
	PT	92	6	49	8	87	8	88	16	116	20	53	12	65	4	56	0	53	8	51	10	
	AFP	0	0	0	0	1	0	0	0	0	0	0	0	3	0	3	0	4	0	6	2	
	AG	22	2	20	0	36	0	32	10	12	8	20	0	14	0	4	0	29	0	7	0	
	RD	37	8	30	10	41	8	36	10	54	8	12	0	13	6	9	2	15	0	0	0	
	Outdoor	EJ	76	36	97	10	104	50	67	24	35	14	62	30	72	12	60	16	42	8	55	8
		CT	35	6	49	0	50	16	48	6	28	20	39	8	20	3	18	9	29	11	30	6
		PB	16	10	32	12	68	32	21	13	23	18	16	8	3	3	3	3	7	7	2	2
PT		33	18	34	20	84	42	40	40	36	34	28	10	28	23	34	18	30	28	37	6	
AFP		2	2	0	0	0	0	1	1	1	1	2	2	1	1	0	0	0	0	2	2	
ANC		5	4	3	2	1	0	10	8	1	1	1	0	2	0	18	18	2	2	1	1	
WPP		125	14	115	20	324	26	328	14	326	14	70	0	6	2	35	6	88	2	20	4	
CL		8	6	5	5	19	18	43	14	15	6	2	2	0	0	8	6	2	0	5	1	
TC		18	11	18	18	71	44	44	26	51	28	61	26	22	10	62	16	29	5	60	17	
AG		1	1	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	
CS	22	2	2	2	161	34	26	26	49	15	29	5	9	0	46	0	4	4	41	0		
RTKSC	5	2	5	5	9	6	2	0	2	2	0	0	0	0	0	0	0	0	0	0		
CMC	7	0	2	2	2	2	1	0	1	1	1	1	0	0	0	0	1	1	1	1		

T = total containers, P = positive containers. EJ = earthen jars, CT = cement tanks, PB = plastic buckets, PT = plastic tanks, AFP = animal feeding pans, AG = anti-guards, RD = refrigerator drainages, ANC = areca nut containers, WPP = water plant pots, CL = container lids, TC = trash containers, CS = coconut shells, RTKSC = rubber tapping knife sharpening containers, and CMC = cement mixing containers.

Table 3. Positive and total containers in the wet and dry seasons in the five sub-districts

Season	Sub-district	Indoor	Outdoor	% of outdoor	Artificial	Natural	% of natural	Dark	Light	% of light	With lids	Without lids	% of without lids
Positive containers													
Wet	Khaokaew	28	112	80	134	6	9	116	24	17	22	118	89
	Lansaka	28	96	77	122	2	2	106	18	14	6	118	95
	Thadi	54	270	83	280	44	13	262	62	19	32	292	90
	Kamlon	52	172	77	198	26	11	164	60	27	18	206	92
	Khunthale	58	156	73	200	14	6	174	40	19	2	212	99
Dry	Khaokaew	42	92	69	130	4	3	98	36	27	14	120	89
	Lansaka	20	54	73	74	0	0	52	22	30	4	70	95
	Thadi	12	92	88	104	0	0	72	32	31	4	100	96
	Kamlon	20	68	23	84	4	5	72	16	18	6	82	93
	Khunthale	24	48	67	72	0	0	34	38	53	2	70	97
Total containers													
Wet	Khaokaew	213	353	62	544	22	4	485	81	14	70	496	88
	Lansaka	160	362	69	520	2	0.4	442	80	15	82	440	84
	Thadi	237	893	79	969	161	14	983	147	13	79	1051	93
	Kamlon	211	631	75	816	26	3	722	120	14	68	774	92
	Khunthale	233	570	71	754	49	6	655	148	18	70	733	91
Dry	Khaokaew	146	311	68	428	29	6	317	140	31	62	395	86
	Lansaka	157	163	51	311	9	3	229	91	28	74	246	77
	Thadi	129	284	69	367	46	11	322	91	22	62	351	85
	Kamlon	163	234	59	393	4	1	329	68	17	52	345	87
	Khunthale	145	254	64	358	41	10	252	147	37	47	352	88

al., 2007; Wongkoon *et al.*, 2007a). Rozilawati *et al.* (2007) observed that the average egg numbers of *Ae. albopictus* were higher in the wet season than in the dry season in Penang, Malaysia. In southern Thailand, during the rainy season, residents prefer to use rain and well-water for cooking, bathing, and drinking purposes and, for these reasons, they collect water in various types of containers, both inside and outside of their houses (Wongkoon *et al.*, 2007b). In this study, we observed that the total numbers of containers and positive containers were higher in the wet season than in the dry season. A possible explanation for this could be that, during the wet season, rain water can get into outdoor containers easily; however, this is not the case in the dry season. This suggests that coconut shells and used tyres around houses would become suitable breeding sites for *Ae. albopictus* in the wet season, compared to the dry season.

Ae. aegypti larvae numbers and larval indices were higher in the dry season than in the wet season in most sub-districts. Similar results were observed in American Samoa, Argentina, and rice paddy areas in Southern Thailand (Lambdin *et al.*, 2009; Micieli & Campos, 2003; Preechaporn *et al.*, 2007). There are four possible explanations for this. First, *Ae. aegypti* ovipositioning activity increases during the autumn and the summer (the dry season) if the relative humidity becomes 60% or higher (Micieli and Campos, 2003). In Lansaka district, the average humidity was more than 80% in 2015 (data collected by CoE, Walailak University). Second, as the number of water storage containers were fewer in the dry season than in the wet season, *Ae. aegypti* females had less choice in where to lay eggs; therefore, they laid eggs in water containers that they found during the dry season. Third, due to water shortage in the dry season, local villagers store water for a long time in the same containers without cleaning them; as a result, *Ae. aegypti* larvae would have a higher chance of remaining in the water containers for longer in the dry season, compared to in the wet season. Fourth, in Southern Thailand, the average temperature in the dry season is 28.2°C. High temperatures would shorten the larval and pupal stages of

mosquitoes, and produce smaller females, which take and digest blood more often, produce more offspring, and transmit diseases more frequently (Githeko *et al.*, 2000; Promprou *et al.*, 2005; Kiarie-Makara *et al.*, 2015).

We observed that the habits of people were different among the five sub-districts. Based on HI, 85% of houses in the wet season and 42% of houses in the dry season in Thadi sub-district were *Aedes* positive. People in Thadi sub-district had the more dengue risk than other sub-districts due to several reasons. First, there were more positive trash containers and coconut shells around the houses in Thadi, compared to other sub-districts. The Thadi Administrative Organisation does not provide trash collection services. Thadi people throw their trashes outside their houses and burn the resulting pile once every two weeks. Because of this habit, many trashes such as used cans, styrofoam cups, plastic boxes, broken jars, and plastic cups appear in their trash piles. Additionally, in the wet season, these trash piles can be too wet to burn, so may remain as they are for many weeks before being burnt. Thadi people raise free-range chicken and feed them with coconut meat inside the coconut shells. Once the chickens finish eating the meat, the shells are left unattended around houses and become a key breeding site for mosquitoes. Second, Thadi people have the habit of storing water in high numbers of water storing containers (e.g., earthen jars, plastic buckets, and tanks) in and around their houses, due to a lack of a reliable water supply. Third, Thadi sub-district contains much of the areca plantation land for this province. During the dry season, Thadi people preserve areca nuts in earthen containers. We found higher numbers of areca nut container (18 areca nut containers) in Thadi than in other sub-districts, and all areca nut containers were positive for higher number of mosquito larvae (some being as high as 100 larvae per container). These areca nuts deposited a large amount of organic matter in the water, providing nutrients for mosquito larvae to feed on.

In the case of indoor and outdoor containers, *Ae. albopictus* larvae numbers were higher in outdoor containers than in indoor containers, and *Ae. aegypti* larvae numbers were higher in indoor containers than in outdoor containers, in both seasons. We observed that outdoor containers were higher in number than indoor containers were. Similar results were observed by Wongkoon *et al.* (2007b) in Nakhon Si Thammarat. Higher numbers of outdoor containers could be related to higher numbers of *Ae. albopictus* larvae, as this mosquito species prefers to breed more in outdoor containers than in indoor containers (Preechaporn *et al.*, 2006; Lim *et al.*, 2010; Norzahira *et al.*, 2011; Rozilawati *et al.*, 2015). Outdoor water containers are the preferred breeding sites for *Ae. albopictus* because of the greater amounts of organic debris found in them, which are important for larval development (Rattanarithikul & Panthusiri, 1994; Dom *et al.*, 2013). On the other hand, *Ae. aegypti* prefers to breed more in indoor containers than in outdoor containers (Romero-Vivas & Falconar, 2005; Chen *et al.*, 2006; El-Badry & Al-Ali, 2010), as it is highly anthropophilic and frequently bites, and thrives in close proximity to humans (WHO, 2009).

In the case of natural and artificial containers, *Aedes* larvae numbers did not differ between natural or artificial containers in either season but, in the case of *Ae. albopictus*, the interaction of season and container type showed some effects. This indicates that *Ae. albopictus* larvae numbers between natural and artificial containers showed opposite trends between seasons (i.e., higher in artificial containers in the wet season, but lower in artificial containers in the dry season). Our results clearly demonstrate the effects of socio-cultural practices on the breeding sites of *Ae. albopictus*. In the dry season, local villagers usually burn their trash piles outside of their houses, but in the wet season, the trash piles gets wet and cannot be burnt easily. These trashes (e.g., used cans, styrofoam cups, plastic boxes, broken jars, plastic cups, etc.) can contain rain water and become key breeding sites for *Ae. albopictus*. On the

other hand, in the dry season, fewer potential outdoor breeding sites are available for *Ae. albopictus* due to the trash piles being burnt.

Interestingly, *Ae. albopictus* larvae numbers were higher in natural containers in the dry season, but lower in the wet season. In the wet season, due to frequent and heavy rainfall, water in shallow natural containers (i.e. coconut shells) can be changed daily, and mosquito larvae can be flooded out of the coconut shells during heavy rains. However, in the dry season, coconut shells contain water for a longer period, with a high organic content from the coconut meat, and mosquito larvae have a higher chance of remaining and completing their larval development. This might explain why *Ae. albopictus* larvae were more often found in natural containers in the dry season, compared to the wet season.

In the case of dark and light coloured containers, in the wet season, the *Aedes* larvae numbers did not differ between dark or light coloured containers; however, in the dry season, larvae numbers were higher in light coloured containers than in dark coloured containers. Previous studies (Chua *et al.*, 2004; Wongkoon *et al.*, 2007b) observed that *Aedes* spp. preferred dark-coloured containers more than light coloured containers for breeding.

In the case of lid and without lid containers, *Ae. aegypti* larvae numbers did not differ between containers with or without lids in either season. However, *Ae. albopictus* larval numbers did not differ between containers with or without lids in the wet season, but *Aedes* larvae numbers were higher in containers without lids than in containers with lids in the dry season. In Lansaka district, the number of containers without lids (both total and positive containers) was more than the number of containers with lids. Similar results were observed by Phuanukoannon *et al.* (2005), Wongkoon *et al.* (2007b), Wong *et al.* (2011), Getachew *et al.* (2015), and Vannavong *et al.* (2017); *Aedes* females prefer to lay eggs in containers without lids than in containers with lids. In addition, in Wongkoon *et al.* (2007b), lids may prevent the falling of leaf litter, insects, and other organic materials

inside water containers and, therefore, these containers contain fewer nutrients than containers without lids. Higher nutrients in containers without lids may attract ovipositioning females more than containers with lids.

We observed that mosquito larvae numbers increased with increasing of container numbers in all sub-districts. To our knowledge, no study has shown the direct relationship between container numbers and mosquito larvae numbers. Snow & Medlock (2006) suggested that increasing numbers of water butts (containers used to collect rainwater) might increase the numbers of mosquitoes that breed in them. The results of our study show that number of containers also has an effect on the number of mosquito larvae, along with container types and seasons.

Based on our findings, in Lansaka district, *Ae. aegypti* breeds more in the dry season than in the wet season, and prefers to breed in indoor and light coloured containers. On the other hand, *Ae. albopictus* breeds more in the wet season than in the dry season, and prefers to breed in outdoor, light coloured, and without lid containers. Mosquito larvae numbers increased with increasing of container numbers. The differences in *Aedes* larval numbers between seasons and among sub-districts occurred due to the use of different types of water containers by local people from different sub-districts in Lansaka district. Local people in Lansaka district should take initiatives to prevent DHF in both the dry and wet seasons by reducing the number of indoor/outdoor, light coloured, and without lid containers.

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