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Species diversity and distribution of mosquito vectors in coastal habitats of Samut Songkhram province, Thailand

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Abstract. This study assessed species diversity and distribution of mosquito vectors in coastal habitats dividing it to three areas according to the distance from the sea. We also described a comprehensive analysis on factors associated with natural habitat preference of mosquito larvae in the Samut Songkhram province, Thailand. Adult and larval mosquito survey was conducted using black light traps and standard mosquito dipper during rainy season between October to November 2015. A total of 1,764 mosquitoes belonging to 3 genera with 5 species were captured. The most abundant species was *Anopheles epiroticus* accounting for 37.13% followed by *Culex sitiens* (34.92%), *Cx. quinquefasciatus* (27.66%), *Aedes aegypti* (0.23%) and *Cx. gelidus* (0.06%) respectively. *Cx. sitiens* and *An. epiroticus* larvae were collected from available breeding habitats. Highest density of mosquito larvae (603 larvae) was found at 2 kilometers distance from the sea whereas at 4 kilometers distance from the sea found the least of larvae numbers (331)s. Pearson correlation showed association the factors associated with breeding habitat preference of mosquito larvae. *Cx. sitiens* larvae were significantly higher in permanent, temporary water resources ($p<0.01$) and mangrove trees ($p<0.05$) than those of *An. epiroticus*. *An. epiroticus* larvae that significantly prefer water with green algae ($p<0.05$). These results provided important information with regards to mosquito vectors in coastal habitats of Samut Songkhram province, Thailand. It also provided information on species diversity, distribution and factors associated with breeding habitat, preference for surveillance and control to prevent the spread of mosquito-borne diseases to the population of the coastal communities. These findings fulfil knowledge of mosquito ecology and support mosquito control strategies that can be applied in coastal areas of Thailand in the future.

INTRODUCTION

Mosquito is the most important insect that transmit vector-borne diseases (Gubler, 1998). Mosquito-borne diseases are important medical problems and are responsible for about 1.4 million deaths per year and 17.0% of all infectious diseases worldwide (Rueda, 2008). For instance dengue hemorrhagic fever, chikungunya, malaria and filariasis are serious mosquito-borne diseases in many tropical and subtropical areas (Jude *et al.*, 2012). Each mosquito species has many different behaviors such as biting behavior, feeding behavior, resting behavior (Tainchum *et al.*, 2015). Species diversity of mosquito vectors is a crucial role for transmission of diseases (Tainchum *et al.*, 2014).

Thailand is one of countries with problems of mosquito-borne diseases (Brusich *et al.*, 2015). Samut Songkhram is the smallest coastal province in Thailand and there are a lot of migrant workers who play an important role in the transmission of mosquito-borne diseases. According to the Bureau of Epidemiology, Samut Songkhram reported the highest number of cases of mosquito-borne diseases. In 2014, the most

infectious disease was dengue hemorrhagic fever, there were 507 cases of DHF or morbidity rate of 101.58 per 100,000 populations. Second and third were malaria and filariasis respectively with few patients. Thus, the population has a high risk of getting infected with mosquito-borne diseases in the province.

Species diversity and distribution of mosquito vector in the areas are important information to develop vector monitoring and control strategies, which depends on mosquito species present in each state for effective implementation (Rueda, 2008). There is currently a lack of information regarding the mosquito vectors in Samut Songkhram province especially the coastal habitats, where ecology differs from other areas.

Therefore in this study, we assessed species diversity and distribution of mosquito vectors in coastal habitats dividing it to three areas according to the distance from the sea of Samut Songkhram province. These information will be useful for planning a high efficient surveillance and control of mosquito-borne diseases in each coastal area. We also described a comprehensive analysis on factors associated with natural habitat preference of mosquito larvae in Samut Songkhram provinces. The mosquito species interactions are ecology of breeding habitat related to mosquito surveillance are also targeted by mosquito control measures involving improve sanitation to decrease numbers of mosquito vector (Rochlin *et al.*, 2009).

MATERIALS AND METHODS

Study areas

We classified study sites into 3 zones according to a distance from the sea (Figure 1). Firstly, 200 meters away from the sea (13°23'31.57"N 100°1'59.36"E) where there are no people in the areas. This area covered with mangrove forests and sea salt farms. Secondly, 2 kilometres away from the sea (13°25'11.82" N 100° 2'19.64"E) where there are some communities. This area is almost covered by sea salt farms and abandoned farms. Lastly, 4 kilometers away from the sea (13°24'32.52"N 100° 0'41.40"E) where there are crowded people in particular part of the area.

Figure 1. Study areas (A: 200 meters away from the sea, B: 2 kilometers away from the sea, and C: 4 kilometers away from the sea).

Mosquito collection
Mosquito collection was conducted to collect adult and immature stage of mosquitoes during rainy season in October to November 2015. The collection was done once a week for the two-month period in 3 study areas.

Adult mosquito collection
Adult mosquitoes were collected in all coastal habitats between 6:00 PM and 6:00 AM by black light traps (IS-003, P.M.C. industrial company, Thailand). A total of 12 traps were set (4 traps per area) for 3 study areas. Mosquito samples were collected from the trap in the morning. Consequently, they were recorded date, time and place. All collected mosquitoes were morphological identification in a laboratory by available keys.

Larval mosquito collection
Larval mosquito samples were collected in 4 habitats presented in each study site. To collect the sample, we used a standard mosquito dipper (Mwangangi *et al.*, 2010) that 20 dips were taken from available breeding places in each study site. All mosquito larval samples were collected into plastic bottles. They were counted, labeled collection date, time and place. Physical and biological factors of their breeding habitats were recorded including water quality (salinity, pH and temperature), vegetation around their habitats to characterize mosquito breeding places. Water quality was measured in the field using portable water quality meter (Lutron WA-2017SD, Multi parameter Display System, Taiwan).

Mosquito identification
All mosquito samples were transported to the laboratory at college of Allied Health Sciences, Suan Sunandha University. Collected larval and adult mosquitoes were identified to genus or species by microscopic examination of morphological characters using established taxonomic keys; Illustrated keys to the mosquitoes of Thailand (Rattanarithikul, Harrison, Panthusiri & Coleman, 2005).

Data Analysis
Collected mosquito samples derived from each collection site were measured number, percentage and mean to estimate mosquito fauna and distribution. Pearson correlation was used to determine the association between factors of breeding habitats and species of mosquito larvae. Species diversity of mosquito was calculated by Shannon-Weiner diversity index (Gordon, McMahon, Finlayson, Gippel, & Nathan, 1993).

$$H = \sum_{i=1}^{s} -(P_i)(\ln P_i)$$

Substitute;
H is species diversity index
s is number of species
pi is proportion of all species in samples

RESULTS

Species diversity and distribution of mosquito vector

Adult mosquito surveys
A total of 1,764 mosquitoes belonging to 3 genera within 5 species were collected by black light trap between October and November 2015 in the coastal habitats of Samut Songkhram province. The most abundance species was *An. epiroticus* accounting for 37.13% (n = 655). Other collected mosquito species were *Cx. sitiens* with 34.92% (n = 616), *Cx. quinquefasciatus* with 27.66% (n = 488), *Ae. aegypti* with 0.23% (n = 4) and *Cx. gelidus* with 0.06% (n = 1) respectively (Figure 2).

Species diversity and distribution of mosquitoes in each coastal habitat according to the distance from the sea of Samut Songkhram province were shown in Table 1.

The highest number of mosquitoes was found in 4 kilometers far from the sea which species diversity index was 0.86. Five species of mosquitoes were found in this area; *Cx. quinquefasciatus* was predominant

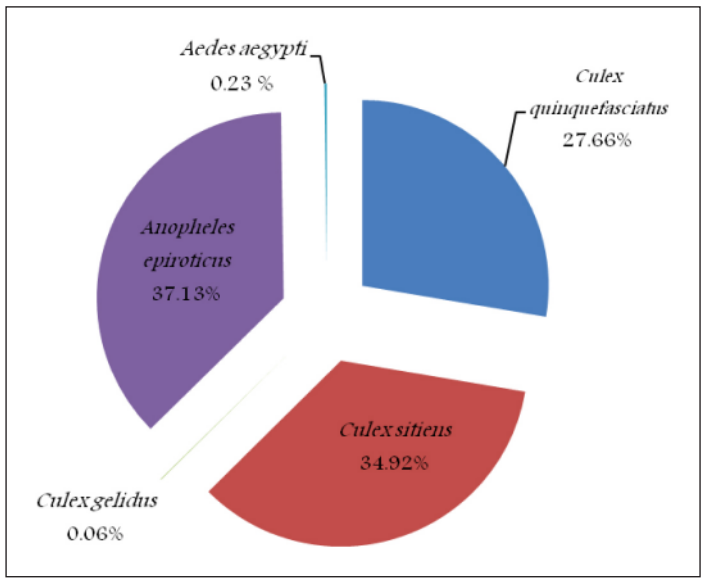

Figure 2. Percentage of mosquito species at the coastal habitats of Samut Songkhram province since October to November 2015.

Table 1. Species diversity and distribution of mosquito in the coastal habitat according to the distance from the sea

Species	Coastal habitats			Total
	Distance from the sea 200 meters	Distance from the sea 2 kilometers	Distance from the sea 4 kilometers	
Cx. quinquefasciatus	11 (2.19%)	57 (9.88%)	420 (61.40%)	488
Cx. sitiens	282 (56.06%)	295 (51.13%)	39 (5.70%)	616
Cx. gelidus	0	0	1 (0.15%)	1
An. epiroticus	210 (41.75%)	225 (38.99%)	220 (32.16%)	655
Ae. aegypti	0	0	4 (0.59%)	4
Total (%)	503 (100%)	577 (100%)	684 (100%)	1,764
Species diversity index	0.61	0.94	0.86	

species with 61.40% (420 individuals). The followed species found was *An. epiroticus* as 32.16% (n = 220), *Cx. sitiens* as 5.70% (n = 39), *Ae. aegypti* as 0.59% (n = 4) and *Cx. gelidus* as 0.15% (n = 1) respectively. At 200 meters distance from the sea, 3 species were present; *Cx. Sitiens* with 56.06% (n = 282), *An. epiroticus* with 41.75% (n = 210) and *Cx. quinquefasciatus* with 2.19% (n = 11). Species diversity index was 0.61. We also found 3 species of mosquitoes at 200 meters distance from the sea and species diversity index was 0.94. According to the result, we found that *Cx. sitiens* was more specific to the seashore (200 meters and 2 kilometers away from the sea) than *Cx. quinquefasciatus* (4 kilometers away from the sea).

Larval mosquito surveys

We surveyed mosquito larvae from four available breeding places in each coastal habitat. A difference in biological and physical factors was showed in Table 2.

We found 2 larval species in coastal habitat including *Cx. sitiens* and *An. epiroticus* as shown in Table 3. The breeding habitat where found the highest number of

Table 2. Characters on biological and physical factors of larval collection place in coastal habitat according to the distance from the sea

Coastal habitats	Larval collection places	Biological Factors			Physical Factors			
		PH	SALT (ppt)	TEM (°C)	Type of water resources		Type of plants	
					Permanent of water resources	Temporary of water resources	Mangrove trees	Green algae
Distance from sea 200 meters	1	5.82±0.11	33±2	31.8±2.5	No	Yes	No	No
	2	6.43±0.15	33±2	31.5±2.9	No	Yes	No	No
	3	5.7±0.09	30±3	32.6±3.1	Yes	No	No	Yes
	4	5.25±0.12	34±2	32.1±2.5	Yes	No	Yes	No
Distance from sea 2 kilometers	1	6.14±0.14	18±2	34.4±3.2	No	Yes	No	Yes
	2	5.93±0.6	30±2	32.6±2.2	Yes	No	Yes	No
	3	6.25±0.8	26±3	34.5±3.2	No	Yes	No	Yes
	4	6.48±0.11	15±3	35.5±3.8	No	Yes	No	Yes
Distance from sea 4 kilometers	1	5.39±0.13	8±3	31.4±3.6	No	Yes	No	Yes
	2	5.25±0.12	18±3	32.4±2.1	Yes	No	Yes	No
	3	5.31±0.11	18±3	31.4±2.5	No	No	No	No
	4	5.5±0.06	32±2	33.1±2.4	Yes	No	Yes	No

Yes = There is/are above category/categories in the larvae collection place, No = There is no any above category in the larval collection place.

Table 3. Numbers of mosquito larvae in the coastal habitat according to the distance from the sea

Coastal habitat	Larvae collection place	Species	
		Cx. sitiens	*An. epiroticus*
Distance from the sea 200 meters	1	159 (34.94%)	0
	2	280 (61.54%)	0
	3	8 (1.76%)	62 (95.38%)
	4	8 (1.76%)	3 (4.62%)
	Total (%)	455 (100%)	65 (100%)
Distance from the sea 2 kilometers	1	180 (33.64%)	41 (60.29%)
	2	18 (3.36%)	2 (2.94%)
	3	305 (57.01%)	22 (32.35%)
	4	32 (5.99%)	3 (4.42%)
	Total (%)	535 (100%)	68 (100%)
Distance from the sea 4 kilometers	1	17 (6.99%)	58 (65.91%)
	2	8 (3.29%)	28 (31.81%)
	3	216 (88.89%)	1 (1.14%)
	4	2 (0.83%)	1 (1.14%)
	Total (%)	243 (100%)	88 (100%)

larvae population was the 2 kilometers distance from the sea as 603 individuals including 535 of *Cx. sitiens* and 68 of *An. epiroticus*. The breeding habitat where found the secondary most of larvae population was the distance from the sea 200 meters with 520 individuals identified to 455 *Cx. sitiens* and 65 *An. epiroticus*. The distance from the sea 4 kilometers found the least of larvae numbers when compare to the other areas. There were 331 larvae including 243 *Cx. sitiens* and 88 *An. epiroticus*.

Table 4. Factors associated with habitat preference of mosquito larvae in coastal areas

Species		PH	SALT (ppt)	TEM (°C)	Permanent	Temporary	Mangrove trees	Green algae
Cx. sitiens	Pearson Correlation	.499	.038	.501	-.708**	.708**	-.591*	.043
	Sig. (2-tailed)	.098	.761	.311	.010	.010	.043	.895
An. epiroticus	Pearson Correlation	-.159	-.457	-.563	.029	-.029	-.311	.703*
	Sig. (2-tailed)	.621	.135	.245	.928	.928	.326	.011

* = Correlation is significant at the 0.05 level (2-tailed).
** = Correlation is significant at the 0.01 level (2-tailed).

Factors associated with habitat preference of mosquito larvae in coastal habitats

Pearson correlation analysis showed association on factors associated with habitat preference of mosquito larvae. The relation between factors and larval mosquito vector, *Cx. sitiens* larvae were significantly higher with permanent, temporary of water resources (p<0.01) and mangrove trees in breeding places (p<0.05). *An. epiroticus* larvae were significantly with green algae (p<0.05) (Table 4).

DISCUSSION

This study relates to species diversity and distribution of mosquito vectors in coastal habitat. The diversity index showed little difference (0.61 – 0.94) according to the difference of each habitat. From a distance of 200 meters and 2 kilometers from the sea, we found 3 species namely *An. epiroticus*, *Cx. sitiens* and *Cx. Quinquefasciatus*. The predominant mosquitoes was *Cx. sitiens*. In coastal habitat, at a distance of 4 kilometers from the sea, we found 5 species of mosquitoes; namely *An. epiroticus*, *Cx. sitiens*, *Cx. quinquefasciatus*, *Ae. aegypti and Cx. gelidus* of which *Cx. quinquefasciatus* was highest recorded. This result showed difference in numbers of mosquito species in relation to the distance of the coastal areas because mosquitoes have diverse habitats to colonize different kinds of environments (Rueda, 2008). In a habitat that is 200 meters and 2 kilometers from the sea, there are many salt farms that lead to increasing salinity of groundwater. The environmental characters of these coastal habitats are brackish and saline waters that suitable for breeding of *Cx. Sitiens* and *An. epiroticus* due to their capacity to breed in brackish water (Harinasuta *et al*, 1974). Only few numbers of *Cx. quinquefasciatus* were involved in households and occur in low numbers as their breeding habitat is household waste water (Rattanarithikul *et al.*, 2005: Rueda, 2008). The residential areas and household density are important factors influencing the increase numbers of some species of mosquito. The habitat of mosquitoes 4 kilometers from the sea to the residential areas was found high number of *Cx. quinquefasciatus*. Other species found in this area includes *Cx. gelidus* which is found in flooding or in fresh water resource areas and *Ae. aegypti* which lives around human habitations (Rodrigues *et al.*, 2015). However, mosquitoes in this study were collected at night time (6:00 PM to 6:00 AM), but *Aedes* spp. is active and bite during the daytime (Rodrigues *et al.*, 2015). Therefore, *Ae. aegypti* cannot be represented in this study. *An. epiroticus* was equally found in coastal areas. There is a report that shows that *An. epiroticus* could live in brackish and salt water habitats (Linton *et al.*, 2005).

The results of larvae survey in the natural water resource at coastal habitats found 2 species; *Cx. sitiens* and *An. epiroticus*. The number of *Cx. sitiens* larvae was higher than *An. epiroticus* in all areas because *Culex* larvae has higher numbers of eggs, grows quickly, and has strength and adaptive survivor abilities (Prummongkol *et al.*, 2012) than those *of Anopheles* larvae. Most of larvae

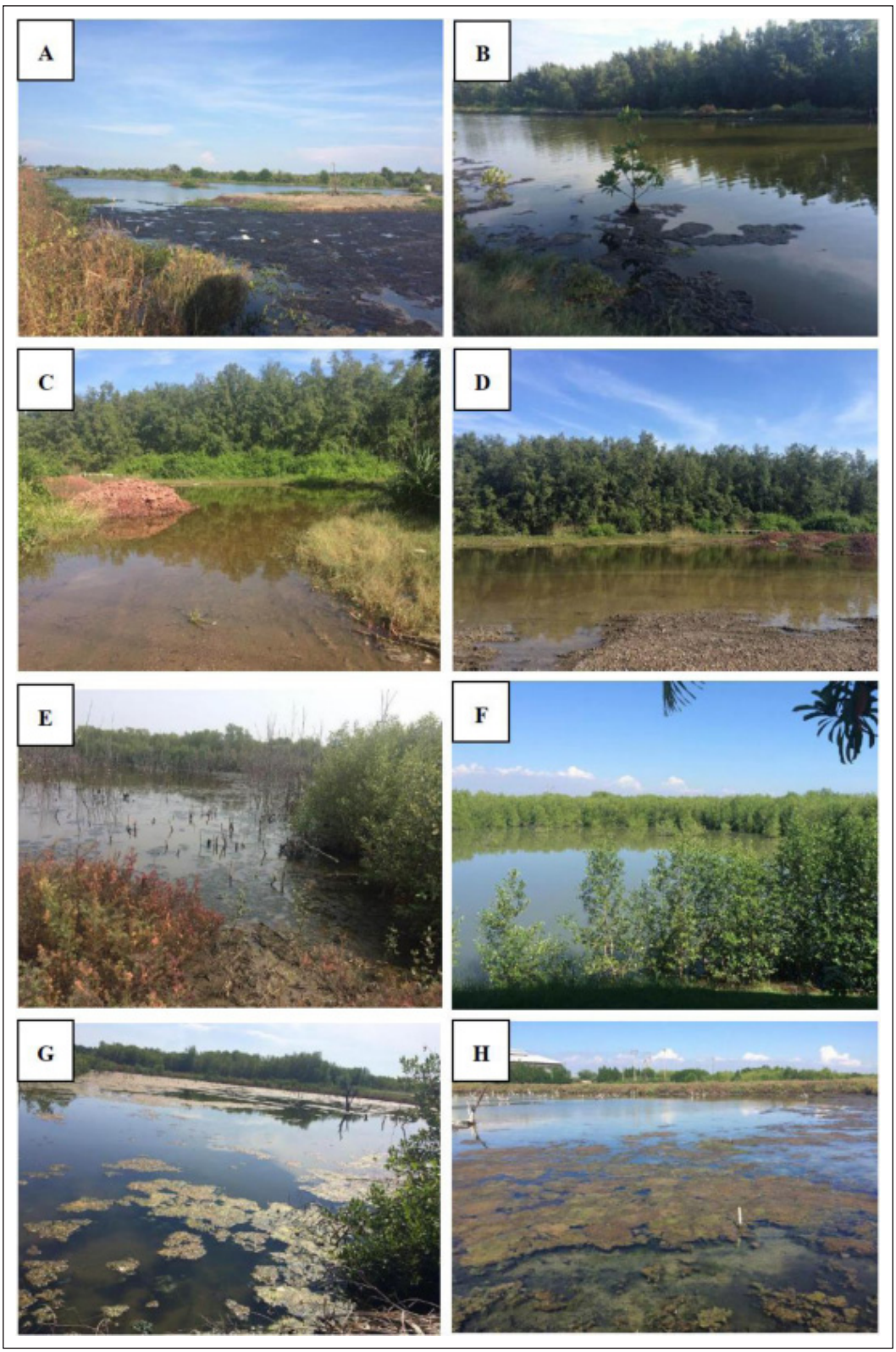

Figure 3. Physical factors in natural coastal habitats, A and B: permanent habitats, C and D: temporary habitats, E and F: mangrove trees in habitats, G and H: floating green algae in habitats.

collection places found both species except for those from 200 meters distance from the sea, we did not find any *An. epiroticus* larvae in 1st and 2nd collection places because these places are water puddles caused by tidal current which is not suitable for their breeding.

Factors of larval habitats measured in this study were pH, salinity, water temperature, permanent and temporary water resources, mangroves and floating green algae. *Cx. sitiens* larvae have relation with mangrove, permanent and temporary water resources. These results were consistent to Prummongkol's researches which explain *Cx sitiens* breeding place is in big pounds or fresh, brackish and salty water resources nearby the coastal area and mangroves are common *Cx. Sitiens* habitats (Prummongkol *et al.*, 2012). *An. epiroticus* larvae have relation with floating green algae that are a shelter or a food resource for survival (Sumruayphol *et al.*, 2010).

This study was conducted to evaluate mosquito vectors in the coastal areas of Samut Songkhram province. Our surveys found a lot of *An. epiroticus* that should be monitored because they transmit malaria to human (Linton *et al.*, 2005). Numbers of malaria cases in Samut Songkhram province in 2014 (Bureau of Epidemiology) reported 6 cases, thus malaria can occur in this area. Other findings inclued *Cx. sitiens* and *Cx. gelidus* which can transmit Japanese encephalitis and filariasis (Prummongkol *et al.*, 2012). However, no cases of Japanese encephalitis was reported but 7 cases of filariasis was reported during 5 years in Samut Songkhram province. Even though there are no any outbreaks event before in this area, it is very necessary for surveillance and control mosquito population within areas. Therefore, this research showed important information of mosquito vectors in coastal habitats of Samut Songkhram province, Thailand including species diversity, distribution and factors associated with habitat preference for surveillance and control to prevent mosquito-borne diseases spread in population of the coastal communities.

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