

ORIGINAL ARTICLE

THE EVALUATION OF THE *Aedes albopictus* ENTOMOLOGICAL INDEX BASED ON THE TOPOGRAPHY TO COMPUTE THE ESSENTIAL REQUIREMENTS FOR DENGUE TRANSMISSION SURVEILLANCE IN NAKHON SI THAMMARAT, THAILAND

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ABSTRACT

An entomological index is widely used for determining dengue transmission and mosquito populations. In this study, we determined the association between *Aedes albopictus* mosquito counts in three areas with different topographical features from six districts in Nakhon Si Thammarat and infected patients reported by the Ministry of Public Health's Department of Disease Control, using entomological indices, such as the sticky pad index and the sweep net index. The sampling region was selected from the top six districts of the province based on the number of reported dengue cases per 100,000 people. The regions were Khanom, Mueang, Pak Phanang, Phrom Khiri, Thung Song, and Thung Yai. They were divided into three categories of geographical features, including city, beach, and mountain. The mosquitoes were collected by walking in a star form inside a 5 m diameter circle. The mosquitoes were separated into groups based on their species, and the percentile of the index was computed. We found that the number of mosquitoes was the highest in Pak Phanang and lowest in Khanom. The two indices and the number of *A. albopictus* collected from the six sampling locations were not significantly different ($t = 1.31$, $p > 0.05$). The coastline region had the highest number of mosquitoes and was substantially different from the other two regions (cosmopolitan and mountain) ($F = 4.30$; $p < 0.05$). By performing Pearson's correlation analysis, we found a negative correlation between dengue cases and the entomological index ($r = -0.472$; $N = 36$). Similarly, the number of patients showed a negative correlation with relative humidity ($r = -0.355$) but a positive correlation with temperature ($r = 0.064$). The epidemic pattern shifted throughout the year, and the number of cases then increased 2-4 weeks after the insect was found.

Keywords: *Aedes*; dengue; entomological index; surveillance; sweep net; sticky pad

INTRODUCTION

The topography of, Nakhon Si Thammarat (NST) is classified into three categories, i.e., beach, hill, and city. The region is humid and hot and receives rain for eight months. Temperature and topography are ideal for the development and reproduction of plants and animals¹⁻³. Mosquitoes are abundant in the area and transmit various diseases that affect humans. The *Aedes* mosquitoes (*Aedes aegypti* or *Aedes albopictus*) transmit dengue fever and other vector-borne infections^{1, 2}. The number of mosquitoes in an area influences the intensity of a dengue epidemic, and the mosquito population is influenced by several environmental variables^{4,6}, such as the movement of humans, their location, and climatic conditions⁷⁻⁹. The number of people, houses, and mosquitoes has increased due to urbanization. Mosquitoes are drawn to stagnant water containers that serve as the breeding grounds of mosquitoes, and thus, the presence of these water containers promotes egg-laying behavior¹⁰⁻¹². The boundaries of mosquito development area, water bodies, rainfall, temperature, and humidity

are critical characteristics in every dengue-endemic ecosystem^{8, 9}. Since the habitat of each stage of the mosquito differs, which is employed in the primary survey approach, the prevalence of *Aedes* mosquitos may be investigated in a variety of ways.^{13, 14}

In this study, we determined which stage of the mosquito life cycle might be studied and what equipment should be used. In general, the procedure should be simple, cost-effective, and easy to apply¹⁵. Entomological indicators might be used for investigating mosquito epidemiology¹⁶. Laboratory testing is necessary for species identification, and epidemiological studies are conducted using four indices: the House (premise) index (HI), the Container index (CI), the Breteau index (BI), and the Trap index (TI)¹⁶. However, a larval survey can only provide information on contact frequency, and determining the number of larvae is not possible. Although human bait harvesting can be applied to conduct an adult mosquito study, at least three people are necessary

for the mosquito bite survey time^{13, 17}. Surveyors are also at danger of developing dengue fever due to prolonged contact with mosquitoes.^{1, 6, 8}. The number of components required to calculate the adult mosquito survey index might be reduced. In non-residential study areas, swing indexing and the sweep net index (Net index) are viable options¹⁸. Some studies that used adult mosquitoes as an entomological index applied the sticky trap technique. A black device is placed in a darkened region for a long time to identify the species^{19, 20}. However, determining the index value is time-consuming. For studying the mosquitoes in real-time, the number and kinds of species need to be monitored. The technique might be able to track mosquito-borne infections. Designing a study using available materials and then calculating the number of the mosquito per area is faster in the community.

For dengue surveillance, understanding the pattern of disease spread and its advancement across time and geographical boundaries is necessary. Surveillance is required for any dengue prevention and control program because it provides information that can be used for risk assessment, epidemic response, and program evaluation²¹. Data can be collected through surveillance using both passive and active techniques. To conduct surveillance effectively, depending on the circumstances, many data sources can be used, which can provide accurate epidemiological information on the risk of transmission^{9, 22}.

The entomological index related to the geographical characteristics of the studies published every year is extremely important. The index serves as a preventive guideline, helps to minimize disease spread, and determines the course of the outbreak. The Department of Disease Control documented nine fatalities caused by dengue fever in 2020, out of 10,000 that occurred across Thailand. The Nakhon Si Thammarat Province has the fourth highest number of dengue fever cases in the country, the highest number in the southern area, and the highest number in the prevalence of dengue fever patients^{23, 24}. When the trajectory of the incidence of dengue fever was examined, it was found to rise gradually and change with the topography and climate²⁵. In this study, we examined the distribution of dengue vector mosquitos in a specific region based on the relationship between the entomological index and geographical characteristics.

METHODS

Sampling area

The Nakhon Si Thammarat Province contains 23 districts. Instead of sampling the areas at random, we selected areas with a significant number of reported instances²³⁻²⁵. Thus, we performed purposeful sampling. We sampled areas with three

topographical features, including mountains, beaches, and towns, from six districts with the highest number of dengue cases per 100,000 people. These areas included Khanom (KN), Mueang Nakhon Si Thammarat (NST), Pak Phanang (PP), Phrom Khiri (PK), Thung Song (TS), and Thung Yai (TY).

The samples were collected from the west to east and from the north to south across the area of Nakhon Si Thammarat (NST) province. NST has an annual rainfall of about 6.12 ± 13.75 mm. The rainy season lasts from mid-May to mid-October, and floods occur from September to December. The average annual temperature and annual relative humidity are $27.51 \pm 1.09^\circ\text{C}$ and $86.04 \pm 3.80\%$, respectively. The Mueang district is a two-place community area consisting of a densely populated metropolitan area, and the university community of the Nakhon Si Thammarat Rajabhat University (NSTRU), which is surrounded by low hills. Thung Song has a public park with a plant breeding ground at the center, surrounded by roads. Pak Phanang is an apocalypse parallel to the coast, and the residents have a career of coconut groves that dig a trench parallel line inside the region. Phrom Khiri has plains with small valleys mixed with steep mountains; water is available throughout the year in this region. Khanom has narrow coastal plains, and the coastline is damaged in several places. Thung Yai is a plateau with rubber plantations.

Sampling method

Mosquito samples were collected once a month and twice a day, between 9:00 and 11:00 a.m. and 1:00 and 2:30 p.m. The sticky pad (square paper pad: 20 cm x 20 cm) method and the sweep net (30 cm in diameter) method were used to capture adult mosquitoes^{26, 27}. The data were collected by imagining a star-shaped walking route with five points and multiplying it by five to find the center of a circle (5 m in diameter) using a GPS (UTM) device. The mosquitoes were collected at three different sites by swinging the apparatus 60 cm over the ground. The mosquitoes were caught and recorded.

Species identification

Initially, spectroscopy was performed to classify mosquitos into physical groups based on comparable external characteristics. The entomological procedure was used to prepare mosquito samples, which were then identified by a taxonomy expert. Briefly, by affixing the label (a small triangular piece of paper measuring 1 cm x 1.4 cm) to the entomological needle, the mosquito specimen was retained at the tip of the paper to identify the species²². The two labeling signals located below were used to represent the data. To prevent the samples from being damaged by moisture and other

insects, the tips of the samples were painted with a drop of transparent nail polish and allowed to dry. Then, they were stored in an airtight container.

Determining the sticky pad index and the sweep net index.

The population relative density technique was used to determine the density of dengue-carrying mosquitoes. The population number obtained through sampling is a population index that can only be compared to the genuine population. The density per unit of any given unit is obtained by multiplying the number of mosquitoes caught per area investigated by the density per unit of any fixed unit. The formula is presented in Equation 1. Sampling was performed by walking along an imaginary star-shaped path with five points in the survey area, which was a circle with a diameter of 5 m^{28, 29}.

$$index = \left[\frac{\text{total number of mosquitoes caught}}{\text{total mosquito catching areas} \times 100} \right] \times 100$$

*Total mosquito catching area is $A = \pi r^2$, r = circle radius

Secondary data on dengue cases

Dengue case records were obtained from the Health Data Center (HDC) of the Nakhon Si Thammarat Provincial Public Health Office for five consecutive years (2016-2020)²⁵.

Statistical analysis

The R statistical analysis program was used to calculate the variance. The data were analyzed by performing dependent paired t-tests and one-way ANOVA. Finally, a multiple-comparison analysis was performed using Tukey's technique. All differences among and between groups were considered to be statistically significant at $p < 0.05$.

RESULTS

Distribution of mosquitoes

Mosquito samples were collected and mapped from six districts, covering three unique topographical zones, as shown in Figure 1.

The coastal areas and the PP district had the highest percentage of both indices, whereas other locations had relatively lower index values (Table 2).

Geography related to the mosquito number

We hypothesized that the distribution of dengue-carrying mosquitoes was similar across study sites. In three geographical zones, the number of

mosquitos in each of the six districts was compared. The average number of mosquitos in the sampling sites of Khanom (KN), Nakhon Si Thammarat (NST), Pak Panang (PP), Prom Khiri (PK), Thong Song (TS), and Thong Yai (TY) was 27.0, 24.8, 370.8, 34.4, 30.2, and 46.0, respectively (Table 3). We performed ANOVA and found that the F-value was 3.95 ($p = 0.009$). Our results indicated that the mean mosquito populations differed significantly in at least one pair of sites. Tukey's multiple comparisons test was conducted to further determine the differences in the mean number of mosquitoes. The number of mosquitoes in the PP region was significantly different from the other five areas; ($p = 0.019$ (PP-NST), 0.020 (PP-KN), 0.021 (PP-TS), 0.024 (PP-PK), and 0.031 (PP-TY)).

The data showed a normal distribution (Figures 2a and 2c). There were substantial differences in the assumptions at the 95% confidence intervals (Figure 2b), and the effect of discrepancies was within the allowed range (Figure 2d).

The average number of mosquitos in each region, i.e., city, coast, and mountain, was 20.7, 161.9, and 3.27, respectively. The results of the ANOVA showed that the average number of mosquitoes differed significantly between at least two regions ($F = 4.30$, $p = 0.020$). The results of Tukey's multiple comparisons test showed a significant difference between coast-city ($p = 0.046$) and coast-mountain ($p < 0.023$) (Table 4).

The entomological index linked to a dengue disease case

To determine the association between mosquitoes in the region and secondary data from dengue-infected patients recorded in the same year by the Department of Disease Control (2020), Pearson's correlation test was performed, and the relationship between dengue cases and the three factors was investigated. The occurrences were positively related to temperature ($r = 0.064$) and negatively related to relative humidity ($r = -0.355$) (Figure 3a). The mosquito population was significantly related to the prevalence of dengue fever ($p = 0.01$; (2-tailed)), although the correlation was negative ($r = -0.472$; $N = 36$) (Figure 3b). We elucidated the pandemic pattern by evaluating secondary data from cases that rose to index levels (2018-2020). Dengue fever follows a yearly pattern, where the frequency starts increasing in late April and peaks in July and August each year due to the rainy season that persists for the entire month (Figure 3b).

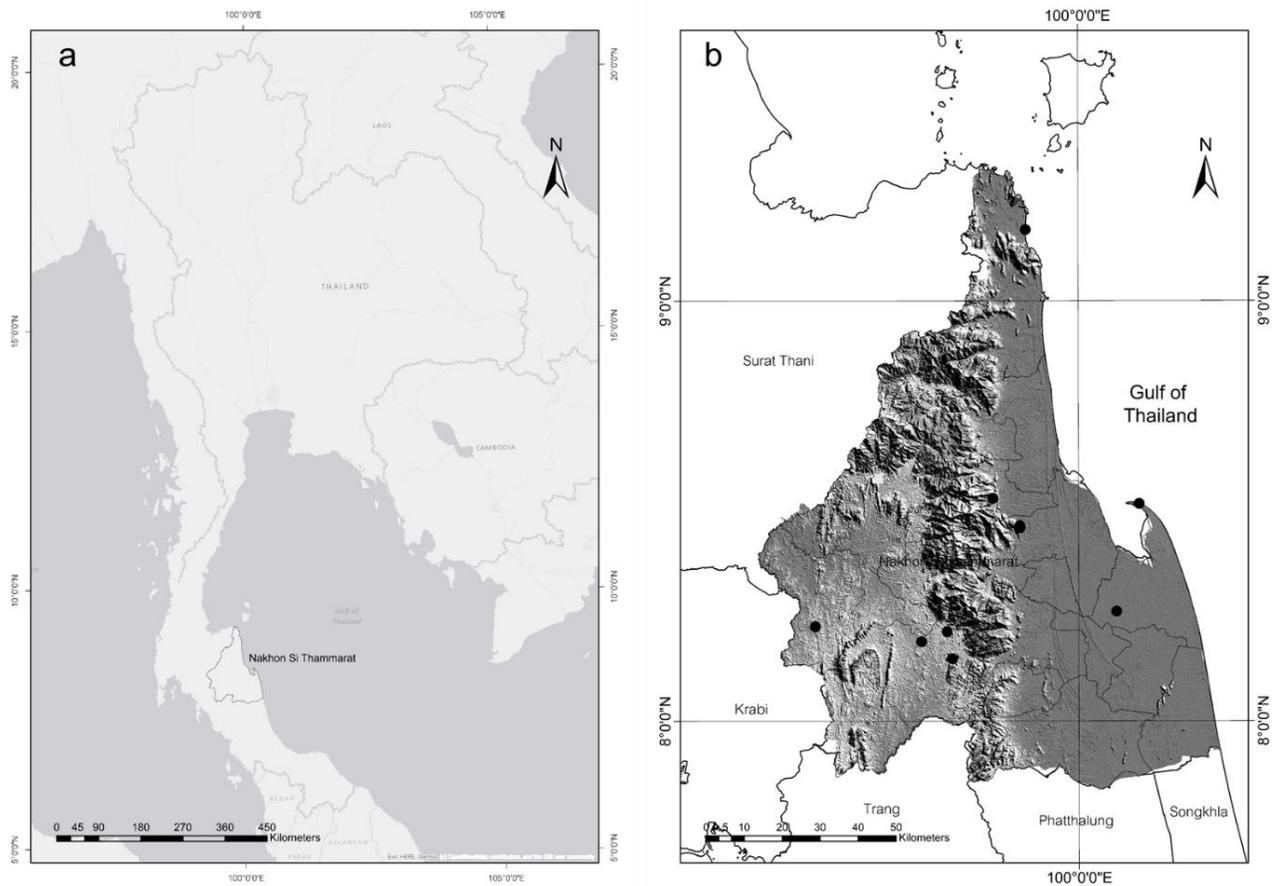


Figure 1: The sites from which mosquitos were collected in Nakhon Si Thammarat. The map (a) represents the map of NST with latitude and longitude. On map (b), black dots represent sampling sites and light gray lines represent district boundaries. The maps were created using ArcGIS (version 10.8.1).

Table 1: The relative density index was related to the studied regions (dependent paired t-tests; n = 6).

Location (district)	Method	
	Sticky pad (mean±SE)	Sweep net(mean±SE)
Khanom (KN)	0.82 ±5.33	1.48 ±6.34
Mueang-Nakhon Si Thammarat (NST)	1.63 ±8.33	3.16 ±1.53
Pak Phanang (PP)	33.06 ±183.33	61.38 ±72.97
Phrom Khiri (PK)	1.32 ±8.67	2.65 ±5.86
Thung Song (TS)	2.19 ±6.00	4.43 ±1.73
Thung Yai (TY)	1.99 ±13.00	3.67 ±7.00

Note: There was no significant difference at a p-value of 0.05.

Table 2: The percentage of both indices in different districts and geographical areas.

Location	Method	
	Sticky pad index (%)	Sweep net index (%)
District:		
Khanom (KN)	1.92	1.99
Mueang-Nakhon Si Thammarat (NST)	5.77	5.34
Geographical area:		
City	4.11	3.98
Coast	86.99	87.45
Mountain	8.89	8.57

Table 3: The average distribution of mosquitoes in an area (district) (n = 6). A one-way ANOVA was performed, followed by Tukey’s multiple comparisons test if the difference was significant (p < 0.05).

Location (District)	No. of mosquito (mean ±SE)
Khanom (KN)	27.0 ±7.00 ^b
Nakhon Si Thammarat (NST)	25.8 ±7.00 ^b
Pak Phanang (PP)	370.8 ±7.00 ^a
Phrom Khiri (PK)	34.4 ±7.00 ^b
Thung Song (TS)	30.2 ±7.00 ^b
Thung Yai (TY)	46.0 ±7.00 ^b

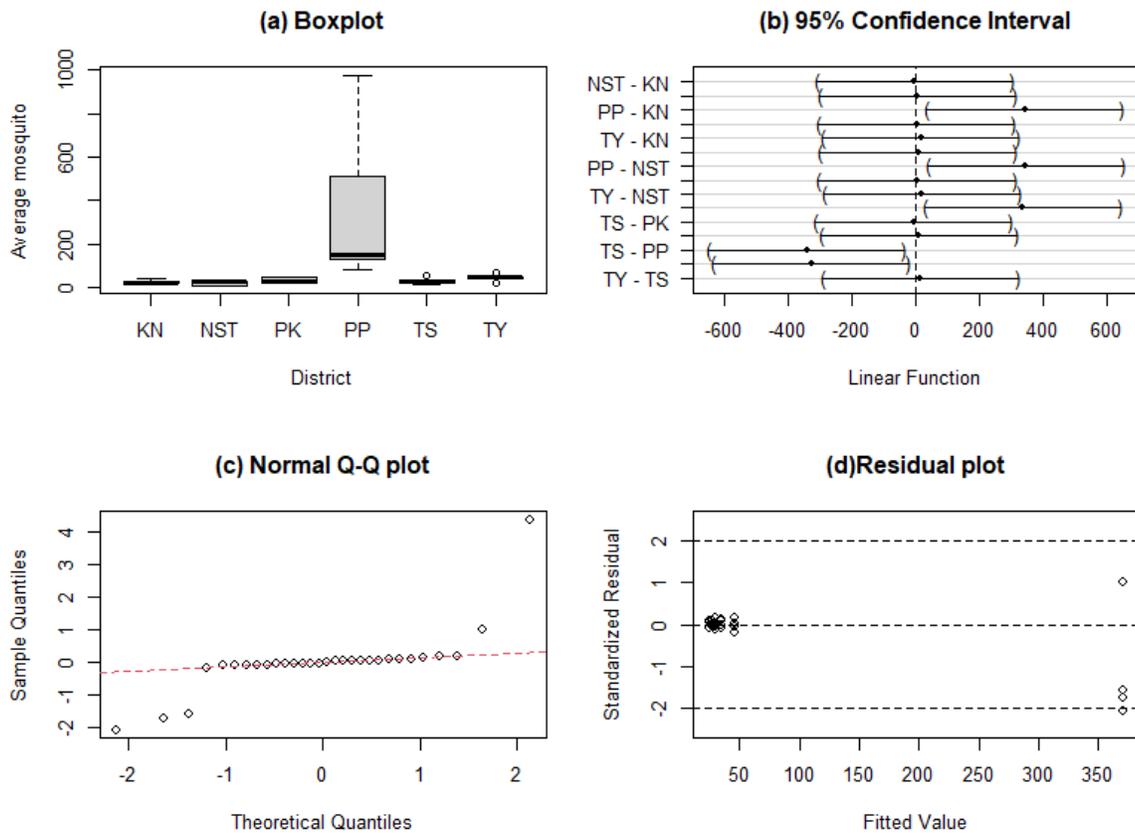


Figure 2: The data analysis in a randomized study comparing the district area to mosquito dispersal. (a) The average level of mosquitoes by region is shown using boxplots. (b) The fitted linear of the confidence interval. (c) The distribution of the residual values. (d) The remainder of the value of the approximation equation.

Table 4: The average distribution of mosquitoes in areas with different geographical features. The one-way ANOVA was performed, followed by Tukey’s multiple comparisons tests (significant differences at $p < 0.05$).

Geographical feature	No. of mosquito (mean \pm SE)
City (n = 10)	20.70 \pm 57.423 ^b
Coast (n = 10)	161.90 \pm 47.779 ^a
Mountain (n = 26)	30.27 \pm 47.779 ^b

The data were found to be normally distributed, based on the analysis of the mean of the mosquito distribution across geographical features (Figures 3a and 3c). Significant differences were found

based on the assumptions at 95% confidence intervals (Figures 3b), and a randomly distributed discrepancy was found around the zero point (Figure 3d).

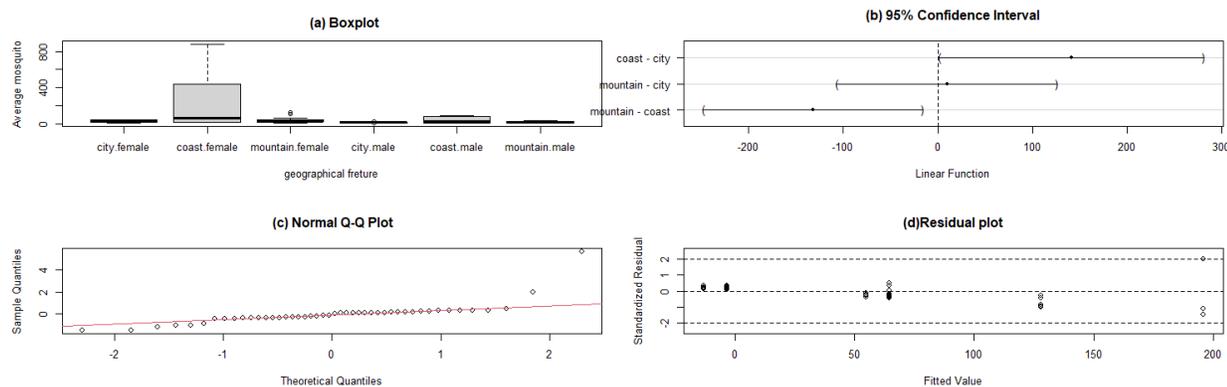


Figure 3: The data analysis in a randomized study comparing the geographical features to mosquito dispersal. (a) The boxplots denote the average level of mosquitoes by geographical features. (b) The fitted linear of the confidence interval. (c) The distribution of the residual values and (d) the remainder of the value of the approximation equation.

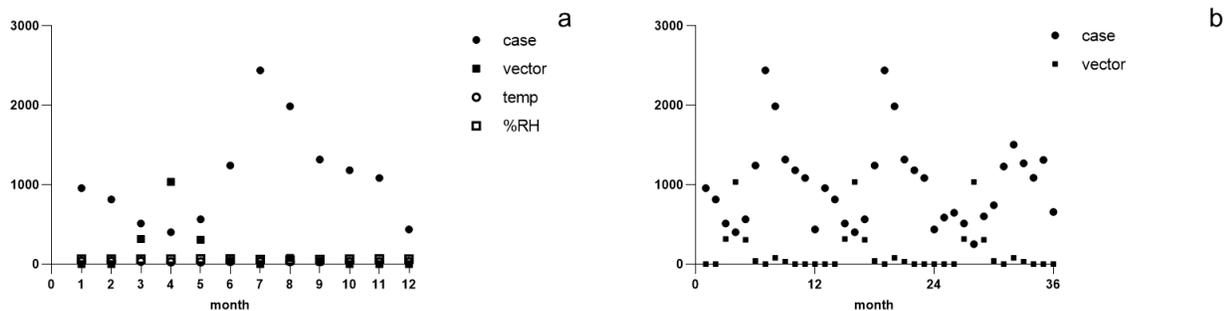


Figure 4: The mean of the mosquito distribution. (a) The correlation between mosquito numbers and the parameters in 2020 and (b) the association of mosquito numbers with the recorded dengue cases in three consecutive years (2018-2020).

DISCUSSION

Aedes albopictus was predominant in our randomly selected areas, while other species were either detected in very small numbers or were absent. *Aedes albopictus* lives and reproduces in both tropical and subtropical environments, as well as, at temperatures below 30° C. It transmits a strain of virus that causes a type of dengue fever that is alarming for the Ministry of Public Health³⁰⁻³¹. We used adhesive pads, similar to the use of swings, to

collect samples. However, the pads were portable and comfortable to use, and the number of insects could be counted instantly using this technique. Previous studies used different methods; for example, they used a mosquito swing to identify species, used people or animals as bait, and used different kinds of traps, which requires long time for sample collection and the samples are difficult to transport³²⁻³⁴. For adult mosquitoes, an entomological index survey might be better than

other indices since it can immediately identify the mosquito vector, making it possible to accurately predict diseases with the potential to lead to an epidemic³⁵. Adult samples might be used in the field to monitor the prevalence of the virus in the community, assess the transmission serotype, and determine the population per unit of area estimate³⁶⁻³⁸. Adult mosquitoes were used as an entomological indicator in other studies, and an index ranging from 8 to 35% was found using sticky traps³⁹⁻⁴². The index was found to be 2-88% using a sticky pad and a sweep net. This index is very important on the beach and can also be used to determine the species, sex, and parity status based on movement direction⁸. The sample from this study will be used in another study to determine the distribution of the dengue serotype.

The number of mosquitos was linked to the geographical characteristics of the sampling sites, particularly coastal locations. The coastline near the coast is swamped and left stagnant by the sea. The distribution was found to be higher along the shore, with fewer people per square mile than in other study areas²⁵. A marshy area near the beach is ideal for nesting⁴³. This might explain why this site had the highest index value. The second highest number of mosquitos was in the mountainous terrain with multiple reservoirs and temperature and humidity favorable for the growth of mosquitoes. In the urban and university areas, the mosquito breeding reservoir might be smaller, or the index approach might not be suited for collecting samples. Mosquito vectors in cities prefer being inside houses rather than outdoors.

The prevalence of dengue fever was inversely proportional to the number of mosquito vectors. This might be due to the length of the incubation period following mosquito bites. There is a discrepancy in mosquito life expectancy. Thus, the timing of case identification and reporting differed from the number of mosquitoes at the time of reporting. The cause of the outbreak in a significant number of cases might be infected mosquitoes biting the prey in the same area, as well as locations with high indices but low cases, despite the fact that there is no victim or people nearby.⁸ During the wet season, the transmission of the virus might be accelerated. Additionally, people are more inclined to spend most of their time indoors during the monsoon. This increases the spread of dengue fever by infected mosquitoes²². Several studies have shown a weak and nonlinear association between the incidence of dengue and the local microclimate. The incidence of dengue fever was negatively correlated with maximum temperature, minimum temperature, and maximum humidity⁴⁴⁻⁴⁶.

The entomological index we determined here was an estimate, where a high index value indicated

that the event occurred within a month. Simple randomization of the adult mosquito populations might be performed to prepare the community for the outbreak of dengue. A community warning about the possibility and danger of an epidemic might be issued. Adoption by the general public might use the method of swinging and calculating mosquito population per area, especially in areas with a history of disease outbreaks. This might require lesser materials, generate lesser waste, and take lesser time than that required for surveying mosquito larvae breeding sites by walking around houses. The procedure was simpler and faster. In contrast, the entomological index is the most basic information available for community awareness and is insufficient for monitoring and planning. Determining the distribution of the virus, as well as, molecular typing of dengue serotypes, might improve the efficiency of preventive and monitoring activities by the government.

CONCLUSION

The two categories of the entomological index differed considerably in the six districts (three distinct geographical features), and the highest index value was associated with the shore. The entomological parameters showed a negative association with dengue cases. The incidence of dengue infection was positively correlated with the temperature and negatively correlated with relative humidity. The pattern of epidemics was associated with adult mosquito vectors. This could have occurred after the mosquitoes identified in the epidemic region were present for 2-4 weeks. Thus, both entomological indices might be used as a tool for identifying mosquito vectors and developing a monitoring strategy. Additionally, they might be used for warning (standard level) people before an epidemic. Future studies might use genetic methods to determine the distribution of dengue serotypes in an area, making prevention and surveillance easier.

ACKNOWLEDGMENTS

Dr. Piti Mongkolrangkun, a public health scholar at the Division of Vector-Borne Diseases, Department of Disease Control, Ministry of Public Health, who contributed to identifying mosquito taxonomy, is thanked by the authors. Onuma Raksachon and Phimwimon Sisuksai, the members of the Specialized Research Unit on Insects and Herbs, Faculty of Science and Technology, Nakhon Si Thammarat Rajabhat University, for their assistance in collecting mosquito samples.

Funding

“This research received no external funding.”

Conflicts of Interest

“The authors declare no conflict of interest.” PR was in charge of the region’s research methodology, data collection, data analysis, interpretation, paper authoring, mapping, and GIS data acquisition for this study. SP aided with data collection and statistical analysis. SS will evaluate the data, select a publication for it, and then write and prepare the report. The authors helped with research, reporting, article writing, editing, and, ultimately, publishing approval.

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