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Seasonal and Topographical Factors Affecting Breeding Sites of *Culex* Larvae in Nakhon Si Thammarat, Thailand

Warabhorn PREECHAPORN, Mullica JAROENSUTASINEE, Krisanadej JAROENSUTASINEE and Jirawat SAETON

CX-KURUE & Computational Science Graduate Program, School of Science, Walailak University, Nakhon Si Thammarat 80161, Thailand

ABSTRACT

This study investigated how the seasons affect the key breeding sites of *Culex* larvae in three topographical areas: mangrove, rice paddy and mountainous areas. We examined how the number of *Culex* larvae varied in different types of water containers. Water containers were categorised into the following groups: indoor/outdoor containers, artificial/natural containers, earthen/plastic containers, containers with/without lids and dark/light coloured containers. Samples were collected from 300 households in both the wet and dry seasons from three topographical areas in Nakhon Si Thammarat province with 100 households/topographical area. *Culex* larvae were found in 19 out of 29 types of water containers in mangrove, rice paddy and mountainous areas. *Culex* females laid eggs in different container types depending on the season and topographical areas. *Culex* larvae were found in highest numbers in metal boxes in all three topographical areas and for both wet and dry seasons. The number of positive containers was higher in outdoor containers than indoor containers, artificial containers than natural containers, earthen containers than plastic containers, and dark coloured containers than light coloured containers Furthermore, the number of positive containers was found to be higher in containers without lids than containers with lids. The number of *Culex* larvae differed among the three topographical areas and between the wet and dry seasons.

Keywords: *Culex* larvae, season, topography, breeding site

INTRODUCTION

West Nile virus Mosquitoes (i.e. *Culex theileri*) serve as intermediate hosts in the transmission of several important human diseases e.g. malaria, yellow fever, dengue, Japanese encephalitis and filariasis [1,2]. Especially in recent years, the spatial distribution of both mosquitoes and mosquito-borne diseases has been changing and expanding for several reasons such as increasing rates of environmental corruption, climatic changes, vector and pathogen resistance to insecticides and drugs, progressive urbanisation, and population movement [1]. Therefore, much research has been conducted, particularly on vector and vector-suspected mosquitoes. Vector factors comprise of mosquito density, vector behaviour, vector competence, food level, duration of development, size at emergence, flight range and survival and biting activity [3-6]. Because preventative care is an increasingly important part of the strategy, topographical factors that influence key breeding sites of *Culex* larvae must be more closely investigated [3,7].

Mosquitoes utilise a wide variety of larval habitat types [8-13]. Artificial containers are a major source of breeding habitats for mosquitoes worldwide. These artificial containers include tyres, plastic bottles, metal boxes, and cans [7,13,14]. There are several examples of how each mosquito species prefers different breeding sites. For example, *Culex pervigilans* were observed at highest densities in containers with the smallest surface area [13]. In addition, previous studies showed that *Culex pervigilans* were present throughout the year and their larvae are even alive under ice [8,9].

Container-breeding mosquitoes use a variety of physical and chemical cues when selecting oviposition substrates. Many mosquito species show preferences for specific habitat colours. Most investigations of mosquito response to habitat colour have been lab-based, and colour preferences have not been studied for other taxa that typically coexist with mosquitoes in the field. Although mosquitoes generally dominate the macrofauna of typical phytotelmata, several other insect taxa are also common in these habitats. Little is known about the effects of habitat colour on colonisation by mosquitoes [15].

In recent decades, epidemiologists have increasingly investigated the relationship between faith-based communities, topography, season and disease risk areas on the key breeding sites of mosquitoes [7,14,15]. However, most previous studies have focused on key breeding sites of *Aedes* mosquitoes and little is known of *Culex* breeding sites. A thorough understanding of the *Culex* biology, ecology, and behaviour is necessary to help predict, identify, and manage the epidemics [16].

This study aimed to determine the effect of season and topographical areas on key breeding sites of *Culex* in Nakhon Si Thammarat province, Thailand. The investigation was conducted in the wet and dry seasons among three topographical areas: mangrove, rice paddy and mountainous areas. We predicted that (1) the number of positive outdoor containers should be higher than indoor containers; (2) the number of positive artificial containers should be higher than natural containers; (3) the number of positive earthen containers should be higher than plastic containers; (4) the number of positive containers without lids should be higher than containers with lids; (5) the number of positive dark coloured containers should be higher than light coloured containers; and (6) the number of *Culex* larvae should be higher in the wet season than the dry season.

MATERIALS AND METHODS

Data Collection

Mosquito larval survey was conducted in Nakhon Si Thammarat province located at 8° 32' 16.5" N latitude and 99° 56' 50.7" E longitude in the dry season (i.e. March-April 2006) and in the wet season (i.e. October-November 2006). We studied three topographical areas: mangrove, rice paddy and mountainous areas (**Figure 1**). These three topographical areas differed in terms of main occupations. In the mangrove area, the main occupation of the people is fishery. On the other hand, in the rice paddy and mountainous areas, the main occupation of the people is mostly agricultural related careers.

Samples were collected in households from all sub-districts in nine districts using stratified simple random sampling. Nine districts were divided into three categories: mangrove, rice paddy and mountainous areas. Mangrove areas included Khanom, Sichon and Pakpanang districts; rice paddy areas included Praprom, Chalermprakiet and Huasai districts; and mountainous area included Thungyai, Bangkhan and Thungsong districts (**Figures 1a** and **b**). 100 sampling units were assigned to each topographical area. Communities were assigned as stratums, one person in the collected household identified as a sample unit. A structured questionnaire was constructed to obtain the information by personal interview. The questionnaire included the family details, and mosquito protection practices. There were a total of 300 households sampled in the dry season. The same 300 households were then resampled in the wet season in order to compare the difference in the number of *Culex* larvae in various water containers between seasons.

(a) (b)

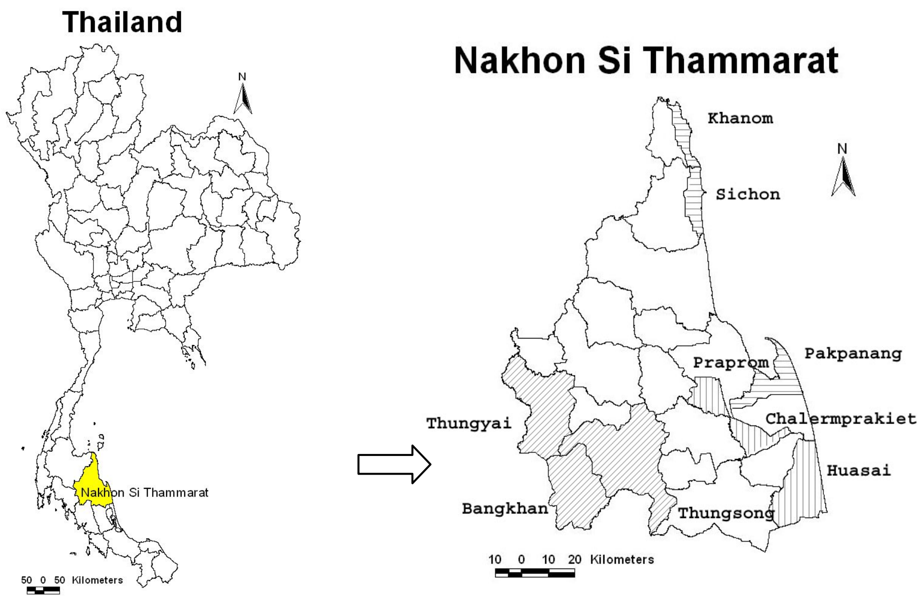

Figure 1 (a) Map of Thailand, and (b) Map of three topographical areas: ▤ Mangrove, ▥ rice paddy and ▨ mountainous areas

Entomological Studies

We collected all mosquito larvae from both indoor and outdoor containers using fishnets. The outdoor larval surveys were conducted within 15 m of the houses [3,7,14]. Very small water containers were emptied out through the fishnet. Larger water containers were sampled by dipping the net in the water, starting at the top of the container and continuing to the bottom in a swirling motion that sampled all edges of the container [7,14,17]. All live mosquito larvae were collected in plastic bags, taken to the Vector Borne Disease Control Centre, 11.2 Nakhon Si Thammarat laboratory. All mosquito larvae were preserved and identified up to genus level using Rattanarithikul and Panthusiri's [18] keys. In this study, the first, second instars and pupae were discarded because immature mosquitoes at these stages could not be identified.

There were a total of 29 container categories in this study. Water containers were categorised into the following groups: indoor/outdoor containers, artificial/natural containers, earthen/plastic containers, containers with/without lids and dark/light coloured containers. Indoor/outdoor containers were the containers in which water for drinking and/or washing was stored including earthen jars, cement tanks, plastic containers, flower vases, ant guards, ornament pots and refrigerators with plates. Earthen jars were classified into two categories: small earthen jars with a volume of <100 L and large earthen jars with a volume of >100 L. Plastic water containers were also divided into two categories: large plastic containers used for water storage (>100 L) and plastic bottles (i.e. 0.5 - 2.0 L water bottles). Artificial containers were composed of earthen jars, flower vases, used cans, discarded tyres, metal boxes, preserved areca jars and foam containers. Natural containers were composed of areca/coconut husks, banana trees, coconut shells, tree holes and bamboo clumps. Earthen containers were composed of small/large earthen jars, cement tanks, plant pot saucers, plant pots, and preserved areca jars. Plastic containers were composed of plastic containers, plastic bottles and plastic buckets. Lids were made of cement, plastic, wood and metal. The dark coloured containers were containers that made of black, brown, dark grey, deep green, deep blue and red. On the other hand, the light coloured containers were containers that made of white, light pink, light blue and yellow.

Statistical Analysis

All variables were tested for normality using the Komogorov-Smirnov test. The equality of variances was evaluated using Levene's test. Descriptive statistics of the data were analysed. Chi-square tests were used to test the differences between the two seasons and (1) indoor/outdoor containers, (2) artificial/natural containers, (3) earthen/plastic containers (4) containers with lids and without lids, and (5) dark/light coloured containers in all three topographical areas, two seasons and their interaction terms. All significant tests were two-tailed.

RESULTS

The number of *Culex* larvae and container types

Culex larvae were found in 19 out of 29 types of water containers (**Figures 2a-c**). There were eight types of indoor containers in three topographical areas including small earthen jars, large earthen jars, cement tanks, plastic containers, ant guards, vases, ornament plant pots and refrigerators with plates. There were no *Culex* larvae found in eight types of indoor containers in mangrove areas (**Figure 2a**). There were some *Culex* larvae found in one out of eight types of indoor containers (i.e. small earthen jars) in both rice paddy and mountainous areas (**Figures 2b** and **c**). There were 21 types of outdoor containers in three topographical areas. *Culex* larvae were found in 18 out of 29 types of outdoor containers (**Figures 2a-c**). *Culex* larvae were found mostly in metal boxes in three topographical areas in both the wet and dry seasons (**Figures 2a-c**).

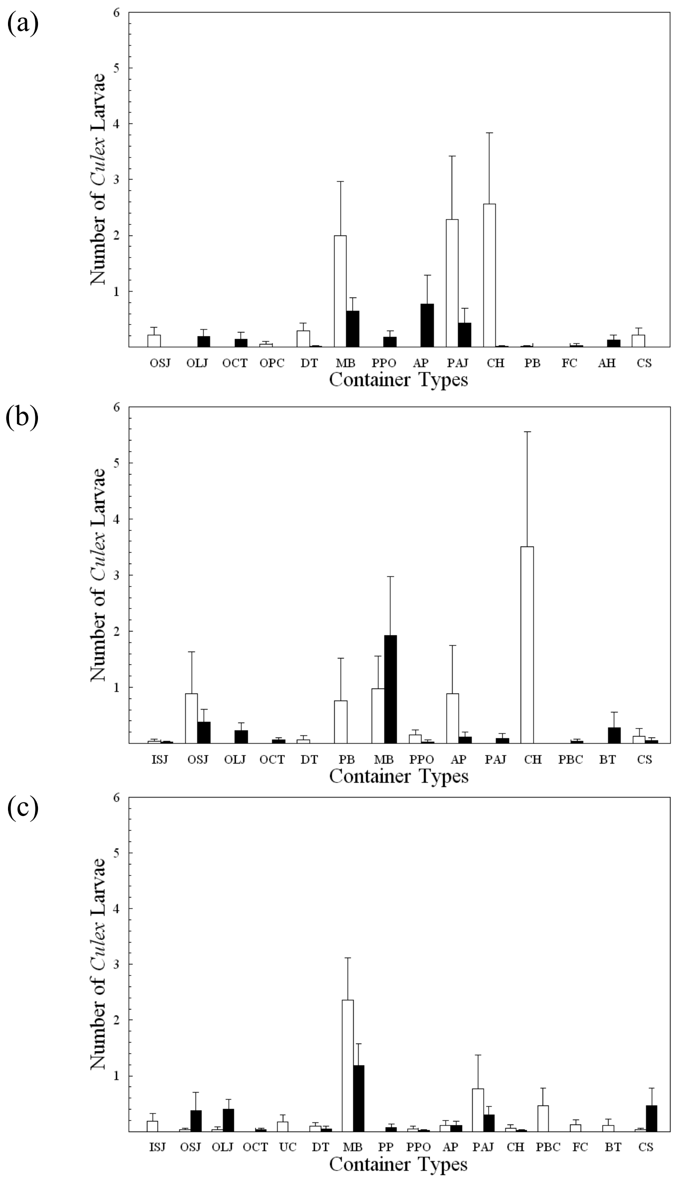

Figure 2 *Culex* larval occurrence in three topographical areas (a) mangrove area, (b) rice paddy area, and (c) mountainous area where ISJ, OSJ, OLJ, OCT, OPC, UC, DT, PB, MB, PP, PPO, AP, PAJ, CH, PBC, FC, AH, BT and CS represent indoor small earthen jar, outdoor small earthen jar, outdoor large earthen jar, outdoor cement tank, outdoor plastic container, used can, discarded tyre, plastic bottle, metal box, plant pot saucers, plant pot, animal pan, preserved areca jar, cement hole, plastic bucket, foam container, areca husk, banana tree and coconut shell, respectively.

The number of *Culex* larvae was higher in the wet season than the dry season, higher in mangrove areas than rice paddy and mountainous areas, and there was some interaction between topographical areas and seasons (**Table 2**).

The number of positive indoor/outdoor containers

The number of positive containers was higher in outdoor than indoor containers with little difference between wet and dry seasons and there was no interaction between indoor/outdoor containers and the season (**Table 3**). The number of positive containers was higher in outdoor than indoor containers, higher in mountainous areas than mangrove and rice paddy areas and there was no interaction between indoor/outdoor containers and topography (**Table 4**).

The number of positive artificial/natural containers

The number of positive containers was higher in artificial containers than natural containers and did not differ between wet and dry seasons. Moreover, there was no interaction between artificial and natural containers and the season (**Table 3**). The number of positive containers was higher in artificial than natural containers, higher in mountainous areas than mangrove and rice paddy areas and there was no interaction between indoor/outdoor containers and topography (**Table 4**).

The number of positive earthen/plastic containers

The number of positive containers was higher in earthen containers than plastic containers, did not differ between wet and dry seasons and there was no interaction between earthen and plastic containers and season (**Table 3**). The number of positive containers was higher in earthen containers than plastic containers, did not differ among the three topographical areas and there was no interaction between earthen and plastic containers and topography (**Table 4**).

The number of positive containers with lids/without lids

The number of positive containers did not differ between containers without lids than with lids, was higher in the dry than in the wet seasons and there was no interaction between containers with lids/without lids and the season (**Table 3**). The number of positive containers did not differ between containers without lids than with lids, among the three topographical areas and there was no interaction between containers with lids/without lids and topography (**Table 4**).

Table 1 The number of positive households and positive containers in mangrove, rice paddy and mountainous areas.

	Mangrove area		**Rice paddy area**		**Mountainous area**	
	Wet	**Dry**	**Wet**	**Dry**	**Wet**	**Dry**
No. of households	100	100	100	100	100	100
No. of positive households	33	29	19	28	33	42
No. of containers	1880	1847	1519	1347	1229	903
No. of positive containers	34	33	23	36	42	51

Table 2 The number of *Culex* larvae in three topographical areas during both wet and dry seasons.

Season	**Topographical area**			**Statistical test**		
	Mangrove area	**Rice paddy area**	**Mountainous area**	χ_2^2 **Topography**	χ_1^2 **Season**	χ_1^2 **interaction**
Wet	761	742	462	$\chi_2^2 = 54.134$ ***	$\chi_1^2 = 415.606$ ***	$\chi_1^2 = 44.191$ ***
Dry	253	322	303			

*** P<0.001

Table 3 The number of positive containers in wet and dry seasons.

Containers	Season		Statistical test		
	wet	dry	χ_1^2 Season	χ_1^2 Container	χ_1^2 interaction
Indoor	3	1	$\chi_1^2 = 0.288$	$\chi_1^2 = 206.288$ ***	$\chi_1^2 = 1.172$
Outdoor	104	114			
Artificial	82	79	$\chi_1^2 = 0.202$	$\chi_1^2 = 116.494$ ***	$\chi_1^2 = 0.384$
Natural	10	7			
Earthen	55	50	$\chi_1^2 = 1.241$	$\chi_1^2 = 76.172$ ***	$\chi_1^2 = 3.489$
Plastic	9	2			
With lid	4	14	$\chi_1^2 = 8.100$ **	$\chi_1^2 = 0.400$	$\chi_1^2 = 0.457$
Without lid	7	15			
Dark coloured	31	30	$\chi_1^2 = 0.253$	$\chi_1^2 = 5.343$ *	$\chi_1^2 = 0.185$
Light coloured	21	17			

* P<0.05
** P<0.01
*** P<0.001

Table 4 The number of positive containers in three topographical areas.

Containers	Topographical area			Statistical test		
	Mangrove area	**Rice paddy area**	**Mountainous area**	χ^2_2 **Topography**	χ^2_1 **Container**	χ^2_1 **interaction**
Indoor	0	2	2	$\chi^2_2 = 16.865$***	$\chi^2_1 = 206.288$***	$\chi^2_1 = 2.330$
Outdoor	66	52	100			
Artificial	53	43	65	$\chi^2_2 = 6.146$*	$\chi^2_1 = 116.494$***	$\chi^2_1 = 0.682$
Natural	6	3	8			
Earthen	37	34	34	$\chi^2_2 = 0.276$	$\chi^2_1 = 76.172$***	$\chi^2_1 = 2.249$
Plastic	3	2	6			
With lid	5	7	6	$\chi^2_2 = 2.150$	$\chi^2_1 = 0.400$	$\chi^2_1 = 0.567$
Without lid	4	9	9			
Dark coloured	16	14	31	$\chi^2_2 = 10.364$**	$\chi^2_1 = 5.343$*	$\chi^2_1 = 1.526$
Light coloured	8	13	17			

* $P<0.05$
** $P<0.01$
*** $P<0.001$

The number of positive dark/light coloured containers

The number of positive containers was higher in the dark coloured containers than light coloured containers, did not differ between the wet and the dry seasons and there was no interaction between dark/light coloured containers and the season (**Table 3**). The number of positive containers was higher in the dark coloured containers than light coloured containers, higher in mountainous area than mangrove and rice paddy areas and there was no interaction between dark/light coloured containers and topography (**Table 4**).

DISCUSSION

The number of *Culex* larvae and container types

This study clearly demonstrates that *Culex* females laid eggs in different container types depending on the season and topography. Das *et al* [19] collected *Culex* larvae from potential breeding habitats and found that *Culex* larvae were highly abundant in nursery paddy beds, paddy fields and other large water bodies in the area. Our results confirm their findings such that when we summed the number of *Culex* larvae in both wet and dry seasons, the rice paddy area had the highest number of *Culex* larvae. This may be because rice paddy areas have a high number of large freshwater bodies for females to oviposit [20] and more cattlesheds and pigs for *Culex* females to feed [20].

It has been reported that *Culex quinquefasciatus* larvae are found in several water container types including ceramic vessels, plastic vessels, metal vessels, tuckerboxes, plastic water barrels, metal water barrels and concrete water tanks with most larvae in metal vessels [21,22]. Our findings support these findings with *Culex* larvae found in various container types but mostly in metal containers. Understanding their key breeding types would be useful for prevention and control.

The number of positive indoor/outdoor containers

The results obtained from 29 types of containers showed that *Culex* larvae were found mostly in outdoor metal boxes in both wet and dry seasons in all three topographical areas. This is because *Culex* species have been shown to prefer to rest and breed outdoors [19]. Das *et al* [19] collected all adult *Culex* mosquitoes in Andhra Pradesh state, India and found three adult *Culex* species rested only outdoors (i.e. *Cx. triaeniorhynchu, Cx. bitaeniorhynchus,* and *Cx. gelidus*) and only adult *Cx. quinquefasciatus* rested indoors.

When we looked at the effect of topography on indoor/outdoor containers, we found that there were a higher number of positive containers in mountainous areas than rice paddy and mangrove areas. This could be because in mountainous areas, there were a high number of rubber plantations around the human dwellings. A high number of rubber plantations could be responsible for high number of *Culex* larvae for two possible reasons.

First, during the rainy season, it is common practice to suspend tapping temporarily in rubber plantations leading to accumulation of rainwater in sap-collecting containers, therefore proving an ideal breeding site for *Culex* mosquitoes. Sumodan's study [23] showed that *Aedes albopictus* laid eggs in sap-collecting containers and their eggs could fully develop into mature adults during that period. However, no work has been conducted on how sap-collecting containers in rubber plantations affect *Culex* breeding sites and larval development.

Second, the rubber tree canopy can contribute to a significantly larger number of *Culex* mosquitoes. This has been shown for *Cx. pipiens* which are mostly captured in the tree canopy rather than near the ground [24]. This suggests to us that relatively large numbers of *Cx. pipiens* inhabit the tree canopy.

The number of positive artificial/natural containers

This study clearly demonstrates that the number of *Culex* larvae was higher in the artificial containers than natural containers, and did not differ between the wet and dry seasons. This could be because there were more artificial containers available than natural containers. *Culex* mosquitoes are versatile breeders varying from clean to polluted water in both natural and artificial containers. Even though *Cx. quinquefasciatus* and *Cx. pipien* tend to breed in stagnant water with high organic content in an open yard setting, these natural containers are very rare. In order to become a versitile breeder, *Culex* mosquitoes have to have two major adaptations. First, *Culex* eggs are often laid on the walls of the containers just above the water-line. When the next rain event occurs the eggs become submerged and hatch shortly thereafter. Second, *Culex* mosquitoes are capable of many generations per season [25,26]. This multiple generation means that there could be a large number of mosquitoes in a short peroid of time and therefore, this could cause an outbreak.

The number of positive earthen/plastic containers

Our results showed that the number of *Culex* larvae was higher in the earthen containers than plastic containers, and did not differ between the wet and dry seasons. This could be because people in Nakhon Si Thammarat prefer to use earthen containers to plastic containers for water storage. Therefore, there are a lot of earthen containers to store water for drinking and washing providing suitable sites for mosquito larvae. Although there is a piped water supply in most residential areas, many water-storage containers are still kept in and around the house for collection and storage of rain as well as tap water. To supplant the precarious source of domestic water supply, people catch and store rain water in small to large earthen jars. This multitude of water storage containers provides preferred developmental sites for mosquito larvae [3].

The number of positive containers with lids/without lids

Our results revealed that the number of *Culex* larvae was higher in the containers without lids than containers with lids, and there was some difference between the wet and dry seasons. This finding supports Schneider *et al*'s [26] study that mosquito larvae were most commonly found in water storage containers without lids. Lids could prevent gravid mosquito females from ovipositing in water storage containers. Therefore, local health care services should encourage people to put lids on water storage containers at all times because this will be the most effective and cheapest way to prevent female mosquitoes from ovipositing in water storage containers.

The number of positive dark/light coloured containers

A significant amount of research has been done on colour cues for oviposition behaviour in mosquitoes [27]. For example, *Toxorhynchites r. septentroinalis*, *Tx. r. rutilus*, *Tx. moctezuma* and *Tx. Amboinensis* prefer to oviposit in black rather than white containers [27-30]. In addition, Yonoviak's study [15] investigated how the colour of water containers affects mosquito diversity and abundance and found that black and red coloured artificial treeholes attracted higher mosquito species diversity and abundance than green coloured artificial containers. Our results support previous findings that the number of *Culex* larvae was higher in dark coloured containers. Therefore, in order to minimise the number of *Culex* larvae in water storage containers, people should use light coloured water storage containers in their houses.

CONCLUSIONS

The key breeding sites of *Culex* mosqitoes were outdoor metal boxes. *Culex* mosquitoes also prefer to oviposite in dark coloured and earthen containers without lids. Mountainous areas had the lowest number of water containers. However, mountainous areas had highest number of positive dark coloured outdoor and artificial containers. The determination of breeding sites is of great benefit for mosquito control. For the prevention of vector borne disease outbreak, container management to reduce the sources of breeding habitats is one of the best approaches. If the containers are considered to be useless or nonessential, removal and destruction is desirable. If the population considers the containers to be useful or essential, the strategy employed will be the prevention of *Culex* breeding in containers rather than the destruction or removal of the containers.

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บทคัดย่อ

วราภรณ์ ปรีชาพร มัลลิกา เจริญสุธาสินี กฤษณะเดช เจริญสุธาสินี และ จิรวัฒน์ แซ่ตัน

ปัจจัยด้านภูมิประเทศและฤดูกาลมีผลต่อแหล่งเพาะพันธุ์ยุงรำคาญ ในจังหวัดนครศรีธรรมราช ภาคใต้ของประเทศไทย

งานวิจัยนี้เป็นการศึกษาว่าฤดูกาลมีผลต่อแหล่งเพาะพันธุ์ยุงรำคาญ ได้แก่ ภาชนะภายในบ้าน หรือภายนอกบ้าน ภาชนะที่ทำด้วยดิน หรือภาชนะที่เป็นพลาสติก ภาชนะที่ผลิตขึ้น หรือภาชนะในธรรมชาติ ภาชนะที่มีฝาปิด หรือภาชนะที่ไม่มีฝาปิด และภาชนะสีเข้ม หรือภาชนะสีอ่อนในภูมิประเทศที่เป็น พื้นที่ป่าชายเลน นาข้าว และบริเวณภูเขา ในจังหวัดนครศรีธรรมราช โดยการเก็บตัวอย่างลูกน้ำยุงจาก 300 ครัวเรือน ในพื้นที่ที่เป็นป่าชายเลน นาข้าว และบริเวณภูเขา เก็บตัวอย่างพื้นที่ละ 100 ครัวเรือน ทั้ง 2 ฤดูกาล กล่าวคือ ฤดูแล้งระหว่าง เดือนมีนาคม และเดือนเมษายน ส่วนฤดูฝนระหว่างเดือนตุลาคม และเดือนพฤศจิกายน โดยจำแนกภาชนะออกเป็น 29 ชนิดภาชนะ ผลจากการศึกษาพบลูกน้ำยุงรำคาญใน 19 ชนิดภาชนะ ซึ่งยุงรำคาญตัวเมียวางไข่ในภาชนะชนิดต่างๆ ขึ้นอยู่กับภูมิประเทศ พบลูกน้ำยุงรำคาญชุกชุมทั้ง 3 พื้นที่ ในฤดูฝนและฤดูแล้ง และพบลูกน้ำยุงรำคาญชุกชุมมากที่สุดในภาชนะประเภทโลหะนอกจากนี้ยังพบว่า ภาชนะซึ่งอยู่ภายนอกบ้านที่พบลูกน้ำยุงรำคาญมีจำนวนมากกว่าภาชนะซึ่งอยู่ภายในบ้าน ภาชนะซึ่งผลิตขึ้นที่พบลูกน้ำยุงรำคาญมีจำนวนมากกว่าภาชนะในธรรมชาติ ภาชนะดินที่พบลูกน้ำยุงรำคาญมีจำนวนมากกว่าภาชนะซึ่งเป็นพลาสติก ภาชนะซึ่งไม่มีฝาปิดที่พบลูกน้ำยุงรำคาญมีจำนวนมากกว่าภาชนะซึ่งมีฝาปิด ภาชนะซึ่งมีสีเข้มที่พบลูกน้ำยุงรำคาญมีจำนวนมากกว่าภาชนะซึ่งมีสีอ่อน และจำนวนลูกน้ำยุงรำคาญแตกต่างกันใน 3 พื้นที่ ทั้ง 2 ฤดูกาล

สำนักวิชาวิทยาศาสตร์ มหาวิทยาลัยวลัยลักษณ์ อำเภอท่าศาลา จังหวัดนครศรีธรรมราช 80161