

SOIL ANALYSIS AROUND ANOPHELINE BREEDING HABITATS IN NORTH-WESTERN THAILAND

Prasan Kankaew¹, Somporn Krasaesub² and Ratana Sithiprasasna¹

¹Department of Entomology, ²Department of Administration, US Army Medical Component, Armed Forces Research, Institute of Medical Sciences, Bangkok, Thailand

Abstract. The epidemiology of malaria is largely dependent on its vector habitat. Each species of *Anopheles* larvae has a specific habitat requirement for its development. Anopheline mosquitoes are common throughout Thailand and utilize a wide variety of habitats. The dominant malaria vectors in Thailand are *An. dirus*, *An. maculatus*, and *An. minimus*. The relationship between soil chemical components and the particular species of anopheline in their specific aquatic habitats was studied from September 2002 to July 2003 at Ban Khun Huay, Ban Pa Dae, and Ban Tham Seau in the Mae Sot district, Tak Province, Thailand. Mapping of each habitat was performed using a Global Positioning System unit. A total count of 2,130 laboratory reared adult *Anopheles* were collected from 138 habitats categorized into 11 different types identified into 18 species from larval sampling in three villages. *An. dirus*, *An. maculatus*, and *An. minimus* were found 5.26%, 10.70%, and 55.31%, respectively, along with other minor species. Drainage and/or season seemed to be associated with the presence of *An. dirus*, *An. maculatus*, *An. minimus*, *An. jamesii*, *An. sawadwongporni*, and *An. peditaeniatus*. Chemical tests: pH, aluminum, magnesium, calcium, and ferric iron showed some associations with the presence of *Anopheles*. Only drainage was found to be a parameter associated with the presence of *An. minimus*.

INTRODUCTION

Thailand is located between 6-21 degrees north latitude and 98-105 degrees east latitude, where anopheline mosquitoes are vectors for transmitting human malaria (Harrison and Scanlon, 1975). The epidemiology of malaria depends on the habitats of the vector. The population density of a species is largely dependent on its larval ecology. Generally, anophelines prefer clean, unpolluted water for oviposition. However, some are found in highly polluted water, such as *An. barbirostris*, found in habitats with high concentrations of buffalo dung and urine (Ratanarithikul and Panthusiri, 1994). *Anopheles* larvae are found in many different types of habitats ranging from small/temporary to large/more permanent habitats and from fresh to brackish water. Common anopheline aquatic habitats include the margin of ponds, lakes, or slow-flow-

ing streams; temporary bodies of water produced by rain, river flooding, or drying rivers and streams; and even water found in such "containers" as rain water cisterns, household water receptacles, and water trapped by the leaf axils of plants, such as bromeliads (Beaty and Marquardt, 1996). Many species, particularly some major malaria vectors, are found in aquatic habitats created by the activities of man, such as flooded agricultural fields, irrigation and seepage ditches, and flooded borrow pits (Beaty and Marquardt, 1996). Each species of *Anopheles* larvae has specific habitat requirements for its development. Some prefer habitats with aquatic vegetation, while others prefer no vegetation. Some species like exposed sunlit waters, while others prefer more shaded breeding places. *Anopheles* larvae are surface-feeders that ingest bacteria, yeasts, protozoans, diatoms, desmids and other microflora and microfauna as well as non-living, very fine, suspended matter (Lane and Crosskey, 1993).

In Thailand, anopheline mosquitoes are observed at altitudes ranging from the lowland areas of the central valley to the high mountains of the north. They are frequently associated with

Correspondence: Ratana Sithiprasasna, Department of Entomology, US Army Medical Component, Armed Forces Research Institute of Medical Sciences, 315/6 Rajvithi Road, Bangkok 10400, Thailand.
Tel: 66 (0) 2644-488 ext 2724; 66 (0) 2354 7885
E-mail: Ratanas@afirms.org

a variety of types of forest cover, including primary and secondary tropical rain forests, wet to dry evergreen forests, and secondary evergreen and deciduous forests. Anopheline mosquitoes are common throughout Thailand and utilize a wide variety of habitats. The primary vector in Thailand is *An. dirus*, a forest-dwelling mosquito (Ratanarithikul and Panthusiri, 1994). The other two important vectors are *An. minimus* and *An. maculatus*. The former usually breeds along the edges of slowly moving streams, whereas the latter breeds in puddles (Ratanarithikul and Panthusiri, 1994). Both species are often found at the margins of the forest.

For many years, scientists have been searching to answer why mosquitoes, not only anophelines but other species, have particular habitats. By performing environmental investigation including physical and chemical studies and/or using the Geographic Information System (GIS), some interesting outcomes have been observed in different aspects. Remote sensing and GIS technologies have been demonstrated to study the epidemiology of dengue hemorrhagic fever (Sithiprasasna *et al*, 1997a), to identify the breeding habitats of major malaria vectors and their distribution (Sithiprasasna *et al*, 2003a,b), to survey for dengue virus-infected *Aedes* mosquitoes (Sithiprasasna *et al*, 2004), and to predict malaria transmission risk (Sithiprasasna *et al*, 2005). GIS, employing toposheets and satellite image thematic maps on water table, water quality, hydro-geomorphology, soil type, relief, and irrigation channels as a tool to study malaria in India, revealed that high levels of malaria in villages of Nadiad were due to a high water table, soil type, irrigation, and water quality (Srivastava *et al*, 1999). Development of a dynamic hydrology model to predict mosquito abundances in flood and swamp water in New Jersey utilized historical meteorological, topographic, soil, and vegetation data. As a result, surface wetness was positively associated with the subsequent abundance of the dominant floodwater mosquito species, *Aedes vexans*, and the swamp water species, *Anopheles walkeri*. The subsequent abundance of *Culex pipiens*, a species that breeds in polluted, eutrophic waters, was negatively correlated with local mod-

eled surface wetness (Shaman *et al*, 2002).

Environmental investigation in areas where *Anopheles anthropophagus* distributed in Hubei Province in China showed that it was distributed in point, flaky, or belt form in low hills or hilly plains. These areas were full of vegetation and the source water pH ranged from 6.1-7.7. The principal crop was rice, and the soil texture was yellow, containing 2.72% organic material (Huang *et al*, 2001). Study of the agricultural, environmental, and institutional determinants of malaria in Azerbaijan in 1999 suggested that irrigation water use and soil salinity were significantly associated with malaria incidence (Temel, 2004). Particular types of soil, such as acid sulfate soil containing iron sulfides, were associated with intertidal mosquito breeding habitats in Australia (Saffigna and Dale, 1999). Study of the environmental factors associated with oviposition by *Aedes caspius* and *Aedes detritus* along a transect in Algeria showed the interesting result that neither species in North Africa was tied to seasonal or climate conditions, but instead resulted from habitat preference (Metge and Hussaine, 1998).

Water chemistry is also clearly important in the selection of oviposition sites. Halophilous species, such as *Aedes natronius* in East Africa, breed in inland salt-waters. Sometimes, volatile chemicals produced by decomposition of organic debris are the principal attractants. For example, log-ponds are particularly attractive oviposition sites for *Culex tarsalis* and *Cx. quinquefasciatus* in North America, and waters contaminated with chicken manure or rice-straw infusions are very attractive to *Culex pallens* (Lane and Crosskey, 1993). Apart from acquiring food and surviving in an aquatic habitat, the surrounding environment may be involved in the biochemical processes and survival of *Anopheles* larvae. One of the possible factors is soil surrounding the aquatic habitat. The larvae may acquire essential nutrient elements from soil through its decomposition and release into the water. There are three types of nutrients: macronutrients, micronutrients, and trace nutrients (LaMotte, 2001). The major essential macronutrients supplied through the soil are nitrogen (N), phosphorus (P), potassium (K), calcium (Ca),

magnesium (Mg), and sulfur (S). Other major nutrients, Carbon (C), hydrogen (H), and oxygen (O), come from water and atmospheric carbon dioxide. Common micronutrients are manganese, iron, boron, copper, zinc, molybdenum, and chlorine. Possible trace nutrients present in the soil are cobalt, iodine, fluorine, sodium, lithium, and aluminum.

The purpose of this study was to study the relationship between chemical components of soil and the existence of particular species of anophelines in specific anopheline aquatic habitats. The study was conducted from September 2002 to July 2003 in Ban Khun Huay, Ban Padae, and Ban Tham Seau of Mae Sot district, Tak Province, Thailand.

MATERIALS AND METHODS

Larval sampling

A minimum number of ten larvae of anopheline mosquitoes were collected from Ban Khun Huay, Ban Padae, and Ban Tham Seau of Mae Sot district, Tak Province, Thailand and their vicinities, to rear to adulthood and identify the species in the laboratory from September 2002 to July 2003. Mapping of each habitat was performed using a GPS unit (Trimble Navigation, Sunnyvale, CA, USA).

Larval habitat soil analysis

Soil sampling and preparation was performed following the LaMotte Soil Handbook with some modifications. A garden trowel was used to collect samples to a depth of approximately 30 cm. Four soil samples were collected from each habitat to produce average samples representing the natural habitat. Two soil samples were collected 30 cm on each side of

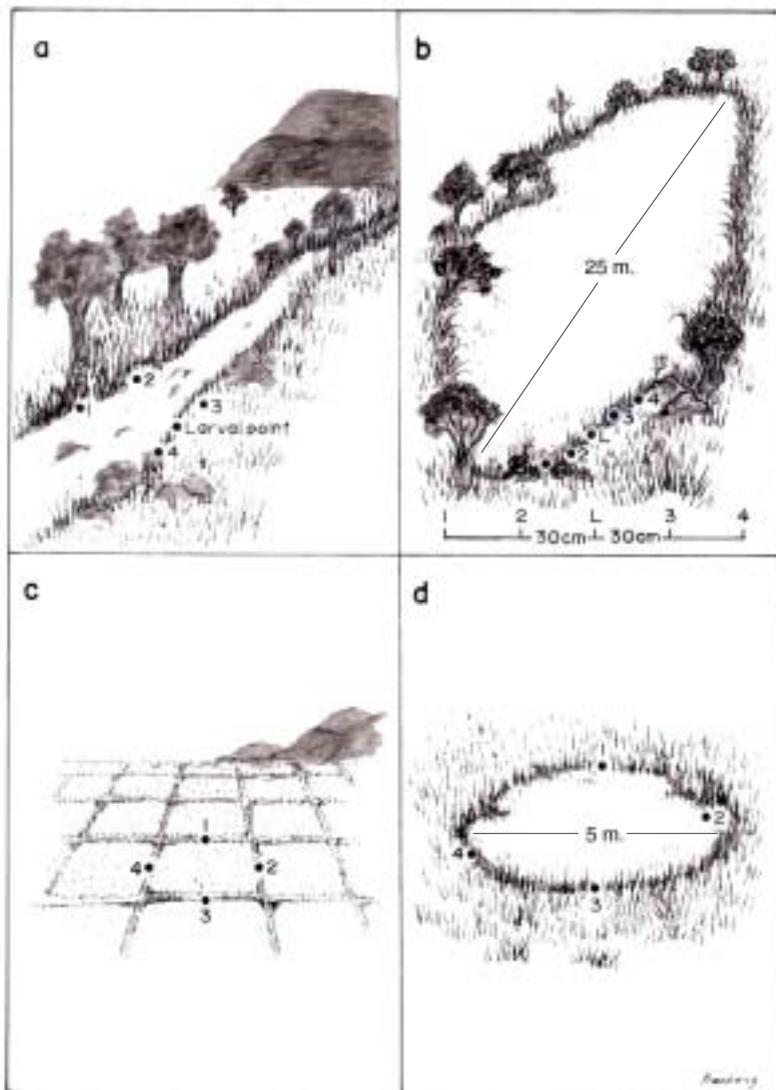
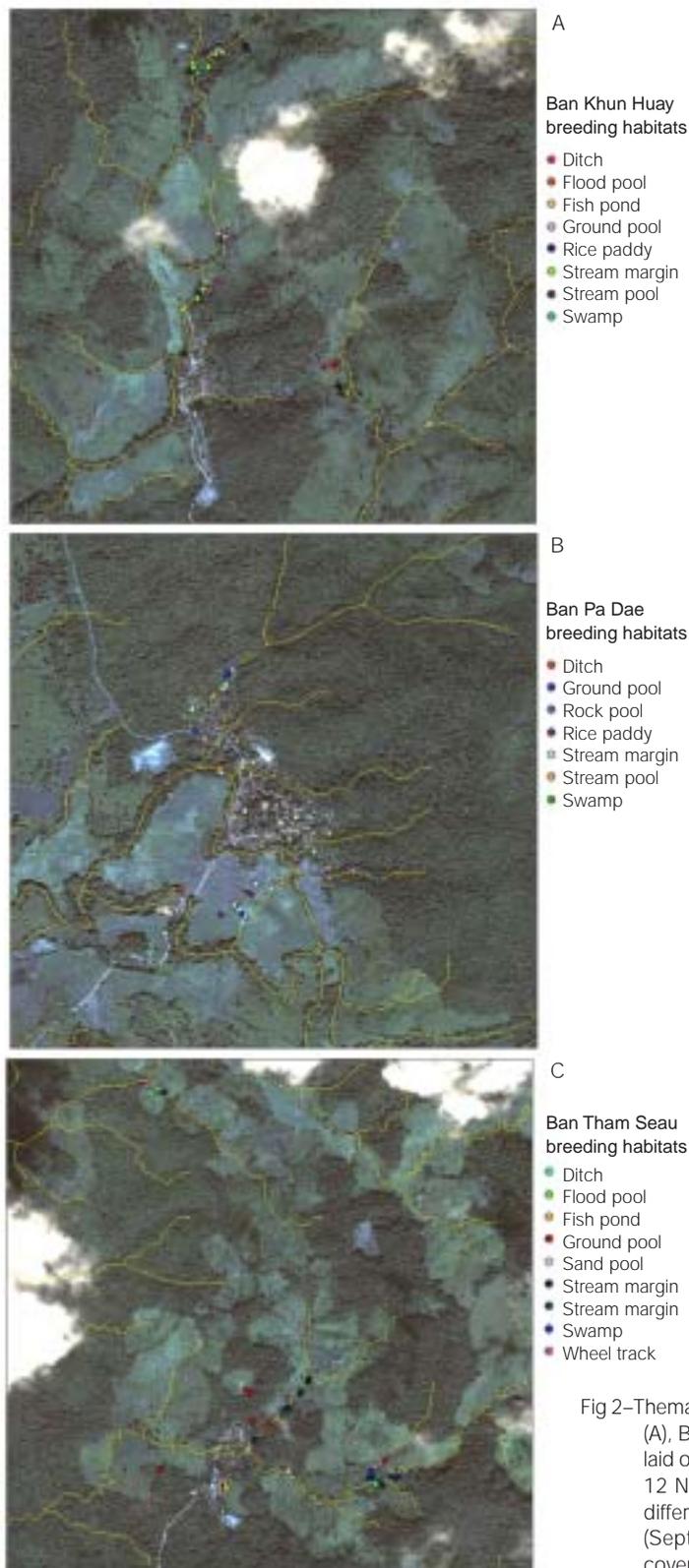


Fig 1—Soil sample collection from stream margin (a), swamp (b), square-shaped (c), and round-shaped (d) *Anopheles* larval habitats from September 2002 to July 2003.

the *Anopheles* larvae on each side of the stream or ditch. The width observed for the stream and ditch varied from approximately 0.2 to 2.5 m. For habitats with shape of a rectangle, square, or circle, soil samples were collected at the midpoint of each side. The observed width varied from 0.3 to 10 m. These criteria applied to all the habitats except the swamp with a diameter of 25 m. Two soil samples were collected 30 centimeters apart on each side of the positive location of the *Anopheles* larvae (Fig 1).



Four soil samples from each habitat were mixed together by turning the soil from the outside toward the center a number of times until all layers were thoroughly mixed. Roots, stones, or any other foreign material were removed. Some samples were air-dried on the field while other samples were transported and air-dried in the laboratory depending on the season. Air-dried soil samples were ground by a stone mortar and screened through a wired sifter. The fine soil samples were stored in Wheaton screw-cap vials with proper labels for the chemical tests.

Fine soil samples were extracted and tested by following the LaMotte model STH series Instruction Manual with some modifications. The LaMotte pH meter was used to measure pH instead of the colorimetric test. Wheaton screw-cap vials were used instead of the LaMotte tube for extraction. Double filter papers were used for filtration instead of one. Eleven chemical tests: pH, nitrate nitrogen, potassium, phosphorus, humus, magnesium, calcium, sulfate, aluminum, ferric iron, and manganese, were performed. Three tests: chloride, nitrite nitrogen, and ammonia nitrogen were not performed. Samples were mixed with Vortex.

Data analysis

For analytical purposes, the soil samples were divided into 2 groups according to the specific

Fig 2—Thematic map showing locations of Ban Khun Huay (A), Ban Pa Dae (B), and Ban Tham Seau (C) overlaid on IKONOS (spatial resolution 1 by 1 m) dated 12 November 2001 displayed in true color with different *Anopheles* breeding habitats collected (September 2002 - July 2003) with some cloud cover. Stream network is displayed in yellow lines.

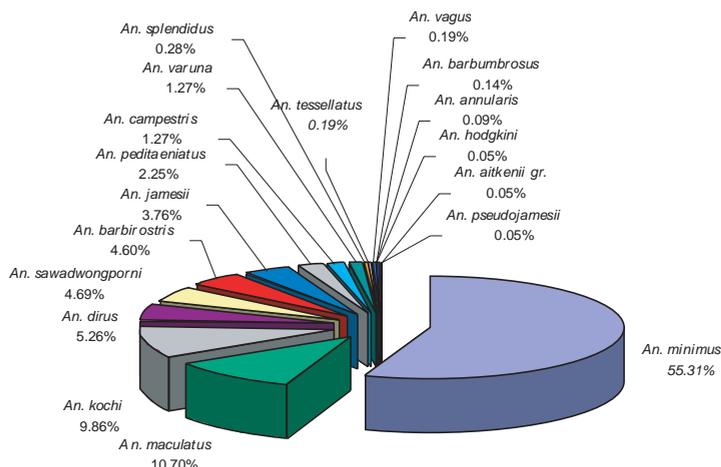


Fig 3—Anopheline mosquitoes collected from 138 breeding habitats in Ban Khun Huay, Ban Pa Dae, and Ban Tham Seau from September 2002 to July 2003.

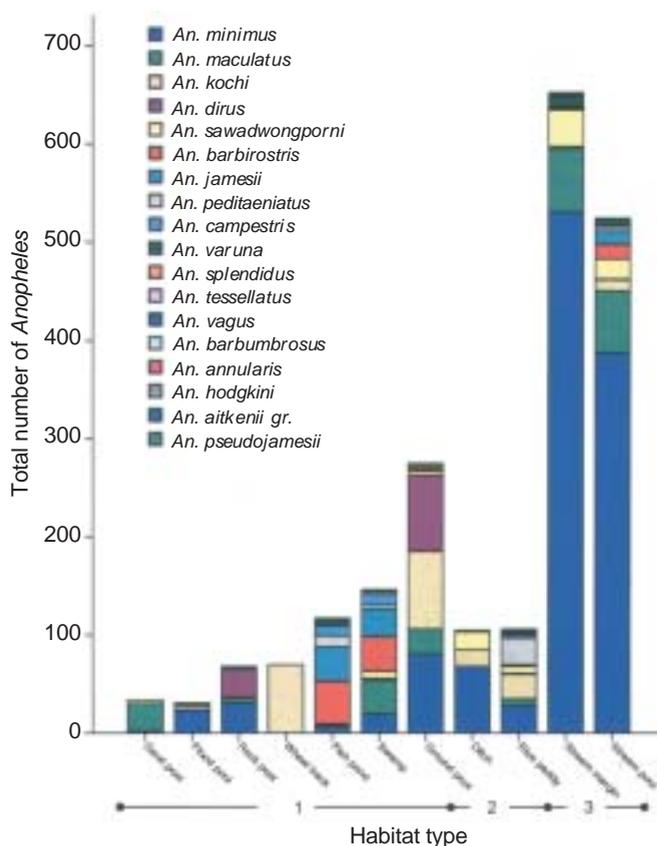


Fig 4—Distribution of anopheline mosquitoes by habitat and drainage types collected from 138 breeding habitats in Ban Khun Huay, Ban Pa Dae, and Ban Tham Seau from September 2002 to July 2003 (1 = non-drainage, 2 = semi-drainage, 3 = flowing-drainage).

species found ($y = 1$). A logistic regression model was used to test the association between chemical components of the soil in conjunction with 2 environment factors: drainage type and season. The SPSS software for windows version 12.0 (SPSS Inc, *et al* 2003) was used for this analysis.

RESULTS

A total count of 2,130 laboratory reared adult *Anopheles* mosquitoes were collected from 138 collection sites categorized into 11 different types of habitats, which were then identified as 18 species from larval sampling in three villages (Fig 2). The dominant malaria vectors in Thailand: *An. dirus*, *An. maculatus*, and *An. minimus* were found in 5.26% (112), 10.70% (228), and 55.31% (1,178) respectively, along with other minor species 28.72% (612) (Fig 3). *An. dirus* were found in rice paddies, ground pools, flood pools, stream pools, swamps, and rock pools whereas *An. maculatus* were found in swamps, stream pools, ground pools, stream margins, rice paddies, flood pools, rock pools, and sand pools. *An. minimus* were found in ditches, fish ponds, ground pools, rice paddies, stream margins, stream pools, swamps, sand pools, rock pools, and flood pools (Fig 4).

Observations of soil morphology showed that collected soil samples ranged from loam to clay loam with medium to fine texture. The colors observed were light brown to dark brown with roots, stones, or other foreign materials. There were three types of drainage observed during larval collection that were associated with habitat. The first one was non-drainage consisting of sand pools, flood pools,

Table 1
Chemical characteristics of soil samples.

Chemical	Valid N	Mean±SD	Median	Interquartile range	Min, Max
pH	138	7.2±0.8	7.5	6.5-7.9	5.1, 8.4
Nitrate nitrogen (ppm)	138	24.8±28.1	10.0	5.0-50.0	5.0, 75.0
Phosphorus (ppm)	138	11.8±12.0	5.0	5.0-12.5	5.0, 100.0
Potassium (ppm)	138	107.9±53.3	90.0	65.0-150.0	15.0, 300.0
Aluminum (ppm)	138	30.5±43.3	5.0	5.0-80.0	5.0, 125.0
Calcium (ppm)	138	9,493.5±4,264.2	7,000	5,000.0-14,000.0	1,400.0, 14,000.0
Ferric iron (ppm)	137	15.8±18.0	7.5	2.5-25.0	2.5, 62.5
Humus (levels)	138	2.2±1.4	2.0	1.0-3.0	1.0, 5.0
Magnesium (ppm)	138	40.6±38.7	25.0	25.0-80.0	10.0, 150.0
Manganese (ppm)	138	14.0±9.6	12.0	5.0-25.0	5.0, 40.0
Sulfate (ppm)	138	122.5±306.3	50.0	50.0-50.0	50.0, 2,000.0

Table 2
Parameters associated and not associated with the presence of the *Anopheles* larvae.

	Total ^a	<i>An. dirus</i>		<i>An. maculatus</i>		<i>An. minimus</i>		
		n ^b	Crude odds ratio (95%CI)	n ^b	Crude odds ratio (95%CI)	n ^b	Crude odds ratio (95%CI)	Adjusted odds ratio (95%CI)
Number of habitats (%)	138 (100)	15 (10.9)		50 (36.2)		108 (78.3)		
Drainage: (vs non drainage)								
Semi drainage	14	1	0.2 (0.0-1.8)	3	0.8 (0.2-3.5)	10	2.4 (0.7-8.7)	2.3 (0.6-9.2)
Flowing drainage	75	1	0.0 (0.0-0.3)*	35	2.7 (1.2-6.0)*	73	35.0 (7.7-158.9)*	30.0 (6.2-145.9)*
Season: (vs dry season)								
Wet	51	12	8.6 (2.3-32.3)*	16	0.7 (0.3-1.5)	35	0.4 (0.2-0.9)*	1.3 (0.4-3.7)
pH: (vs ≤6.5 (acid))								
6.6-7.5 (neutral)	39	5	0.9 (0.2-3.4)	14	1.6 (0.6-4.4)	30	2.2 (0.8-6.1)	1.0 (0.2-4.9)
7.5 + (basic)	64	5	0.5 (0.1-1.9)	27	2.1 (0.9-5.2)	57	5.4 (1.9-15.3)*	2.2 (0.4-12.0)
Aluminum: (vs 5-10 ppm)								
80 - 125	35	5	1.6 (0.5-4.9)	9	0.5 (0.2-1.2)	21	0.3 (0.2-0.6)*	0.8 (0.2-3.3)
Magnesium: (vs 10 -24 ppm)								
25-79	72	5	2.2 (0.2-20.0)	19	0.4 (0.2-1.0)	56	0.8 (0.3-2.4)	
80-150	35	9	10.4 (1.2-87.5)*	17	1.2 (0.4-3.0)	27	0.8 (0.2-2.7)	

*Statistically significant parameter; ^aTotal number of habitat within category; ^bNumber of habitats involved in the analysis for each species

rock pools, wheel tracks, fish ponds, swamps, and ground pools. The second one was semi-drainage consisting of ditches and rice paddies. The last one was flowing-drainage consisting of stream margins and stream pools. Chemical characteristics were determined for all 138 samples, except for one sample for ferric iron due to insufficient number of samples (Table 1).

The study of soil chemicals in conjunction with drainage type and seasons in association with anopheline breeding habitats showed that drainage type, season, pH, aluminum, magnesium, and potassium were associated with the presence of *An. dirus*, *An. maculatus*, and *An. minimus* in nature (Table 2). *An. dirus* presence was significantly associated with drainage type,

Table 3
Parameters associated and not associated with the presence of the Anopheles larvae.

	An. sawadwongporni		An. barbirostris		An. kochi		An. jamesii		An. peditaeniatus	
	Total ^a	n ^b Crude odds ratio (95%CI)								
Number of habitats (%)	138	29 (21.0%)	22 (15.90%)	20 (14.5%)	20 (14.5%)	20 (14.5%)	13 (9.4%)			
Drainage: (vs non drainage)										
Semi drainage	14	4 3.5 (0.8-15.5)	0	-	5 2.5 (0.7-9.2)	2 0.5 (0.1-2.6)	6 8.4 (1.9-36.8)*			
Flowing drainage	75	20 3.2 (1.1-9.2)*	9 0.4 (0.2-1.0)	6 0.4 (0.1-1.2)	6 0.4 (0.1-1.2)	6 0.3 (0.1-0.8)*	3 0.5 (0.1-2.2)			
Season: (vs dry season)										
Wet	51	11 1.0 (0.4-2.4)	6 0.6 (0.2-1.6)	10 1.9 (0.7-4.9)	5 0.5 (0.2-1.5)	9 4.4 (1.3-15.3)*				
pH: (vs ≤ 6.5 (acid))										
6.6-7.5 (neutral)	39	10 2.7 (0.8-9.5)	5 0.4 (0.1-1.4)	6 0.5 (0.2-1.7)	6 0.7 (0.2-2.5)	6 6.2 (0.7-54.2)				
7.5 + (basic)	64	15 2.4 (0.7-7.8)	8 0.4 (0.1-1.2)	5 0.2 (0.1-0.8)*	7 0.5 (0.2-1.5)	6 3.5 (0.4-30.5)				
Aluminum: (vs 5-10 ppm)										
80-125	35	3 0.3 (0.1-1.0)*	10 3.0 (1.2-7.8)*	8 2.2 (0.8-6.1)	9 2.9 (1.1-7.7)	2 0.5 (0.1-2.4)				
Calcium: (vs 1,400-2,800 ppm)										
3,500-14,000	135	28 0.5 (0.0-6.0)	22	-	18 0.1 (0.0-0.9)*	19 0.3 (0.0-3.8)	13			
Ferric iron: (vs 2.5 ppm)										
7.5	48	7 0.2 (0.1-0.7)*	6 0.7 (0.2-2.3)	5 1.1 (0.3-4.4)	4 0.5 (0.1-2.1)	7 1.6 (0.4-6.0)				
≥ 25	47	5 0.2 (0.1-0.5)*	9 1.2 (0.4-3.5)	11 2.9 (0.8-1.0)	10 1.6 (0.5-4.9)	2 0.4 (0.1-2.4)				

*Statistically significant parameter; ^aTotal number of habitats within category; ^bNumber of habitats involved in the analysis for each species

season, and magnesium. Their presence in flowing-drainage was not higher (0.0 time) than non-drainage, but 8.6 times higher in the wet season than in the dry season. They were found at magnesium levels between 80-150 ppm, 10.4 times higher than 10-24 ppm. In *An. maculatus* larval habitats, only flowing-drainage was found at 2.7 times higher than non-drainage. The presence of *An. minimus* was associated with the drainage, season, pH, and aluminum. They were found in flowing-drainage 35.0 times higher than in non-drainage, 0.4 times higher in the wet season than in the dry season, in alkaline (7.5+) soil 5.4 times higher than acidic soil (≤ 6.5), and 0.3 times higher at aluminum levels 80-125 ppm than at 5-10 ppm. However, after taking into consideration all statistically significant parameters, only flowing-drainage had an association with the presence of *An. minimus*.

An. sawadwongporni, *An. barbirostris*, *An. kochi*, *An. jamesii*, *An. peditaeniatus*, *An. campestris*, and *An. varuna*, 7 species out of a total of 15 species on which soil analysis was performed, showed that drainage type, season, pH, aluminum, calcium, and ferric iron were associated with their presence (Table 3). Flowing-drainage of *An. sawadwongporni* was (Fig 3) 3.2 times higher than non-drainage, aluminum levels of 80-125 ppm were 0.3 times higher than at 5-10 ppm. Both ferric iron levels, 7.5 and ≥ 25 ppm, were 0.2 times higher than the 2.5 ppm. The presence of *An. barbirostris* was associated with only aluminum levels, 3.0 times higher at 80-125 ppm than at 5-10 ppm. However, *An. barbirostris* were collected from only 22 habitats that neither

had semi-drainage nor calcium levels between 3,500-14,000 ppm. The presence of *An. kochi* in alkaline soil was 0.2 times higher than in acidic soil, and 0.1 times higher in calcium between 3,500-14,000 ppm than at 1,400-2,800 ppm. *An. jamesii* were found 0.3 times higher in flowing-drainage than in non-drainage. *An. peditaeniatus* were found 8.4 times higher in flowing-drainage than in non-drainage, and 4.4 times higher in the wet season than in the dry season. *An. peditaeniatus* were only collected from 13 habitats that had calcium levels between 3,500-14,000 ppm. In addition, they were only found in habitats that had sulfate and manganese levels between 50-200 ppm and 5-12 ppm, respectively. *An. campestris* and *An. varuna* were only collected at calcium levels between 3,500-14,000 ppm from 10 and 19 habitats, respectively.

DISCUSSION

The soil analysis around anopheline breeding habitats in north-western Thailand was a part of a longitudinal study described elsewhere (Sithiprasasna *et al*, 2003a,b, 2005). It was rather difficult to control some confounding parameters in this study. The nature of the longitudinal study was to perform *Anopheles* larval surveys and adult collections. For larval survey, all possible *Anopheles* habitats were checked and larvae were collected where they were present. Each habitat had a various shape, size, and water depth which led to difficulty in controlling the number of larval dippings. Furthermore, larvae were present in various numbers which were independent from their habitat size. The only possible criteria used were to collect of a minimum number of ten larvae per habitat in order to have enough samples for identification. A total of 2,130 *Anopheles* mosquitoes were recorded based on the number of laboratory-reared larvae. Larval mortality during field rearing process, transportation to AFRIMS, and the laboratory rearing process could have contributed to a reduction in the anopheline numbers. Due to the time limits of this study, only 138 habitats were surveyed with a positive finding for *Anopheles* mosquitoes.

Soil samples were collected at approxi-

mately 30 cm deep from each edge above water level to detect nutrients essential for anopheline larvae that might be dissolved in the water. Four soil samples were collected to provide a sufficient sample to determine the existence of nutrients in each habitat. The LaMotte model STH series was designed for agricultural soil analysis and was used in our study to test 11 chemical characteristics. However, the other available two tests, ammonia nitrogen and chloride, were discarded due to reagent instability. Most of the tests were colorimetric tests and only repeated when the results were in doubt or to minimize laboratory error. The test was not performed in duplicate since the results were clearly observed at one single test. The habitats in our study area were natural habitats, situated away from industrial factories. The only possible source of contamination of N, P, and K elements was from fertilizer used in rice paddies.

Larval survey showed that more than one species was collected in the same habitat and they might interact with each other. However, interaction is often difficult to understand, especially in the field studies. Moreover, it does not always reflect what is going on in the sample and it is always difficult to explain (Browner, 1999). Furthermore, there might be other factors or chemicals that influence the presence of particular species in particular habitats. It is prudent to interpret soil analysis result species by species.

From this study, we found that drainage and/or seasons seemed to be associated with the presence of *An. dirus*, *An. maculatus*, *An. minimus*, *An. jamesii*, *An. sawadwongporni*, and *An. peditaeniatus*. Each chemical test, including pH, aluminum, magnesium, calcium, and ferric iron showed some relationship with the presence of some particular *Anopheles* breeding habitat. After analyzing all the chemical and environmental parameters, only drainage was found to be a parameter associated with the presence of *An. minimus*.

Further research is necessary to better understand chemicals and/or environmental parameters influencing the presence of particular species of anophelines in specific breeding habitats.

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