WATER QUALITY AND BREEDING HABITATS OF ANOPHELINE MOSQUITO IN NORTHWESTERN THAILAND

Ampornpan Kengluecha[1], Pratap Singhasivanon[2], Montip Tiensuwan[3], James W Jones[1] and Ratana Sithiprasasna[1]

[1]Department of Entomology, US Army Medical Component, Armed Forces Research Institute of Medical Sciences, Bangkok; [2]Department of Tropical Hygiene, Faculty of Tropical Medicine, Mahidol University, Bangkok; [3]Mathematics Department, Faculty of Science, Mahidol University, Bangkok, Thailand

Abstract. Malaria transmission is dependent upon many hydrology-driven ecological factors that directly affect the vectorial competence, including the presence of suitable habitats for the development of anopheline larvae. Larval habitats were identified and characterized at three malaria endemic villages (Ban Khun Huay, Ban Pa Dae, and Ban Tham Seau) in Mae Sot district, Tak Province, in northwestern Thailand between July 2002 and June 2003. The Global Positioning System (GPS) was used to provide precise locational data for the spatial distribution of anopheline mosquito larvae and their habitats. Ten habitat categories were identified. Eighteen adult *Anopheles* species were identified from larvae in all the surveyed habitats. *An. minimus* was the most common species throughout the year. The relationship between eight abiotic variables (temperature, hardness, carbon dioxide, dissolved oxygen, nitrate, phosphate, silica and pH) and the abundance of four major species of malaria vectors *(An. (Cel.) dirus, An. (Cel.) minimus, An. (Cel.) maculatus,* and *An. (Cel.) sawadwongporni)*, and six species of non-vectors *(An. (Cel.) kochi*, *An. (Cel.) jamesii, An. (Ano.) peditaeniatus, An. (Ano.) barbirostris, An. (Ano.) campestris,* and *An (Cel.) vagus)* larvae was investigated. The results from the multiple regression models suggest that hardness, water temperature and carbon dioxide are the best predictor variables associated with the abundance of *An. minimus* larvae ($p<0.001$); water pH for *An. dirus* larvae ($p<0.001$); temperature and pH for *An. kochi* larvae ($p<0.01$); temperature and silica concentration for *An. jamesii* larvae ($p<0.001$); dissolved oxygen and silica concentration for *An. campestris* larvae ($p<0.001$); and pH and silica concentration for *An. vagus* larvae ($p<0.001$). We could not identify key environmental variables for *An. maculatus*, *An. sawadwongporni, An. peditaeniatus*, and *An. barbirostris*.

INTRODUCTION

A thorough understanding of the population dynamics of the larval stages of mosquitos is important in the development of sound abatement programs. Successful larval control requires the ability to identify larval habitats and distinguish between sites with high and low vector populations in a timely manner (Wood *et al*, 1992). In Thailand, mosquito control requires prioritization of the areas in need of pesticide application; this can be achieved with larval surveillance. One approach to surveillance is to identify key environmental factors that predict the presence of vector populations, then use these factors as markers to predict the presence of significant larval densities. A quantitative description of larval demography can produce data useful for the development of computer models and evaluation of control efforts. The biological and physico- chemical attributes of the aquatic environment may alter adult vector competence. An important target for malaria vector control is the anopheline larvae. In the US, Israel, and Italy, the key to eradication efforts is source reduction through modification of larval habitats (Kitron and Spielman, 1989).

Our understanding of anopheline larval ecol-

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) 2644-5777; Fax: 66 (0) 2354-7885
E-mail: ratanas@afrims.org

ogy is limited, and the knowledge is insufficient to achieve effective vector control through the means of larval control (Oaks *et al*, 1991). It is unknown what causes heterogeneity in vector distribution and abundance, and how the mosquito larval abundance is regulated in the diverse aquatic habitat. An understanding of the aquatic stages of vectors would be extremely relevant to malaria control (Molineaux, 1997). Claborn *et al* (2002) associated the environmental factors with larval habitats of malaria vectors. Larval habitats of *An. darlingi* and *An. pseudopunctipennis* were characterized by Manguin *et al* (1996 a, b). Reisen *et al* (1989, 1997) found an association between water quality and the vector competence for *Culex tarsalis* to transmit Western Equine Encephalitis and St Louis Encephalitis viruses. Prakash *et al* (2002) discussed the specificity of breeding habitats of *An*. *dirus* in relation to its ecology. Zoppi de Roa *et al* (2002) found an association between the abundance of cyclopoid species, the malaria vector *An. aquasalis*, and certain abiotic parameters and vegetation features. Factors influencing the abundance of Japanese Encephalitis vectors in rice fileds were studied by Sunish and Reuben (2001). Victor and Reuben (2000) conducted a study on the effects of organic and inorganic fertilizers on mosquito populations in rice fields. Guzman and Axtell (1987) studied the effects of temperature and water quality on the infection of *Culex* mosquito larvae by *Lagenidium.* Minakawa *et al* (1999) conducted a study to characterize the larval habitats of anopheline mosquitos and analyze their spatial heterogeneities.

Thailand is situated at a unique zoogeographic crossroads in Southeast Asia. It is the home to approximately 13% of the described mosquito species in the world (Harrison, 1980). Tak Province is located in the northern and western areas of the Oriental Faunal Region (Belkin, 1962), and has a large number of *Anopheles* species. The epidemiological and ecological data on anopheline malaria vectors in northwestern Thailand is complex; related to vegetation distribution and not well understood (Singhasivanon *et al*, 1999). Understanding anopheline habitats is fundamental for efforts in managing malaria through vector control in Thailand. The larval ecology of malaria vectors in Thailand has been neglected. Entomologists have traditionally been reluctant to study larval ecology because of the difficulties involved in larval sampling aquatic habitats in the field, especially when many larval habitats are not permanent (Service, 1976). New tools, such as the Global Positioning System (GPS) and Geographic Information System (GIS), are now available for mapping larval habitats. We used these new tools and characterized anopheline larval habitats in northwestern Thailand. We examined the spatial distribution of malaria vectors and nonvectors and evaluated physico- chemical factors affecting the abundance of anopheline larvae under natural conditions.

MATERIALS AND METHODS

Study area

Ban Khun Huay, Ban Pa Dae, and Ban Tham Seau in the Mae Sot district, Tak Province are Karen (Sgaw) villages, about 20 km east of the city of Mae Sot near the Myanmar border with Thailand (Fig 1). These three villages are located approximately 200 m above sea level in the deciduous woodland of the eastern watershed area of the Moei River, which drains westward into the Salween River. This study was part of a study by Sithiprasasna *et al* (2003a,b).

Larval sampling

Larval collections were made in and around the three villages to identify, quantify and characterize the habitats where anophelines occur. The coordinates for each larval habitat were recorded using a GPS unit (Trimble Navigation, Sunnyvale, CA, USA). Mosquito larvae were reared to adults and identified by species.

Water quality testing

We evaluated eight abiotic factors: water temperature, hardness, carbon dioxide, dissolved oxygen, nitrate, phosphate, silica, and pH, using a water chemistry kit (Model RL-05, LaMott, Chestertown, MD). The LaMotte pH meter was used to measure water temperature

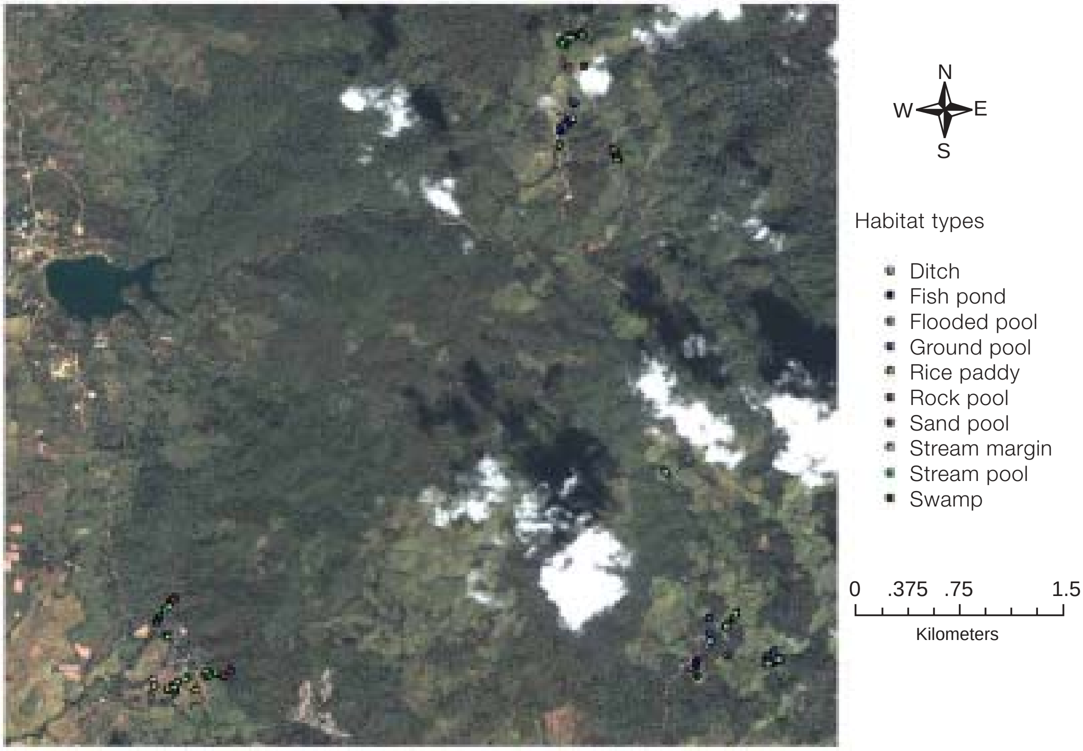

Fig 1–Thematic map showing different categories of breeding habitats of larval anophelines between July 2002 and June 2003 around Ban Khun Huay (top), Ban Pa Dae (bottom left), and Ban Tham Seau (bottom right) displayed on IKONOS satellite image (spatial resolution 1x1 m) in true color, acquired on 12 November 2001, clouds shown in white, cloud-shadows appear as black areas north of clouds, forest appear as dark green color.

and pH in the larval habitats. On each sampling, 200 ml of water was collected from each habitat, using a standard dipper from the sites where the mosquito larvae were sampled. The water samples were returned to the laboratory for immediate analysis. The sample was fixed in the field for estimating the amount of dissolved oxygen present in the water by the Winkler's method. Chemical indicators of water quality were measured using standard methods (Rand *et al*, 1976).

Data analysis

Multiple regression analysis by the backward elimination method was employed to obtain the best predictor variables contributing to the abundance of mosquito larvae for each species. The population of mosquito larvae was analyzed after transformation into log (X+1). SPSS for windows version 7.5 (SPSS, Inc, 1997) was used for the analyses.

RESULTS

Between July 2002 and June 2003, a total of 1,893 anopheline mosquito larvae were collected from 133 larval breeding habitats divided into 10 different types, containing 18 species of *Anopheles* (Table 1). The anopheles species identified were: *An. minimus* (57%), *An. maculatus* (11%), *An. dirus* (6%), *An. kochi* (6%), *An. jamesii* (4%), and *An. sawadwongporni* (4%), *An. barbirostris* (4%), *An. peditaeniatus* (3%), *An. campestris* (2%), *An. vagus* (1%), *An. varuna* (1%), and a combination *of An. aitkenii* gp, *An. annularia, An. barbumbrosus, An. hodgkini, An. pseudojamesii, An. splendidus,* and *An. tessellatus* which comprised 1% (Fig 2). Of the major malaria vectors, *An. minimus* was found in all the habitat types, namely stream margin, stream pool, ground pool, ditch, swamp, rice paddy, rock pool, and fish pond habitats in 41,

Table1
Breeding habitats containing *Anopheles* larvae in Ban Khun Huay, Ban PaDae, and Ban ThamSeau (July 2002-June 2003).

Habitats	#collections	Village	*An. aitkenii* gp	*An. annularis*	*An. barbirostris*	*An. barbumbrosus*	*An. campestris*	*An. dirus*	*An. hodgkini*	*An. jamesii*	*An. kochi*	*An. maculatus*	*An. minimus*	*An. peditaeniatus*	*An. pseudojamesii*	*An. sawadwongporni*	*An.splendidus*	*An. tessellatus*	*An. vagus*	*An. varuna*	Total
Ditch	1	Ban Khun Huay									4		1								5
Ditch	3	Ban Pa Dae									11		59	2							72
Ditch	1	Ban Tham Seau											7								7
Fish pond	5	Ban Khun Huay	1		3					7	2		3	11		1		2			30
Fish pond	3	Ban Tham Seau			18		10			26			3							5	62
Flooded pool	3	Ban Khun Huay						1			4	1	17								23
Flooded pool	2	Ban Tham Seau		1							15		6			2			1		25
Ground pool	4	Ban Khun Huay									20		43				1	1		1	66
Ground pool	5	Ban Pa Dae			2			16			2	1	14				2	1			38
Ground pool	6	Ban Tham Seau						67			13	13	14								107
Rice paddy	7	Ban Khun Huay		2				1		1	25	7	35	19		7				1	98
Rice paddy	4	Ban Pa Dae		1			5			1	1			18					10	1	37
Rock pool	4	Ban Pa Dae						22				5	31							3	61
Sand pool	1	Ban Tham Seau										28	2			4					34
Stream margin	13	Ban Khun Huay			1	2						14	133			13	2			1	166
Stream margin	10	Ban Pa Dae										2	140			5			2	3	152
Stream margin	12	Ban Tham Seau			2		1			1	2	41	165			18				5	235
Stream pool	15	Ban Khun Huay			8		1	1		12	6	48	112	4		15					207
Stream pool	10	Ban Pa Dae			2		1	8		1		15	122			5					154
Stream pool	7	Ban Tham Seau								1	5	7	107			4					124
Swamp	10	Ban Khun Huay			3		8	2		12	2	4	34	5	1		4			1	76
Swamp	2	Ban Pa Dae			1		3													4	8
Swamp	5	Ban Tham Seau			33				1	19		30	15	1		7					106
Total	133	3 villages	1	4	73	2	29	118	1	81	112	216	1,063	60	1	81	9	4	13	25	1,893

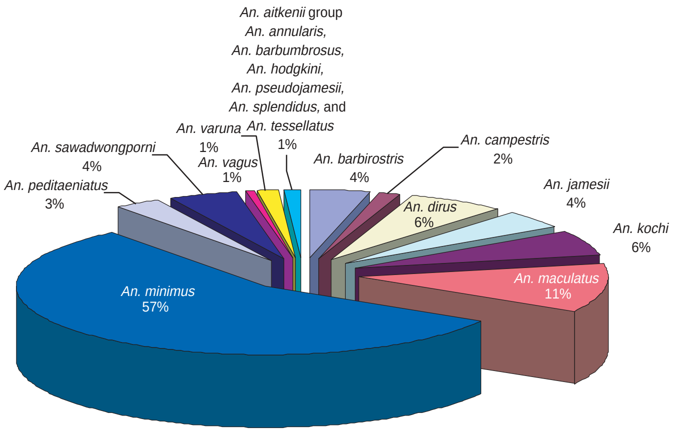

Fig 2–Anopheline larvae collected from breeding habitats in Ban Khun Huay, Ban Pa Dae, and Ban Tham Seau from July 2002 to June 2003.

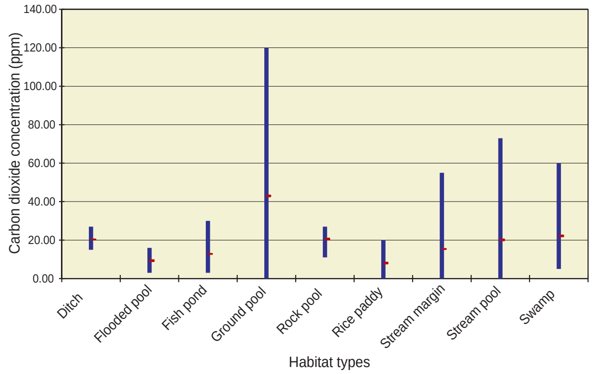

Fig 3–These high-low-close stock plots show the distribution of the data points of carbon dioxide in different habitat types for Anopheline larvae. The horizontal line in the interior of the box is the mean of the data.

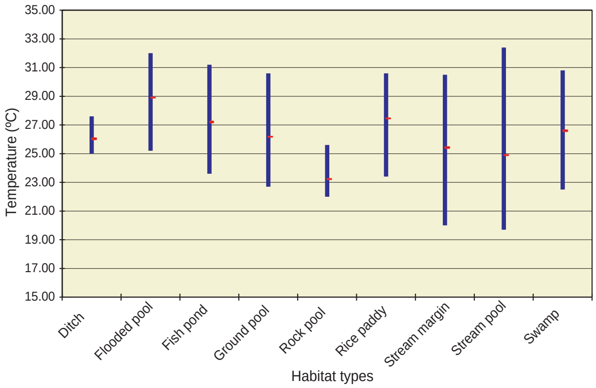

Fig 4–These high-low-close stock plots show the distribution of the data points of water temperature in different habitat types for Anopheline larvae. The horizontal line in the interior of the box is the mean of the data.

32, 7, 6, 5, 3, 3, 2, and 1%, respectively. *An. dirus* was found in ground pool, rock pool, stream pool, swamp, rice paddy and flooded pool habitats in 70, 19, 7, 2, 1, and 1%, respectively. *An. maculatus* was found in stream pool, stream margin, swamp, sand pool, ground pool, rice paddy, rock pool, and flooded pool habitats in 32, 26, 16, 13, 7, 3, 2, and 1%, respectively. *An. sawadwongporni* was found in stream margin, stream pool, swamp, rice paddy, sand pool, flooded pool, and fish pond habitats at 44, 30, 9, 9, 5, 2, and 1% respectively. Among the non-vectors, *An. kochi* was found in ground pool, rice paddy, flooded pool, ditch, stream pool, fish pond, and swamp habitats in 31, 23, 17, 13, 10, 2, and 2%, respectively. *An. jamesii* was found in fish pond, swamp, stream pool, rice paddy, and stream margin habitats in 41, 38, 17, 2, and 1%, respectively. *An. campestris* was found in swamp, fish pond, rice paddy, stream pool, and stream margin habitats in 38, 34, 17, 7, and 3%, respectively. *An. vagus* was found in rice paddy, stream margin, and flooded pool habitats in 77, 15, and 8%, respectively. Physico- chemical ranges for each species is shown in Table 2. Water quality in the habitats where the anopheline larvae occured is shown in Figs 3-6. Multiple regression equations were obtained in order to explain the abiotic factors affecting the population of anopheline larvae (Table 3).

DISCUSSION

Anopheline larvae in northwestern Thailand were present in a wide range of habitats. The densities of 6 species of anopheline larvae varied according to different abiotic factors, namely: temperature, CO_2, dissolved oxygen, hardness, pH, nitrate, phosphate, and silica concentrations. The abundance of the 2 major malaria vector species, *An. minimus* and *An. dirus,* was associated

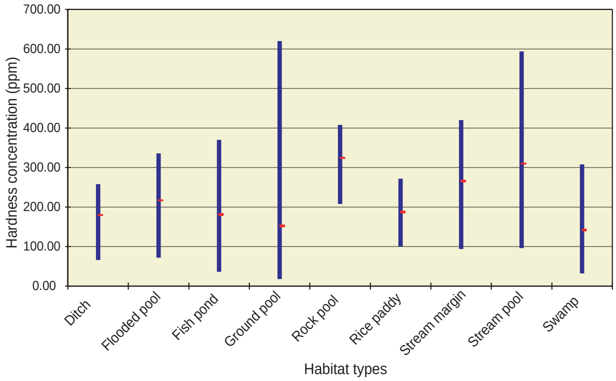

Fig 5–These high-low-close stock plots show the distribution of the data points of hardness in different habitat types for Anopheline larvae. The horizontal line in the interior of the box is the mean of the data.

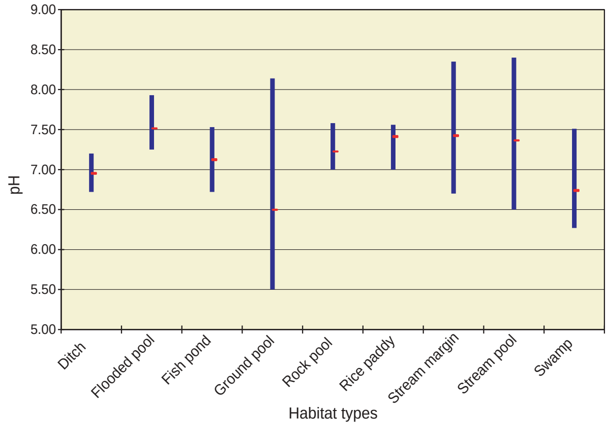

Fig 6–These high-low-close stock plots show the distribution of the data points of pH in different habitat types for Anopheline larvae. The horizontal line in the interior of the box is the mean of the data.

with some parameters. The higher requirement of *An. minimus* for water hardness is probably responsible for its dominance. Lower temperature and carbon dioxide concentrations in the breeding sites led to an increase in population density ($p<0.001$, $R^2=0.253$). Stream margins and stream pools were identified as potential habitats for the development of *An. minimus*. There was a significantly negative relationship between pH and *An. dirus* density ($p<0.001$, $R^2=0.183$). An abundance of *An. dirus* larvae was found in habitats with lower pH values, especially in the ground pools. Other environmental parameters not investigated in this study may have served as limiting factors. Among the non-vectors, there was a significant negative relationship between silica concentration and the population densities of *An. jamesii*, *An. campestris*, and *An. vagus*. A higher temperature requirement was associated with *An. kochi* and *An. jamesii*; while the former species preferred a lower pH ($p<0.01$, $R^2=0.082$) for ground pool, and the latter species preferred environment with a lower silica concentration ($p<0.001$, $R^2=0.151$) for a fish pond. *An. vagus* preferred a higher pH ($p<0.001$, $R^2=0.122$) for rice paddies. *An. campestris* preferred a higher concentration of dissolved oxygen in the swamp ($p<0.001$, $R^2=0.135$). We did not

Table 2
Physico- chemical ranges of the abiotic factors for each anopheline species from the three villages.

Species	Ranges							
	Temperature (°C)	Carbon dioxide (ppm)	Dissolved oxygen (ppm)	Hardness (ppm)	Nitrate (ppm)	pH	Phosphate (ppm)	Silica (ppm)
An. minimus	19.70-32.40	0.00-60.00	1.00-16.10	24.00-462.00	0.00-4.40	5.50-8.40	0.00-0.80	6.00-18.00
An. maculatus	19.70-30.50	0.00-120.00	0.90-16.00	18.00-620.00	0.00-4.40	6.00-8.35	0.00-0.60	10.00-18.00
An. kochi	24.00-32.00	3.00-76.00	0.90-8.20	51.60-330.00	0.00-3.52	5.90-7.59	0.00-0.80	3.00-16.00
An. dirus	20.30-29.40	0.00-120.00	0.90-16.00	18.00-620.00	0.00-0.88	5.50-7.59	0.00-0.50	7.00-16.00
An. jamesii	23.50-32.40	0.00-50.00	1.00-16.10	32.00-370.00	0.00-0.88	6.27-8.40	0.00-0.50	6.00-16.00
An. sawadwongporni	22.10-28.90	0.00-60.00	1.40-16.00	92.00-420.00	0.00-0.88	6.42-8.35	0.00-0.60	10.00-16.00
An. peditaeniatus	23.50-29.40	0.00-39.00	2.50-16.00	62.00-310.00	0.00-1.32	6.50-7.50	0.00-0.60	7.00-16.00
An. barbirostris	20.30-31.20	3.00-73.00	1.90-8.30	32.00-594.00	0.00-1.32	6.42-7.53	0.00-0.60	6.00-16.00
An. campestris	22.50-31.20	0.00-35.00	3.10-9.20	36.00-350.00	0.00-1.32	6.50-7.53	0.00-0.50	6.00-14.00
An. vagus	25.40-32.00	0.00-11.00	4.80-7.10	72.00-204.00	0.00-0.88	7.50-8.31	0.00-0.50	3.00-12.00

Table 3
Multiple regression equations for the estimated anopheline larval abundance in relation to statistically significant parameters in breeding habitat in the three villages.

Anopheles species	Range (average number per collection)	Multiple regression equations	p	R^2
1) *An. minimus*	1-45 (10.63)	$\hat{Y}$ = 3.37-0.09 (temp) + 0.004 (hard)-0.02 (CO_2)	< 0.001	0.253
2) *An. dirus*	1-28 (7.87)	$\hat{Y}$ = 3.90-0.52 (pH)	< 0.001	0.183
3) *An. kochi*	1-16 (5.60)	$\hat{Y}$ = 0.43 + 0.06 (temp)-0.25 (pH)	< 0.01	0.082
4) *An. jamesii*	1-15 (4.26)	$\hat{Y}$ = -0.10 + 0.04 (temp)-0.07 (Sil)	< 0.001	0.151
5) *An. campestris*	1-9 (2.64)	$\hat{Y}$ = 0.45 + 0.03 (DO)-0.04 (Sil)	< 0.001	0.135
6) *An. vagus*	1- 8 (3.25)	$\hat{Y}$ = -0.32 + 0.10 (pH)-0.03 (Sil)	< 0.001	0.122

Variables entered in the equation: temp = temperature (ºC); CO_2 = carbon dioxide (ppm); DO=dissolved oxygen (ppm); hard = hardness (ppm); pH = hydrogen ion concentration; Sil = silica (ppm) and 1) Y = ln (number of *An. minimus* larvae + 1), 2) Y = ln (number of *An. dirus* larvae + 1), 3) Y = ln (number of *An. kochi* larvae + 1), 4) Y = ln (number of *An. jamesii* larvae + 1), 5) Y = ln (number of *An. campestris* larvae + 1), 6) Y = ln (number of *An. vagus* larvae + 1).

identify key environmental variables for *An. maculatus*, *An. sawadwongporni, An. peditaeniatus*, and *An. barbirostris*. Our data did not provide information regarding mosquito species and their predators, which may be important in determining the abundance of anopheline larvae. Our study examined the aquatic habitats that contained mosquito larvae. While such a design allowed us to focus on the distribution of various species of anopheline larvae in their aquatic habitats, it had some limitations, such as only examining the habitats that contained mosquito larvae. Habitats which did not contain mosquito larvae were not studied. We detected habitats heterogeneity among the various species anopheline larvae and identified some key environmental variables that determined their occurrence and relative abundance.

The results suggest that the abundance of anopheline larvae may be determined by many variables, each contributing a small effect. We may not yet have identified the most important variables through our field studies (yielding higher R^2) to better elucidate the association. Multiple regression analysis by the backward elimination method, which we employed in obtaining the best predictor variables contributing to the abundance of mosquito larvae for each species, has the potential to improve the efficacy of the malaria control and surveillance program in Thailand. Further research should examine seasonal variations, with a more detailed analysis of water chemistry and investigate the effects of water quality on the vector competence of adult mosquitos.

ACKNOWLEDGEMENTS

This study would not have been possible without the assistance of the field-work of Mr Somporn Chanaimongkol, Mr Somsak Tiangtrong, Mr Boonsong Jaichpor, and Mr Prasan Kankaew. Funding for this project was provided by the Department of Defense-Global Emerging Infections Surveillance and Response System Unit located at the US Army Medical Component, Armed Forces Research Institute of Medical Sciences (USAMC-AFRIMS).

REFERENCES

Belkin JN. The mosquitos of the South Pacific (Diptera, Culicidae). Berkeley: University of California Press, 1962: 412, 608.

Claborn DM, Hshieh PB, Roberts DR, Klein TA, Zeichner BC, Andre RG. Environmental factors associated with larval habitats of malaria vectors in northern Kyunggi Province, Republic of Korea. *J Am Mosq Control Assoc* 2002; 18: 178-85.

Guzman DR, Axtell RC. Temperature and water quality effects in simulated woodland pools on the infection of *Culex* mosquito larvae by *Lagenidium* *giganteum* (Oomycetes: Lagenidiales) in North Carolina. *J Am Mosq Control Assoc* 1987; 3: 211-8.

Harrison BA. Medical entomology studies. XIII. The Myzomyzia Series of *Anopheles (Cellia)* in Thailand, with emphasis on intra-interspecific variations (Diptera: Culicidae). *Contrib Am Entomol Inst (Ann Arbor)* 1980; 17: 1-195.

Kitron U, Spielman A. Suppression of transmission of malaria through source reduction: antianopheline measures applied in Israel, the United States, and Italy. *Rev Infect Dis* 1989; 11: 391-406.

Manguin S, Roberts DR, Peyton EL, Rejmankova E, Pecor J. Characterization of *Anopheles pseudopunctipennis* larval habitats. *J Am Mosq Control Assoc* 1996a; 12: 619-26.

Manguin S, Roberts DR, Andre RG, Rejmankova E, Hakre S. Characterization of *Anopheles darlingi* (Diptera: Culicidae) larval habitats in Belize, Central America. *J Med Entomol* 1996b; 33: 205-11.

Minakawa N, Mutero CM, Githure JI, Beier JC, Yan G. Spatial distribution and habitat characterization of anopheline mosquito larvae in Western Kenya. *Am J Trop Med Hyg* 1999; 61: 1010-6.

Molineaux L. Malaria and mortality: some epidemiological considerations. *Ann Trop Med Parasitol* 1997; 91: 811-25.

Oaks SC, Mitchell VS, Person GW, Carpenter CJ. Malaria obstacles and opportunities. Washington DC: National Academy Press, 1991.

Prakash A, Bhattacharyya DR, Mohapatra PK, Mahanta J. Physico-chemical characteristics of breeding habitats of *Anopheles dirus* (Diptera: Culicidae) in Assam, India. *J Environ Biol* 2002; 23: 95-100.

Rand MC, Greenberg DE, Taras MJ, Fraivon MA. Standard methods for the examination of water and waste water. 14th ed. Washington, DC: American Public Health Association, 1976.

Reisen WK, Meyer RP, Shields J, Arbolante C. Population ecology of preimaginal *Culex tarsalis* (Diptera: Culicidae) in Kern County, California. *J Med Entomol* 1989; 26: 10-22.

Reisen WK, Hardy JL, Presser SB. Effects of water quality on the vector competence of *Culex tarsalis* (Diptera: Culicidae) for western equine encephalomyelitis (Togaviridae) and St. Louis encephalitis (Flaviviridae) viruses. *J Med Entomol* 1997; 34: 631-43.

Service MW. Mosquito ecology. New York: John Wiley & Sons, 1976.

Singhasivanon P, Thimasarn K, Yimsamran S, *et al*. Malaria in tree crop plantations in south-eastern and western provinces of Thailand. *Southeast Asian J Trop Med Public Health* 1999; 30: 399-404.

Sithiprasasna R, Linthicum KJ, Liu GJ, Jones JW, Singhasivanon P. Use of GIS-based spatial modeling approach to characterize the spatial patterns of malaria mosquito vector breeding habitats in northwestern Thailand. *Southeast Asian J Trop Med Public Health* 2003a; 34: 517-28.

Sithiprasasna R, Linthicum KJ, Liu GJ, Jones JW, Singhasivanon P. Some entomological observations on temporal and spatial distribution of malaria vectors in three villages in northwestern Thailand using a geographic information system. *Southeast Asian J Trop Med Public Health* 2003b; 34: 505-16.

Sunish IP, Reuben R. Factors influencing the abundance of Japanese encephalitis vectors in ricefields in India-I. Abiotic. *Med Vet Entomol* 2002; 16: 223.

Victor TJ, Reuben R. Effects of organic and inorganic fertilisers on mosquito populations in rice fields of southern India. *Med Vet Entomol* 2000; 14: 361-8.

Wood BL, Washino R, Beck L, *et al*. Distinguishing high and low anopheline producing rice fields using remote sensing and GIS technologies. *Prev Vet Med* 1992; 11: 277-82.

Zoppi de Roa E, Gordon E, Montiel E, Delgado L, Berti J, Ramos S. Association of cyclopoid copepods with the habitat of the malaria vector *Anopheles aquasalis* in the peninsula of Paria, Venezuela. *J Am Mosq Control Assoc* 2002; 18: 47-51.