MOSQUITO LARVAE AND ASSOCIATED MACROORGANISMS OCCURRING IN GEM PITS IN SOUTHERN THA MAI DISTRICT, CHANTHABURI PROVINCE, THAILAND

S Kitthawee[1], JD Edman[2] and ES Upatham[1]

[1]Department of Biology, Faculty of Science, Mahidol University, Bangkok, Thailand; [2]Department of Entomology, University of Massachusetts, Amherst, MA 01003, USA

Abstract. Aquatic field studies were conducted in Tha Mai District, Chanthaburi Province, Thailand. Larval habitats of *Anopheles dirus* were examined from November 1986 through June 1988 in 42 man-made gem pits. Larvae were found in pits containing clear water under full or partial shade. The abundance of different kinds of mosquito larvae were related to seasonal changes in these aquatic habitats. Variations in *An. dirus* density and occurrence were related to predators populations, *ie* Notonectidae and fish.

INTRODUCTION

Tha Mai District is in Chanthaburi, a province in southeastern Thailand with a serious malaria problem. In 1980, the Malaria Division (MD Report, 1982) found that the greatest number of cases (> 30,000) in Thailand occurred in this province. Human migration is an important factor in understanding the epidemiology and control of malaria in this region. Control activity has been concentrated along the Thai-Cambodian border. This consists of active case detection and drug treatment of all positive cases. DDT also is used for controlling adult *Anopheles* resting on the walls of human dwellings. Both drug-resistant malaria and DDT-resistant mosquitos are now widespread in this region (Harinasuta *et al*, 1976).

Anopheles dirus is the primary vector of malaria in hilly, forested areas in Thailand, including Tha Mai District (Scanlon and Sandhinand, 1965). *Anopheles dirus* larvae are commonly found in the numerous small pits which have been dug by hand for the recovery of gems in this district. Old gem pits are ideal habitats for larval studies since they hold water for several months of the year and are easy to sample and manipulate. In 1983, some pits in this area were used for biological control studies with guppies and *Tilapia* (Chanthaburi Malaria Center staff, personal communication).

Newly proposed integrated vector control strategies require a better understanding of vector biology, especially of larval populations. Our objective was to study the environmental requirements of *An. dirus* and other associated mosquito larvae in these gem pits. In addition, we compared mosquito populations with other aquatic insects and macroorganisms.

MATERIALS AND METHODS

Collection of pupal mosquitos

The study site comprised two villages (Fig 1). Both gem pits and concrete-lined, open wells which contained rain or ground water were inspected for mosquito larvae. Each habitat was catalogued and marked with a permanent identification code. Initially, forty-one pits were selected but after five months, one pit (pit 00) was destroyed and had to be replaced by a new pit (PP) with similar characteristics. Routine studies were conducted continuously for 13 and 20 month periods between November 1986 and July 1988. Some pits could not be studied for the entire 20-month period because a mechanized mining operations entering the study area and destroyed several of the pits.

Pupal mosquitos were collected from pits with a standard dipper (800 ml) and a standardized dipping protocol (10 dips per pit per day for 10 consecutive days each month). For non-*An. dirus*, the first 20 adult mosquitos emerging from pupae from each pit for each monthly sample were kept for identification. The remainder were identified as to genus (if obvious) or were simply recorded as

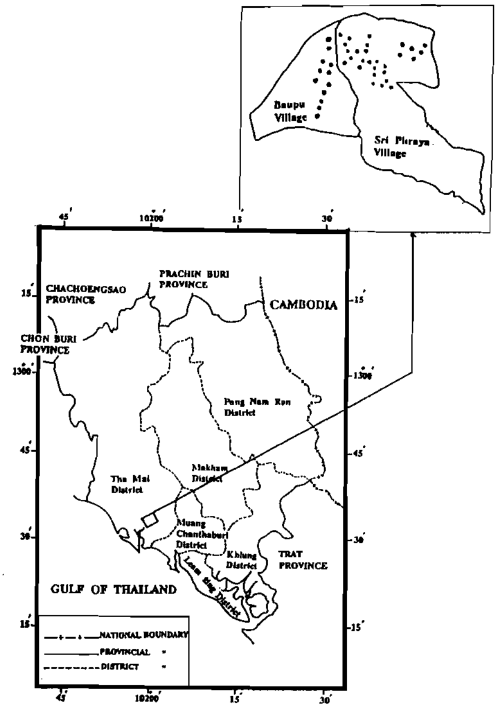

Fig 1—Diagram showing approximate location of 42 test pits in Baupu Village and Sri Phraya Village within Tha Mai District, Chanthaburi Province.

unidentified mosquitos. The following keys were used for identification: Barraud (1934), Bram (1967), Harrison and Scanlon (1975), Sirivanakarn (1976), Reinert (1976), Peyton and Harrison (1979). Other aquatic macroorganisms collected in the first four dips per pit per day were identified to family (using Merritt and Cummins 1984 key) and recorded from November 1987 through June 1988. These data were collected for four consecutive days during each month.

RESULTS

Anopheles dirus were found in large numbers in gem pits during the rainy season. Most pits were partially or fully shaded but a few were exposed to direct sunlight most of the day (Fig 2). Daytime water temperatures in the pits ranged from 22 to 28°C. Pits were between 70 and 190 cm in diameter; all were less than 230 cm in depth, except for the concrete-lined, open wells (depth ranged from 250 to 610 cm) which contained underground water used by villagers for their daily water supply. The mosquitos identified from study pits are listed in Table 1.

Attempts to collect *An. dirus* pupae from study pits during the dry season failed, and so collections were attempted in several neighboring pits during March and April 1988. A few larvae and pupae were found in three heavily shaded pits with fairly clean water that were hidden under dense vegetation (Fig 2a). These pits are listed as extra pits in Table 6.

The aquatic fauna observed represented five orders of insects (Ephemeroptera, Odonata, Hemiptera, Coleoptera and Diptera) as well as other aquatic macroorganisms, *ie* copepods, fish (guppy), tadpoles, snails, prawns and leeches (Tables 2 and 3). Each of these groups of organisms include possible competitors or predators of *An. dirus*.

Mosquito pupae were present in every pit except pit E (Table 1). *Anopheles dirus* pupae were collected from every pit except pits E, T, X, NN and PP; larvae which appeared to be *An. dirus* were found in all pits (Tables 1, 2 and in the pre-survey). Predaceous Notonectidae and fish (guppy) appeared to directly influence the number of mosquito larvae (Tables 4, 5). *Anopheles dirus* pupae were rarely collected from pits having predators and never at the same time that predators were present (Table 4).

Anopheles dirus was the most widely distributed mosquito. It was present in 37 of the 42 experimental pits sampled. *Culex* spp was the second most common group of mosquitos (35 pits) followed by *Uranotaenia* spp (28 pits), and other *Anopheles* (10 pits). These four groups of mosquitos occurred together in 5 of the 42 pits (Table 1). They appeared to vary sequentially with seasonal changes. When *An. dirus* declined in the dry season, *Culex* spp and *Uranotaenia* spp tended to increase (Table 6).

DISCUSSION

Anopheles dirus was historically associated with jungle seepages. The typical breeding habitats

Fig 2—(A) Old, shaded pit under dense vegetation; (B) concrete-lined well in open area under shade trees; (C) exposed pit under full shade; (D) close up of same pit shown in (C) when partially shaded.

were small, clear, shaded pools in clay soil (Colless, 1957; Scanlon and Sandhinand, 1965). At our study site, *An. dirus* larvae were found in gem pits containing underground water or rainwater under varying degrees of shade. The largest number of *An. dirus* pupae were collected from June to October (Table 6). The marked reduction in numbers from November to April may have been caused by both the habitat drying up and the habitat becoming less suitable. However, during the dry season some reproduction was maintained in the heavily shaded, undisturbed pools which were hidden under vegetation. When the rains returned, *An. dirus* reappeared in most of the study pits. Toward the end of the rainy season, we collected humid soil from a suspected oviposition site (*ie*, from pit M) and returned it to the laboratory. First instar *An. dirus* larvae hatched from this artificially flooded soil sample indicating that the eggs of *An. dirus* can tolerate some drying. We observed that 2.5% of laboratory produced eggs were viable after 16-18 days without water but in high humidity (*ie* soaked cotton placed under the filter paper containing the eggs). Only 0.003% remained viable after 33 days. This suggests that the high natural humidity in our study area could support egg viability between rains when ground pools temporarily dried up.

Some pits with many mosquito larvae also produced many pupae. However, other pits containing many larvae produced few pupae. *Anopheles dirus* pupae were most numerous in pits O, M and D, possibly due to the low numbers of predators in these pits. The impact of Notonectidae and fish on *An. dirus* larvae appeared to be absolute for pits E, T, X, NN and PP since pupae were never found in these pits.

The abundance of different kinds of mosquito larvae were related to seasonal changes in the

Table 1

Number of *An. dirus* and other mosquito pupae collected in gem pits. Pits are grouped according to their pupal production of *An. dirus*.

Pit	*An. dirus*	Other *Anopheles*[1]	*Culex* spp[2]	*Uranotaenia* spp	Unidentified species
E					
T			119	22	17
X			2		
NN		2	109	4	144
PP		13	5		1
GG	1		5		
EE	2		175	2	124
KK	2	1	23	1	9
MM	2	15	75	9	129
K	3		38	6	14
II	4		28	12	8
LL	5	9	6		2
V	6	1	3		2
CC	7		233	14	111
FF	9		48	5	29
OO	9		3		1
P	10		1	10	4
F	14				
S	15		11		2
Y	15				
BB	15		25	7	6
JJ	15		17	8	5
I	16	1	135	29	82
B	17		8	1	2
DD	18		21	1	98
G	19		12	10	8
A	20		3	3	1
J	21	1	43	8	22
Q	21				10
HH	21		14	2	6
N	22		169	14	162
R	23				1
W	25		5	1	
Z	27		6	8	6
L	31		76	53	31
U	32	8	62		101
AA	37	4	39	2	15
C	39		1	11	21
H	39		23	3	14
D	51			3	7
M	58		43		16
O	70			23	11

[1] *Anopheles (Anopheles) barbirostris, Anopheles (Anopheles) hyrcanus nigerimus, Anopheles (Anopheles) umbrosus* and *Anopoheles (Cellia) tessellatus*

[2] *Culex (Culex) fuscocephala, Culex (Culex) gelidus, Culex (Culex) mimeticus* group, *Culex (Culex) sitiens, Culex (Culex) tritaeniorhynchus, Culex (Culex) vishnui* subgroup, *Culex (Culiciomyia) nigropunctatus, Culex (Culiciomyia) pallidothorax, Culex (Lophoceraomyia)* spp and *Culex (Lutzia)* spp.

Table 2

Total number of mosquito larvae and other aquatic insects collected from gem pits from November 1987 through June 1988. Grouping of the pits follows Table 1.

Pit	*Anopheles*	*Culex* and *Uranotaenia*	Chironomidae	Ephemeroptera[1]	Odonata[2]	Hemiptera[3]	Coleoptera[4]
E	3	4				>80	
T	7	24		6	3	>255	4
X		1			9	154	8
NN	15	>330		19	9	>560	2
PP	56	1		2		>219	3
GG	1			1	1	34	
EE		3	1			50	
KK	3	2			5	19	1
MM	23	>80	1	3	8	>329	3
K		61			3	30	
II	>47	41		3	5	>285	
LL	4				8	22	
V	9	2		1		>400	3
CC	5	4				62	
FF	4	9				161	
P	11	3	1		8	57	
F	8					14	
S		2		>39		>138	9
Y	7					196	
BB	49	>22		7	5	>246	7
JJ	18	18		4	7	>199	2
I	>41	>89		9	12	>211	
B	51	2				162	
DD	7	15				69	
G	7	20				51	
A	7	1			1	77	
J	30	>65		2	1	>299	
Q	17	1		2		>250	
HH	4	>24			2	>284	
N	2	1	1		3	107	
R	2			15		39	
W	16			1	3	>130	
Z	54	2			14	>190	
L	12	>410			14	>534	2
U	42	>114		15	9	>304	9
AA	22	2		2	1	122	10
C	39		1	8		>194	
H	>100	>46		1	6	138	4
D	4			5		46	
M	2					7	
O	35				1	86	

[1] Ephemeridae
[2] Coenagrionidae and Libellulidae
[3] Notonectidae, Gerridae, Herbidae and Hydrometridae
[4] Dytiscidae

Table 3

Total number of mosquito larvae and other macroorganisms collected from gem pits from November 1987 through June 1988. Grouping of the pits follows Table 1.

Pit	*Anopheles*	*Culex* and *Uranotaenia*	Copepods	Fish (Guppy)	Tadpoles	Snails	Prawns	Leeches
E	3	4		24				
T	7	24	>34		5			
X		1	>96	9	15		22	
NN	15	>330	>410		26	1		
PP	56	1	>14	1	>188		1	
GG	1			5	1			
EE		3	>14	2				7
KK	3	2	>44	1	13			
MM	23	>80	>263	1	28			
K		61	>40	54	15			
II	>47	41	>32		29		1	
LL	4		>33		1			
V	9	2	>10		12			5
CC	5	4	>164		>35			
FF	4	9	>72	1	>40			7
P	11	3						
F	8		1	1			1	
S		2			11			
Y	7				1			
BB	49	>22	>70	1				
JJ	18	18	7	1	12		1	
I	>41	>89	>11		4			
B	51	2			>50	1		
DD	7	15	>51					
G	7	20	>42		1			
A	7	1			3			
J	30	>65	>120		28			
Q	17	1						
HH	4	>24	>162		>52			
N	2	1	2					
R	2					1		
W	16							
Z	54	2			24			
L	12	>410	>138		7			
U	42	>114	>105		>97			
AA	22	2	>280	1				>18
C	39		>30		28			
H	>100	>46	>201		>64			
D	4		>10	1	9	1		
M	2				2			
O	35		>20					

Table 4

The association of predaceous Notonectidae and fish (guppy) with *An. dirus* larvae and pupae in selected pits. See Table 1 for selection and grouping of the particular pits by numbers of *An. dirus* pupae. Letter D indicates pits were dry during the monthly sampling period.

		Nov 1987	Dec	Jan 1988	Feb	Mar	Apr	May	Jun	Total
Pit X	*An. dirus* (L)									0
	An. dirus (P)									0
	Notonectidae	9	1	4	3	9	4	1		31
	Fish (guppy)		2	4	3					9
Pit E	*An. dirus* (L)		3							3
	An. dirus (P)									0
	Notonectidae	1								1
	Fish (guppy)	3	7	4	10					24
Pit T	*An. dirus* (L)		2		5					7
	An. dirus (P)									0
	Notonectidae	1	9		6	3	4	1		24
Pit NN	*An. dirus* (L)		1	1	10		2	1		15
	An. dirus (P)									0
	Notonectidae		3					1		4
Pit PP	*An. dirus* (L)		4	24	2	26				56
	An. dirus (P)									0
	Notonectidae	>21	2	6	14	2			1	>46
Pit C	*An. dirus* (L)		5	25	9		D			39
	An. dirus (P)						D			0
	Notonectidae	11	14	6	4	15	D			50
Pit H	*An. dirus* (L)	19		5	>55		D	21		>100
	An. dirus (P)	5					D	2		7
	Notonectidae		17	3	10	3	D			33*
Pit D	*An. dirus* (L)							4		4
	An. dirus (P)							4		4
	Notonectidae	2	6	4	4					16*
Pit M	*An. dirus* (L)	2	D	D	D	D	D	D		2
	An. dirus (P)		D	D	D	D	D	D	4	4
	Notonectidae		D	D	D	D	D	D		0
Pit O	*An. dirus* (L)	31	4			D	D	D	D	35
	An. dirus (P)	5				D	D	D	D	5
	Notonectidae					D	D	D	D	0*

* none were present during months that *An. dirus* pupae were produced.

aquatic habitats. *Anopheles dirus* dominated from June to October. After October, *An. dirus* decreased but *Culex* and *Uranotaenia* increased (Table 6). After the first rains, the water quality in the study pits were suitable for *An. dirus* (Kitthawee *et al*, submitted). Their density was maintained throughout the rainy season because clean water conditions were maintained. When the rains stopped, the water in the pits became stagnant. These dry season conditions favored *Culex* and *Uranotaenia* to replace *An. dirus* during the dry season.

Table 5

The association of predaceous Notonectidae and fish (guppy) with all mosquito larvae and pupae except *Anopheles* in selected pits. See Table 1 for selection and grouping of the particular pits by numbers of *An. dirus* pupae. Letter D indicates pits were dry during the monthly sampling period.

		Nov 1987	Dec	Jan 1988	Feb	Mar	Apr	May	Jun	Total
Pit X	Mosquitos (L)						1			1
	Mosquitos (P)									0
	Notonectidae	9	1	4	3	9	4	1		31
	Fish (guppy)		2	4	3					9
Pit E	Mosquitos (L)							3	1	4
	Mosquitos (P)									0
	Notonectidae	1								1
	Fish (guppy)	3	7	4	10					24
Pit T	Mosquitos (L)		1	18	4	1				24
	Mosquitos (P)			8					1	9
	Notonectidae	1	9		6	3	4	1		24*
Pit NN	Mosquitos (L)	8	>37	14	>107	>39	33	>77	>15	>330
	Mosquitos (P)	2	27	5	17	5	9	21	2	88
	Notonectidae		3				1			4*
Pit PP	Mosquitos (L)					1				1
	Mosquitos (P)									0
	Notonectidae	>21	2	6	14	2			1	46
Pit C	Mosquitos (L)						D			0
	Mosquitos (P)		1	2			D			3
	Notonectidae	11	14	6	4	15	D			50
Pit H	Mosquitos (L)		4	1	6	2	D	11	>22	46
	Mosquitos (P)			1			D	18		19
	Notonectidae		17	3	10	3	D			33*
Pit D	Mosquitos (L)									0
	Mosquitos (P)									0
	Notonectidae	2	6	4	4					16
Pit M	Mosquitos (L)		D	D	D	D	D	D		0
	Mosquitos (P)	2	D	D	D	D	D	D	2	4
	Notonectidae		D	D	D	D	D	D		0
Pit O	Mosquitos (L)					D	D	D	D	0
	Mosquitos (P)		22		1	D	D	D	D	23
	Notonectidae					D	D	D	D	0

* none were present during months that mosquito pupae were produced.

ACKNOWLEDGEMENTS

We thank Mr Kol Mongkolpanya at the AFRIMS laboratory for the verification of the identification of our mosquitos. This study was supported by the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

Table 6

Adult mosquitos emerging from pupae collected in study gem pit each month.

Mosquitos	1986 Nov	Dec	1987 Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jan	Total
An. dirus	96	36	54	15	1	1	11	55	40	110	66	84	49	35	13	18	*	3**	47	7	741
Other *Anopheles*	1	7	13	5								3	1	14	8	3					55
Culex spp	3	36	83	209	139	355	146	161	48	47	49	16	6	54	57	58	19*	17	51	32	1,586
Uranotaenia spp	4	11	25	37	24	31	11	8		8		2		41	29	12	17	11	1		272
Unidentified	11	17	23	50	31	185	515	227	6	25	8	8	10	26	16	27	13	2	20	2	1,222
Total	115	107	198	316	195	572	683	451	94	190	123	113	66	170	123	118	49	33	119	41	3,876

* +13 *An. dirus* from extra pits : AA, BB, CC
+4 *Culex* and 1 unidentified from pit BB
** +8 *An. dirus* from pit CC

REFERENCES

Barraud PJ. The Fauna of British India including Ceylon and Burma. Diptera Vol V Family Culicidae Tribes Megarhinini and Culicini. London : 1934; pp 463.

Bram RA. Contribution to the mosquito fauna of Southeast Asia-II, The genus *Culex* in Thailand (Diptera: Culicidae). *Contrib Am Entomol Inst* 1967; 12 : 1-296.

Colles DH. The *Anopoheles leucosphyrus* group. *Trans R Entomol Soc Lond* 1957; 108 : 37-116.

Harrison BA, Scanlon JE. Medical entomology studies-II, The subgenus *Anopheles* in Thailand. *Contrib Am Entomol Inst* 1975; 12 : 1-307.

Harinasuta T, Gilles M, Sandosham AA. Malaria in Southeast Asia. *Southeast Asian J Trop Med Public Health* 1976; 7 : 645-78.

Kittawee S, Edman JD, Upatham ES. Effect of seasonal variation in the aquatic habitat of immature *Anopheles dirus* (Diptera: Culicidae) on the size and survivorship of emerging adults. (submitted)

Malaria Division. The Malariology 2525 (in Thai). Malaria Division. Bangkok : Public Health in Thailand Press, 1982; pp 254.

Merritt RW, Cummins KW. An Introduction to the Aquatic Insects of North America. Dubuque, Iowa : Kendall/Hunt Publ 1984; pp 722.

Peyton EL, Harrison BA. *Anopheles (Cellia) dirus*, a new species of the Leucosphyrus group from Thailand (Diptera: Culicidae). *Mosq Syst* 1979; 11 : 40-52.

Reinert JF. Medical entomology studies-V, The subgenus *Rhinoskusea* of the genus *Aedes* (Diptera: Culicidae). *Contrib Am Entomol Inst* 1976; 13 : 1-60.

Scanlon JE, Sandhinand U. The distribution and biology of *Anopheles balabacensis* in Thailand. *J Med Entomol* 1965; 2 : 61-9.

Sirivanakarn S. Medical entomology studies-III, A Revision of the subgenus *Culex* in the oriental region (Diptera: Culicidae). *Contrib Am Entomol Inst* 1976; 12 : pp 272.