



## Breeding habit and habitat of *Anopheles* mosquitoes in forest fringe and plain areas in Myanmar

Maung Maung Mya<sup>1</sup>, Phyo Wai Win<sup>2</sup>, Aye Mya Thanda<sup>3</sup>, Thiha<sup>4</sup>, Maung Maung Gyi<sup>5</sup>, Myin Zu Min<sup>6</sup>, Yan Naung Maung Maung<sup>7</sup>

<sup>1, 4, 7</sup> Medical Entomology Research division, (DMR) Ministry of Health and Sports, Yangon University, Ministry of Education, Myanmar

<sup>2-3, 5-6</sup> Zoology Department, Yangon University, Ministry of Education, Myanmar

### Abstract

Malaria remains a major public health problem in Myanmar. *Anopheles* larvae investigating study was conducted seasonally in Thayetchaung village, Bawbin dam areas, Bago division and Maybinther villages Myothit Township Magwe Division from January to December 2016. Larval survey was done in and around the all possible water bodies such as water pools, sand pools, water pockets, creeks, foot prints, ponds, Dam and pools along the sand banks of creeks and rivers were investigated within 3 kilo meters radius from surrounding areas of both villages. Year round data showed that a total of 513 and 311 habitats were investigated and found to be 96 (18.71%) and 51(16.39%) habitats were positive for *Anopheles* larvae in Bawbin and Maybinther respectively. 87.63 and 92.31% of the larvae collected from survived were emerged to adults. Eight breeding habitats from Bawbin and 5 breeding habitats were observed from Maybinther village respectively. After identification, *An. maculatus*, *An. annularis*, *An. vagus*, *An. culicifacies*, *An. minimus* and *An. stephensi* larvae were observed in both areas but only *An. philippnansis* larvae was collected in water pools, rice field and dam in Bawbin area although *An. philippnansis* and *An. babiostrois* larvae were lacked in Maybinther village in all season. The highest numbers of *Anopheles* larvae were collected in sand pools followed by water pools in cold season. Highest number of *An. vagus* larvae was collected in ponds and foot prints followed by *An. maculatus* in water pools and water pockets in raining season. *An. culicifacies* was found in high density in sand pools of creek in cold season in both areas. High number of *An. annularis* was present in water pools in cold season in Maybinther village. Low number of *An. annularis*, *An. vagus* and *An. culicifacies* larvae were observed in dry season in both areas. The main vector of malaria such as *An. minimus* was observed in rice field and send pool in Bawbin and water pool and sand pool of creek in Maybinther villages in raining and cold season. Density of collected *An. culicifacies* larvae was much higher in Maybinther, 2.9 fold higher than Bawbin village. This study concluded that water pools, water pockets and sand pools creeks might play a significant role in rural areas to breed high number of *Anopheles* mosquitoes in both study areas followed by foot prints, canals, dam, rice fields and ponds. It is hoped that the information about the distribution of larvae in the breeding habitats was gathered from this study will help to broaden the understanding with regards to the geography, biology and ecology of mosquito breeding sites, and thus effective and efficient larval control measures can be applied.

**Keywords:** *Anopheles* larvae, breeding, habit, habitat, identification

### Introduction

Malaria remains a major health problem in Myanmar. According to Ministry of Health (MOH) malaria has been a first and second priority publication health problem in Myanmar and there are 600,000 malaria cases annually. Morbidity rate in 2006 is 9.1/1000 and mortality rate is 2.97/100000 population (Ministry of Health 2006). *Plasmodium falciparum* is the predominant species but now *P. vivax* is gradually rising in Myanmar. (Myint Oo *et al.* 1998). These parasites are transmitted by *Anopheles* mosquitoes. Although more than 37 *Anopheles* mosquitoes species are existing in Myanmar, only few species are involved in the transmission of malaria. *An. dirus* and *An. minimus* are the main vectors of malaria but *An. maculatus*, *An. vagus*, *An. annularis*, *An. philippnensis*, *An. stiphensi* and *An. culicifacies* are secondary or suspected vectors of malaria in Myanmar. The *Anopheles* mosquitoes breeding sites very greatly depending on the climatic conditions and availability of water, this include water pools, water pockets, Dam, canals, ponds, wells, rice fields, man make thanks and water storage containers. Information on

the breeding habit is essential for conduction of anti-larval operation. In Myanmar *An. mimus* and *An. dirus* are considered to be one of the most efficient malaria vectors due to their ability to adapt so rapidly. Previously *An. minimus* was found foothill and forest fringe but now it is found in water pools, sand pools, slowly running water, and rice field in plain areas. *An. dirus* species are also bred in deep forest areas but now their larvae were found in men made wells in Mon State and Thanintheyi Region (Tun Lin *et al.*, 1988, Htay Aung *et al.*, 2005) <sup>[40, 3]</sup>, because certain environmental changes like deforestation and vegetation clearance for crop plantations and also rapid growth of population and installation of new rural areas may lead to an increase abundance of mosquito larval habitats [Rohani *et al.*, 2010, Rahman *et al.*, 1992] <sup>[31]</sup>.

In Malaysia, breeding habitats of *An. maculatus* were located at 100-400 m from human settlement, more than 80% of *Anopheles maculatus* s.s. immature habitats were found in ground pools, rock pools and water pockets within the buffer zone of Pos

Lenjang, Kuala Lipis, Pahang, Malaysia (Rohani *et al.*, 2011)<sup>[31]</sup>. *An. maculatus* are main vector of malaria in Malaysia but it is a secondary vector of malaria in Myanmar and widely distributed in hilly and plain areas of Southeast Asia including Myanmar. *Anopheles culicifacies* species complex are widespread distribution in Asia, from Iran, Afghanistan and Pakistan in west and India to Bangladesh, Myanmar and Thailand in the east. It is also found in Nepal and Southern China in the North and Sri Lanka in South (Subbarao *et al.*, 1988)<sup>[35]</sup>. *An. culicifacies* is a complex of 5 sibling species designated as species A,B,C,D and E. species B is not the vector but all other species transmit malaria, although their vectorial capacity varies greatly (Subbarao and Sharma 1997)<sup>[36]</sup>. It is the vector of Rural and peri urban malaria in the peninsular India (Sharma *et al.*, 1999)<sup>[34]</sup>. Species A and D are efficient malaria vectors, whereas species B is regarded as a poor vector of malaria in India. (Subbarao *et al.*, 1988)<sup>[35]</sup>. In Sri Lanka, however, species B has long been considered to be an important malaria vector. *An. culicifacies* of A-E have been detected in Sri Lanka with species E being incriminated as the major malaria vector of *P. falciparum* and *P. vivax* (Surendran *et al.* 2000, Surendran *et al.* 2006)<sup>[37, 38]</sup>. *An. culicifacies* is the secondary vector of malaria (Giles 1901) and widely distribution in Myanmar (Khin Maung Kyi 1972, Myo Paing *et al.*, 1990a,<sup>[12, 23]</sup>). Each sibling species may have its own distribution and biology. Large number of sibling species B of *An. culicifacies* adult and larvae were found in hyper-endemic foothill areas of Paukaung Township, Bago Regional Division in Myanmar (Maung Maung Mya *et al.*, 2009)<sup>[19]</sup>. *An. culicifacies* is a fresh water breeder in Myanmar (Khin Maung Kyi 1972)<sup>[12]</sup> and India (Sharma 1999)<sup>[34]</sup> but in Sri Lanka *An. culicifacies* sibling species E is breeding in Brackish waters (Jude *et al.*, 2010)<sup>[9]</sup>. The present study is done to search breeding habitats and distribution of breeding sources of *Anopheles* mosquitoes in Myanmar.

## Materials and Methods

### Study areas

Maypyinthar village, Myothit Township Magway Region was selected for the collection of *Anopheles* larvae. Maypyinthar village is situated beside Magwe Naypyidaw road. The village is 10 kilometer away from Son dam. Myaypyinthar Myothit car road is across the village. The population is about 2000, 90% of the people are plantation workers, remaining are government workers, hunters, fishers and school teachers. One primary school and one monastery school are situated in the village.

Thayet Chaung village is situated beside the Bowbin dam; it is 15 kilometer away from Pyiy –Paukaung road. The population is about 1000, 90% of the populations are farmers remaining are fishers, Government dam staff, and school teachers. One primary school and one monastic primary school are situated in the village. The study was conducted seasonally for one year.

### Study periods

*Anopheles* larvae were collected from two different areas during January to December 2016.

### Study design

The study was done in combination with field bases *Anopheles* larvae collection and laboratory based larvae rearing and species identification. This is a descriptive study.

## Larva sampling method

The study was done seasonally from January to December 2016. Larval survey was done in and around the all possible bodies of water such as water pools, sand pools, water pockets, creeks, foot prints, ponds, Dam and pools along the sand banks of creeks and rivers were sampled within 3 kilo meters radius from surrounding areas of both Thayetchaung village, Bawbin dam area, Bago division and Maybinther villages Myothit Township Magwe Division for 5 days. All potential habitats were inspected systematically for the presence of mosquito larvae. When mosquito larvae were present, a standard mosquito dipper was used to collect the larvae by lowering it gently into the water. The water was poured in 14 x 12 inch white plastic tray and carefully observed for the presence of mosquito larvae. Then, larvae were collected alive by means of a pipette and transferred to a labeled plastic bag. Each bag was filled oxygen by oxygen pump then covered tightly to prevent spillage and to ensure that the larvae collected remained alive and undamaged as they were transported to the insectarium. The larvae collected were reared in the insectarium in white plastic tray on a diet of DMR larva food + ground ox liver. Mosquito larvae collected in the survey were reared to adults and emerged adults from survey were identified by species using standard taxonomic keys. 95.2% of the collected larvae were survived to adults.

## Mosquitoes species identification

Adult mosquitoes emerged from larva survey from different breeding places were identified by morphological methods according to Barraud 1934<sup>[3]</sup>, Raid 1967<sup>[27]</sup>, Deifinado 1966, Harrison 1980, and Myo Paing *et al.*, 1990a, b<sup>[23]</sup>.

## Analysis of data

Data entry and analysis were carried out using Microsoft excel software.

## Results

Table (1) shows the distribution of *Anopheline* mosquito breeding places in two study areas seasonally. In Bawbin area, out of 562 breeding habitats were examined and found that 38/233(16.3%), 39/211(18.4%) and 18/118(15.25%) were positive for *Anopheles* larvae in raining, cold and dry season respectively and in Maybinther village, out of 316 breeding habitats were examined 16/132(12.12%) in raining season, 27/119(22.68%) in cold season and 6/65(9.23%) in dry season were positive for *Anopheles* larvae. *Anopheline* larvae were found in 95 habitats in Baw bin and 51 in Maybinther villages, of which 92 (96.84%) and 50 (98.04%) of these had only *Anopheline*. *Culicine* larvae were found in 7 habitats in Bawbin and 5 habitats in Maybinther village, and 5 (71.4%) and 4(80%) of these habitats had only *Culicine*. Both *Anopheline* and *Culicine* larvae were found in 2(22.22%) habitats in Bawbin and 1 (16.67%) habitat in Maybinther, suggesting that the mosquito larvae from the subfamilies *Culicinae* and *Anopheline* coexisted in some of the habitats surveyed. Out of 9 species of *Anopheline* larvae were collected, four species (*An. maculatus*, *An. annularis*, *An. vagus* and *An. culicifacies*) were found in both Bawbin and Maybinther in all season during the survey except *An. maculatus* was lacked in dry season in Maybinther and these species coexisted in 3 breeding habitats( Table 2&3). The highest number of breeding site for *Anopheline* mosquito larvae was found in clear water pool

followed by water pocket in Bawbin and sand pools of creek bands followed by water pool in Maybinther. The most common breeding site for *Culicine* larvae was observed in rock pool with full of leafy debris in water. The most common breeding site where *Anophele* and *Culicine* mosquito larvae co-existed was clear rock pool in both areas. *An. maculatus* was present in six out of eight habitat types in Bawbin and 2 out of 6 habitat types in Maybinther in clear rock pools. *An. minimus* was not found in cloudy ground pool, muddy ground pool and cloudy rock pool. The most common larval habitat for *An. minimus* and *An. culicifacies* were clear sand pools.

A total of 238 *Anopheles* larvae 23 *Culex* and 57 *Aedes* larvae were collected from Bowbin Dam area Bago Region and 349 *Anopheles* 17 *Culex* and 43 *Aedes* larvae were collected from Maybinther village, Myothit Township Magwe Regional Division during January 2014- December 2014. Larvae from both areas were carried to DMR laboratory and reared till to adult. Among these emerged adults mosquitoes, 188 *Anopheles*, 18 *Culex* and 31 *Aedes* from Bowbin and 219 *Anopheles* 11 *Culex* and 23 *Aedes* from Myabinther were identified as 8 species of *Anopheles*, 1 species of *Culex* and 2 species of *Aedes* in different breeding places in Bawbin dam areas and 7 *Anopheles*, 1 *Culex* and 2 *Aedes* species were collected in Maybinther village, Myothit Township, Magwe Regional Division Table 2 & 3). Mosquitoes species,

Detail mosquitoes larvae collection in Bawbin dam area, Bago Regional Division and Maybinther village, Myothit Township Magwe Regional Division were shown in Table (1).

Bawbin dam area, Bago Regional Division

Table (2) shows that in Bawbin dam area a total of 188 *Anopheles* mosquitoes emerged from larvae were collected. Of these 76 *Anopheles* from 7 species of *Anopheles* larvae in raining season, 79 *Anopheles* mosquitoes from 8 species of *Anopheles* larvae in cold season and 33 *Anopheles* from 5 species of *Anopheles* larvae in dry season were collected. In raining season, 7 species of *Anopheles* larvae as 18 *An. maculatus*, 7 *An. annularis*, 28 *An. vagus*, 14 *An. culicifacies*, 5 *An. minimus*, and 4 *An. babilosis* were collected in raining season.

8 species of *Anopheles* larvae as 15 *An. maculatus*, 17 *An. annularis*, 7 *An. vagus*, 28 *An. culicifacies*, 6 *An. minimus*, 4 *An. philippnensis*, 4 *An. babilosis* and 1 *An. stephensi* were collected in cold season.

5 species of *Anopheles* larvae as 8 *An. maculatus*, 2 *An. annularis*, 10 *An. vagus*, 11 *An. culicifacies*, and 2 *An. philippnensis* were collected in dry season.

Maybinther village, Myothit Township Magwe Regional Division

In Maybinther village a total of 219 *Anopheles* mosquitoes emerged from larvae were collected. Of these 41 *Anopheles* from 5 species of *Anopheles* larvae in raining season, 166 *Anopheles* mosquitoes from 6 species of *Anopheles* larvae in cold season and 12 *Anopheles* from 3 species of *Anopheles* larvae in dry season were collected. In raining season, 5 species of *Anopheles* larvae as 7 *An. maculatus*, 7 *An. annularis*, 5 *An. vagus*, 15 *An. culicifacies* and 5 *An. minimus*, were collected in raining season. 6 species of *Anopheles* larvae as 4 *An. maculatus*, 26 *An. annularis*, 6 *An. vagus*, 125 *An. culicifacies*, 1 *An. minimus* and 3 *An. babilosis* were collected in cold season.

3 species of *Anopheles* larvae as 2 *An. annularis*, 3 *An. vagus*, and 7 *An. culicifacies*, were collected in dry season.

*Culex quinquefasciatus* larvae were collected from polluted block water pits and polluted water pools in all season. *Aedes aegypti* larvae were collected from house hold used water storage containers and *Aedes albopictus* larvae were collected from bamboo stamps in raining and cold season although in dry season they were found in water pools which were full with bamboo leaves. Sometime *Aedes aegypti* and *Aedes albopictus* larvae were collected together from bamboo stamps and water storage containers made of earthen in both areas.

### Breeding habitat of *Anopheles* mosquitoes

Breeding of *Anopheles*, *Culex* and *Aedes* species in each habitat, as water pools, water pockets, irrigation canals, dam, rice fields, foot prints, ponds, sand pools, and creek for *Anopheles* larvae, polluted water pits, polluted water pools and creeks for *Culex* and water storage containers and bamboo stamps for *Aedes* mosquitoes larvae were investigated within 3 km away from study villages during the whole study periods. Detail of larval density and occurrence in different habitat types were shown in Table (3).

*An. maculatus* : larvae were collected from water pools, water pockets, canals in all season although the larvae were collected from dam and rice field in raining season and sand pools of creek in dry season in Bawbin area. In Maybinther village the larvae were collected from water pool in raining and cold season and water pocket in raining season but the larvae were lacked in dry season in both habitat types.

*An. annularis*: larvae were collected from water pools and water pockets in raining and cold seasons and also collected from dam in raining and dry seasons. The highest number of larvae was collected in from water pool in cold season in Bawbin area. In Maybinther village *annularis* larvae were collected from water pool in all season, the highest numbers of larvae were observed in water pools of rocky area.

*An. vagus*: larvae were found in foot prints and ponds in raining and colds season and also found in water pool and rice fields in raining and water pockets in dry seasons. Highest number of larvae was collected from cloudy pond in raining followed by water pocket in dry season. In Maybinther village the larvae were collected from foot prints in raining season although larvae were found in pond in cold season and water pocket and sand pools of creek in dry season. *An. culicifacies*: the larvae were collected from sand pools of creek in all season in both Bawbin and Maybinther village, although larvae were collected from water pockets in raining and dry season in Bawbin and dry season in Maybinther village. The highest numbers (28 from Bawbin and 125 from Maybinther) of larvae were collected from sand pool of creeks in cold season. *An. minimus*: The larvae were found in sand pools of creeks in raining and cold season from both study areas and also the larvae were collected from rice fields in raining and cold season in Bawbin area but from water pool in raining season in Maybinther village. The highest number of main malaria vector *An. minimus* larvae were collected in sand pool of creek in cold season from Bawbin area.

*An. philippnensis*: one larva from water pools and 3 larvae from dam were collected in cold season and 2 larvae were collected from rice fields in dry season but in Maybinther village the larvae were lacked in all breeding places in all seasons.

*An. babirostris*: the larvae were collected from canal and pond in raining and 1 larva was collected from dam in Bawbin and 3 larvae were collected from pond from Maybinther village.

*An. kawari*: the larvae were absent in all breeding places in all season in both study areas.

*An. stephensi*: one larva each was collected from water pool from Bawbin and footprint from Maybinther in cold season.

Mosquitoes breeding places

Eight types of mosquitoes breeding places were investigated during the study period. Table (2) shows breeding places and breeding habit of *Anopheles* mosquitoes. The highest numbers of anopheles larvae were collected from water pools followed by

pond, water pocket send pool and rice field in raining season but in cold season, the highest number of anopheles larvae were collected from sand pool of creek followed by water pool. In dry season, the highest numbers of larvae were collected from water pocket followed by sand pool in Bawbin dam area. Only four types of *Anopheles* mosquitoes breeding places (as water pool, water pocket, foot print, pond and sand pools) were observed in Maybinther village. The highest numbers of *Anopheles* larvae were collected from sand pools of creeks followed by water pools in raining and cold season but in dry season highest numbers of larvae were collected from water pocket followed by sand pools.

**Table 1:** Seasonal distribution of *Anopheles* breeding habitats and larvae positive habitats in two study areas

Sr. No.	Breeding Habitats and larva positive habitats	Bawbin Dam area by Seasonally (Bago Region)				Maybinthar by Seasonally (Magway Region)			
		Raining	Cold	Dry	Total	Raining	Cold	Dry	Total
1	Water Pool (+ habitats)	36	25	9	70	17	17	11	45
		8	9	3	20	5	7	1	13
2	Water pocket (+ habitats)	56	36	26	118	25	20	16	61
		8	5	4	17	3	--	4	7
3	Canna (+ habitats)	5	5	5	15	--	--	--	--
		2	2	1	5	--	--	--	--
4	Dam (+ habitats)	10	10	10	30	--	--	--	--
		2	3	5	10	--	--	--	--
5	Rice field (+ habitats)	21	22	22	65	--	--	-	--
		6	1	2	9	--	--	-	--
6	Foot prints, (+ habitats)	54	36	12	102	51	35	18	104
		3	2	-	5	3	3	-	6
7	Ponds (+ habitats)	5	5	3	13	3	3	3	9
		3	1	--	4	0	2	--	2
8	Creek (sand-pools) (+ habitats)	26	43	31	100	31	44	17	92
		6	15	5	26	5	15	3	23
Total habitats (+ habitats)		213	182	118	513	127	119	65	311
		38	38	20	96	16	27	8	51

**Table 2:** Breeding habit of *Anopheles* mosquitoes in different breeding place of Bawbin Dam areas, Bago Regional Division and Myanbinthar village, Magwe Regional Division

Sr. No.	Apecies	Bawbin Dam area by Seasonally				Maybinthar by Seasonally			
		Raining	Cold	Dry	Total	Raining	Cold	Dry	Total
1	Water pools	5An. maculatus, 5An.annularis, 8An.vagus	7An. maculatus, 14An.annularis, 1An.philippnensis, 1 An. stiphensi	2An. maculatus, 1An.annularis	14An.maculatus, 20An.annularis, 4An.vagus, 1An.philippnensis, 1An.stiphensi	1An. maculatus, 9An.annularis, 3An.minimus	4An. maculatus, 26An.annularis	2An annularis-	5An. maculatus, 37An.annularis, 3An.minimus
2	Water pocket	6An. maculatus, 1An.annularis, 5An.culicifacies	3An. maculatus, 3An.annularis,	2An. maculatus, 10An.vagus, 3An.culicifacies	11An.maculatus, 14An.annularis, 10An. Vagus, 8An.culicifacies	6 An. maculatus	--	2An. vagus, 4An.culicifacies	6An. maculatus, 2An.vagus, 4An.culicifacies
3	Canna	2An. maculatus, 1An.babirostris	5An. maculatus	1An. maculatus,	8 An.maculatus, 1An.babirostris	--	--	--	--
4	Dam	2An. maculatus, 1An.annularis	3An.philippnensis, 1An.babirostris	1An. annularis	2An.maculatus, 2An.annularis, 3An.philippnensis, 1An.babirostris	--	--	--	--
5	Rice field	3An. maculatus, 5An.vagus, 3An. minimus	1 An. minimus	2An.philippnensis	3An.maculatus, 5An.vagus, 2An.philippnensis, 4An.minimus	--	--	-	--

6	Foot prints,	4 An. vagus	2 An. vagus	-	6An. vagus	5An.vagus	3 An.vagus, 1An.stephensi	-	8An.vagus, 1An.stephensi
7	Ponds	11 An. vagus, 3An.babirostris	5An.vagus	--	16An.vagus, 3An.babirostris	--	3 An. babirostris	--	3 An. babirostris
8	2Creek(sand-pools)	9 An. culicifacies, 2An.minimus	28An.culicifacies, 5An.minimus	3An. maculatus, 8An.culicifacies	3An.maculatus, 45An.culicifacies, 7An.minimus	15An.culicifacies, 2An.minimus	125An.culicifacies, 1An.minimus	3An.culicifacies, 1An.vagus	143 An.culicifacies, 3An.minimus 1An.vagus

**Table 3:** Seasonal breeding places (habitat) and different number of *Anopheles* mosquitoes emerged from larva survey in Bawbin Dam areas, Bago Region and Myanbinthar village, Magway Region

Sr. No.	Species	Bawbin Dam area by Seasonally			Maybinthar by Seasonally		
		Raining	Cold	Dry	Raining	Cold	Dry
1	An. maculatus	5Water pools, 6Water pocket, 2Cannal, 2Dam, 3Ricefield	7 Water pools, 3Water pocket, 5Cannal	2 Water pools, 2Water pocket, 3 Creek(sand-pools) 5Cannal	1Waterpools, 6Water pocket,	4 Water pools	-
2	An. annularis	5Water pools, 1Water pocket, 1Dam,	14Water pools, 3Water pocket,	1Water pools, 1Dam,	9 Water pools	26 Water pools	2Water pools
3	An.vagus	8Water pools, 5Ricefield, 4Foot prints, 11Ponds	2Foot prints, 5Ponds	10Water pocket,	5Foot prints,	3Foot prints, 3Ponds	2Water pocket, 1Creek(sand-pools)
4	An.culicifacies	5Water pocket, 9Creek(sand-pools)	28 Creek(sand-pools)	3Water pocket, 8Creek(sand-pools)	15 Creek(sand-pools)	125 Creek(sand-pools)	4Water pocket, 3Creek(sand-pools)
5	An. minimus	3Ricefields, 2Creek(sand-pools)	1Ricefields, 5Creek(sand-pools)	-	3Water pools, 2Creek(sand-pools)	1Creek(sand-pools)	-
6	An. philippnansis	--	1Water pools, 3Dam,	2 Rice fields	-	-	-
7	An. babirostris	1Cannal, 3Ponds	1 Dam	--	--	--	--
8	An.kawari	--	--	--	--	--	--
9	An. stephensi	-	1 Water pools	-	-	1 Foot print	-

## Discussion

The result of the present study found that 8 types of anopheles species larvae from Bawbin and 7 types of anopheles species larvae from Maybinthar village were observed during the study period. One type of *Culex quinquefasciatus* larvae and 2 types of *Aedes* species as *Aedes aegypti* and *Aedes albopictus* larvae were collected from both study areas. *Anopheles maculatus* was present in six out of eight habitat types in Bawbin and 2 out of 6 habitat types in Maybinthar in clear rock pools. Rohani *et al.*, (2011)<sup>[31]</sup> revealed that *An. maculatus* was highly present in clear water pools followed by clear water pocket, muddy water pocket and muddy rock pool. The main vector, *An. minimus* was not found in cloudy ground pool, muddy ground pool and cloudy rock pool. The most common larval habitat for *An. minimus* and *An. culicifacies* were clear sand pools (Tun Lin *et al.*, 1995, Maung Maung Mya *et al.*, 2009, 2016)<sup>[19]</sup>. *Anopheles vagus* larvae was found in cloudy water habitats as foot prints, rice fields and ponds water and also the larvae were found in clear water of water pools and water pockets. Similar results has been found in Thabyewa village (Tun Lin *et al.*, 1995)<sup>[40]</sup>.

*An. minimus* was found in both areas in raining and cold season but absent in dry season which are main causative vector of malaria in Myanmar (Khin Maung Kyi 1972, Myo Paing *et al.*, 1989, Tun Lin *et al.*, 1995)<sup>[12, 25, 40]</sup>. Other researchers also revealed that *An. minimus* larvae were collected in Gonminsoe village Paukaung Township Bago Division and Madaya Township, Mandalay Division and Katine Htit village Kamamaung Township, Kayin State (Maung Maung Mya *et al.* 2009, 2016)<sup>[19]</sup>. Highest density of *An. culicigacies* larvae were

collected from sand pools of creek bands in Maybinthar village, it was 125/28= 4.46 fold higher than Bowbin in cold season. It may be due to the facts that female *An. culicifacies* mosquitoes are highly favorable to oviposit in sand pools and also found in water pocket in both study areas. Same results had been found in Thabyawa village Oktwin Township, Gonminsoe village Paukaung Township, Bago Division (Myo Paing *et al.*, 1990b, Tun Lin *et al.*, 1995, Maung Maung Mya *et al.*, 2009, 2016)<sup>[24, 40, 19]</sup>. Present study found that *An. minmus* and *An. culicifacies* larvae were bred together in a sand pool from Maybinthar Township in cold season. Same result has been found in Yunsalin creek in Kamamaung Township Kayin State (Maung Maung Mya *et al.*, 2016)<sup>[19]</sup>. *An. culicifacies* are secondary or suspected vector for malaria in Myanmar but the species are major vector of malaria in India, Pakistan and Sri Lanka (Subbarao *et al.*, 1988, Mahmood *et al.*, 1984, Abhayawardana *et al.*, 1996)<sup>[35, 14, 1]</sup>. *An. culicifacies* larvae is generally regarded to be intolerant of salinity (Sabesan *et al.*, 1986)<sup>[33]</sup>, preferring to breed in newly-dug fresh water pits (Russell and Rao 1942)<sup>[32]</sup> domestic wells and pits used for plantation of coconuts and casurina (Briet *et al.*, 2005)<sup>[4]</sup> in India however *An. culicifacies* larvae have been collected from brackish water in Oman and Sri Lanka (Jude *et al.*, 2010)<sup>[9]</sup> although they survived best in fresh water (Robert 1996)<sup>[29]</sup>. Previous study in Sri Lanka have reported that *An. culicifacies* breed only in fresh water bodies confined to the riverine system of the country (Carter 1930, Amerasinghe *et al.*, 1995)<sup>[5, 2]</sup>. Present study found that *An. culicifacies* breed only in fresh water with high dissolved oxygen, pH 7 and 21-27°C in sand pool of creek bands however well established in India (Reuban *et al.*,

1984)<sup>[28]</sup> and Sri Lanka (Kannathasan *et al.*, 2008)<sup>[10]</sup>. The optimum temperature for mosquito survival is in the range of 20-25 °C (Molineaux 1988, Martens *et al.*, 1995)<sup>[21, 15]</sup>. The water temperature of the present study was not much difference from their favorable temperature.

*An. culicifacies* larvae was collected in high number during January to May in Bago division according to the finding of Khin Maung Kyi 1972<sup>[12]</sup>. Larval study of different authors found that a large number of *An. culicifacies* larvae in sand pool along the creek band of Thabyewa creek, Okktwin Township and the creek beside the Gonminsoe village in Paukkaung Township, Bago division and sand bands of along the Sedawgyi canal, Madaya Township, Mandalay Division in non-monsoon months of October to December (Myo Paing 1990a, Tun Lin *et al.*, 1995, Maung Maung Mya *et al.*, 2012)<sup>[23, 40, 18]</sup>.

*An. maculatus*, *An. annularis* and *An. vagus* (which are secondary or suspected vectors of malaria) larvae were collected high number from water pool, water pocket, canals in all season in fresh water pH 7-7.3, and temperature was 21-28 °C in both study areas except *An. maculatus* was absent in hot season in Maybinthar village. It may be due to the breeding places of *An. maculatus* were dried in hot season and some breeding places of water pools, and water pocket had 37-39 °C in hot season which are much higher than mosquitoes favorable temperature (20-25 °C). *An. maculatus* were also found in dam and rice field in Raining season and sand pool of creek in hot season in Bawbin area. *An. maculatus* larvae were found together with *An. annularis* larvae in one of water pool and also found together with *An. vagus* larvae in one of the water pocket and together with *An. culicifacies* larvae in sandy based water pocket in dry season in Bawbin area but *An. maculatus* larvae were found together with *An. minimus* in one breeding place of water pool in raining season in Maybinther village in Magwe regional division. Other researcher found that *An. maculatus* and *An. minimus* larvae were collected together in one small jungle stream in Taikkyi Township Yangon division (Maung Maung Mya *et al.*, 2002)<sup>[16]</sup>. Mostly *An. vagus* larvae were collected from foot print of cow, buffalo and human, ponds, rice field and water pools in cloudy water in raining season although larvae were bred in clean water (sediment water) in foot prints and ponds in cold season but in dry season larvae were found in water pockets and sand pools of the creek due to the fact that dryness of all the foot prints and ponds in hot season. The same types of the *Anopheles* breeding place were observed in Thabyewa village (Myo Paing *et al.*, 1989, Tun Lin *et al.*, 1995)<sup>[25, 40]</sup>. Some researcher observed that *An. maculatus* larvae were found together in well breeding *An. dirus* in Thaninthayee Division (Htay Aung *et al.*, (1999)<sup>[8]</sup>. *An. maculatus* is the principal vector of human malaria in peninsular Malasia (Loong *et al.*, 1988; Vythilingam *et al.*, 1995)<sup>[13, 41]</sup>. But it is a secondary vector of malaria in Myanmar. *An. philippnensis* which are main vector of malaria in Philippine and Malaysia (Delfinado 1966)<sup>[6]</sup>, the larvae were found small number in Dam, water pool in cold season and only 2 larvae in rice fields in dry season Bawbin but the larvae were lacked in all season in Maybinther village. Only one *An. stephensi* larva each was collected in cold season in both areas one was collected from water pool in Bawbin and one was collected from foot print in Maybinther. *Anopheles philippnensis* and *An. stephensi* are suspected vectors of malaria in Myanmar (Khin Maung Kyi 1970)<sup>[11]</sup>. *Anopheles babirostris* larvae were found in both areas

but this species are not vector of malaria (Barraud 1934)<sup>[3]</sup>. The present study carried out all season during the year. Where, the highest numbers of *Anopheles* larvae were collected in cold season followed by raining season but lowest number was collected in dry season in both areas. It is suggested that remaining some rocky water pools, water pockets and creeks provide breeding sites during the dry season. In dry season, *Anopheles* larvae were rarely collected (12 number or 3 *Anopheles* species) than other season in Maybinther village because in hot season the temperature was relatively high (39-41 °C) and most of the mosquitoes breeding grounds were dried up due to hot in Maybinther village Magwe Division. This study concluded that water pools, water pockets and sand pools creeks might play a significant role in rural areas to breed high number of *Anopheles* mosquitoes in both study areas followed by foot prints, canals dam rice fields and ponds. It is hoped that the information about the distribution of larval in the breeding habitats was gathered from this study will help to broaden the understanding with regards to the geography, biology and ecology of mosquito breeding sites, and thus effective and efficient larval control measures can be applied.

## References

1. Abhayawardana TA, Dilrukshi, Wijesuriya RKC. Cytotaxonomical examination for sibling species in the taxon *Anopheles culicifacies* Giles in Sri Lanka. Indian Journal of Malariology. 1996; 33:74-80.
2. Amerasinghe FS, Indrajith NG, Ariyasena TG. Physico-chemical characteristics of mosquito breeding habitats in an irrigation development scheme in Sri Lanka. Ceylon Journal of Science (Bio Sci). 1995; 2:13-29.
3. Barraud PJ. The fauna of British India, including Ceylon and Burma, Diptera V, family *Culicidae*. Tribas Megarhini and Culicini Taylor and Francis, London, 1934.
4. Briet OJT, Galapathy GNL, Amerasinghe PH, Amerasinghe HP. Maps of the Sri Lanka malaria situation Preceding the tsunami and key aspects to be considered in the emergency phase and beyond. Malaria Journal. 2005; 4:8.
5. Carter HF. Future observation on the transmission of malaria by *Anopheles* mosquitoes in Ceylon. Ceylon Journal of Science. 1930; 2:159-176.
6. Delfinado MD. The *Culicine* mosquitoes of Philippines tribe *Cilicini* (Diptera: *Culicidae*) Memoirs of the Amer. Entomol. Inst. 1966; 21(1):1-307.
7. Harrison BA, Scanlon JE. Medical entomology studies II. The subgenus *Anopheles* in Thailand. Contribution of American Entomological Institute. 1975; 12(1):305.
8. Htay Aung S, Minn S, Thaug M, Tun Lin MM, Mya SM, Than T, Hlaing Soe Soe P. Druilhe, Quecuche Well breeding *Anopheles dirus* and their role in malaria transmission in Myanmar. Southeast Asia Journal of Tropical Medicine and Public Health. 1999; 30:447-453.
9. Jude P, Dharshini S, Vinobaba M, Surendran SM, Ramasamy R. *Anopheles culicifacies* breeding in brackish waters in Sri Lanka and implication for malaria control. Malaria journal. 2010; 9(1):106-110.
10. Kannathasan S, Antonyrajan A, Srikrishnaraj KA, Karunaratne SHPP, Karunaweera ND, Surendran SN. Studies on prevalence of anopheline species and community

- perception of malaria in Jaffna district, Sri Lanka. *Journal of Vector Borne Diseases*. 2008; 45:231-239.
11. Khin Maung Kyi. malaria vectors in Burma *Anopheles balabacensis* balabacensis Baisas, 1936. *Union of Burma Journal Life Science*. 1970; 3:217-225.
  12. Khin Maung Kyi. Malaria vector of Burma 4. *Anopheles culicifacies* Giles, 1901, *Union of Burma Journal of Life Sciences*. 1972; 5:209-227.
  13. Loong KP, Chiang GL, Yap HH. Field study of the bionomics of *Anopheles maculatus* and its in malaria transmission in Malaysia. *Southeast Asian J Trop Med Public Health*. 1988; 19:724-8.
  14. Mahmood F, Sakai RK, Akhtar K. Vector incrimination studies and observations on species A and B of the taxon *Anopheles culicifacies* in Pakistan, *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 1984; 78:607-616.
  15. Martens WJM, Niessen LW, Rotmans J, Jetten TH, McMichael AJ. Potential impact of Global climate change on malaria risk. *Environ Health Perspect*. 1995; 103:458-464.
  16. Maung Maung Mya, Saxena RK, Paing Soe. Study of malaria in a village of lower Myanmar. *Indian Journal of Malariology*. 2002; 39:96-102.
  17. Maung Maung Mya, Myat Phone Kyaw, Sein Thuang, Tin Tin Aung, Yan Naung Maung. Vector bionomics and potential vectors of malaria in Kamamaung Township, Phapun District, Kayin State. 44<sup>th</sup> Myanmar Health Research Congress Programme and Abstracts, 2016, pp. 77.
  18. Maung Maung Mya, Pe Than Htun, Sein Min, Saxena RK. Cytotaxonomical studies of *Anopheles culicifacies* species complex and its malaria vectoral capacity in Myanmar. *International Journal of Contemporary Research in Engineering and Technology*. 2012; 2(2):17-26.
  19. Maung Maung Mya, Pe Than Htun, Sein Min, Sein Thuang. Development of cytogenetic method for identification of *Anopheles culicifacies* species complex and its applications at malaria endemic areas of Myanmar. *Myanmar Health Science Research Journal*. 2009; 21(3):157-163.
  20. Ministry of Health. Annual public health statistics, Ministry of Health, 2006.
  21. Molineaux. The epidemiology of human malaria as an explanation of its distribution of its control. In : Wernsdorfer WH, McGregor I. eds. *Malaria, principles and practice of malariology*, New York: Churchill Livingstone, 1988, 913-98.
  22. Myint Oo, Than Swe, Ye Htut, Tin Shwe, Nyunt Win, Aung Khin, Khin Hla Aye *et al*. The changing incidence of *Plasmodium vivax* infection in subjects with malaria. *Myanmar Health Science Research Journal*, 1992.
  23. Myo Paing, Thi Thi Naing, Sein Min, Zaw Myint. *Anopheline* mosquitoes of Myanmar III. *Anopheles* (*Cellia*) *Philippines* Ludlow 1902 and *Anopheles* (*Cellia*) *nivipes*. Theobald 1903 on Myanmar and their differentiating character. *Myanmar Health Science Research Journal*. 1990a; 2:37-38.
  24. Myo Paing, Sein Min, Zaw Myint, Thi Thi Naing. Comparison of malaria situation in a forested foothill area of Bago division between monsoon and cold dry season. *Myanmar Health Science Research Journal*. 1990b; 2:88-90.
  25. Myo Paing, Tun Lin W, Sein Min, Zaw Myint. Malaria situation in a forested foothill area of Pegu. *Burma Health Science Research Journal*. 1989; 1:52-54.
  26. Rahman WA, Abu Hassan A, Adanan CR, Raza MR, Hamid AK. Malaria transmission in a remote village located in northern peninsular Malaysia near the Malaysia-Thailand border. *Trop Biomed*. 1992; 9:83-89.
  27. Raid JA. The *Anopheline* mosquitoes of Malaya and Borneo, studies of the Institute for Medical Research, Malaya. 1967; 31:1-520.
  28. Reuban R, Kalyanasundaram M, Suguna G. (Salinity tolerance of sibling species in the taxon *Anopheles subpictus Grassi*, 1899. *Indian Journal of Medical Research*. 1984; 80:67-70.
  29. Roberts D. Mosquitoes (Diptera: *Culicidae*) Breeding in brackish water; Female ovipositional preference or larval survival? *Journal of Medical Entomology*. 1996; 33:525-530.
  30. Rohani A, Wan Najdah WMA, Zamree I, Azahari AH, Mohd Noor I, Rahimi H, Lee HL. Habitat characterization and mapping of *Anopheles maculatus* (Theobald) mosquito larvae in malaria endemic areas in Kuala Lipis, Pahang, Malaysia. *Southeast Asian J Trop Med Public Health*. 2010; 41:821-830.
  31. Rohani Ahmad, Wan NWM Ali, Zurainee M Nor, Zamree Ismail, Azahari A Hadi, Mohd N Ibrahim *et al*. Mapping of mosquito breeding sites in malaria endemic areas in Pos Lenjang, Kuala Lipis, Pahang, Malaysia. *Malaria Journal*. 2011; 10:361.
  32. Russell PF, Rao TR. On the ecology of larvae of *Anopheles culicifacies* Giles, in borrow-pits. *Bull Entomol Res*. 1942; 32:341-361.
  33. Sabesan S, Krishnamoorthy K, Jambulingam K, Rajendran G, Kumar PN, Rajagopalan PK. Breeding habitats of *Anopheles culicifacies* in Rameshwaram Island. *Indian Journal of Medical Research*. 1986; 84:44-52.
  34. Sharma VP. Current scenario of malaria in India. *Parassitologia*. 1999; 41(1-3):349-353.
  35. Subbarao SK, Adak K, Vasantha K, Joshi H, Raghvendra K, Cochrane AH *et al*. Susceptibility of *Anopheles culicifacies* species A and B to *Plasmodium falciparum* as determined by immunoradiometric assay. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 1988; 82:394-397.
  36. Subbarao SK, Sharma VP. *Anopheline* species complexes and malaria control. *Indian Journal of Medical Research*. 1997; 160:164-173.
  37. Surendran SN, Abhayawardana TA, De silva BGDNK, Ramasamy MS, Ramasamy R. *Anopheles culicifacies* Y chromosome dimorphism indicates the presence of sibling species (B and E) with different malaria vector potential in Sri Lanka. *Medical Veterinary Entomology*. 2000; 14:437-440.
  38. Surendran SN, Ramasamy MS, De silva BGDNK, Ramasamy R. *Anopheles culicifacies* sibling species B and E in Sri Lanka differ in longevity and in their susceptibility to malaria parasite infection and common insecticides. *Medical Veterinary Entomology*. 2006; 20:153-156.
  39. Tun Lin W, Myat Myat Thu, Sein Maung Than, Maung Maung Mya. hyper- endemic malaria in a forested hilly

- Myanmar village. *Journal of American Mosquitoes Control Association*. 1995; 11(4):401-407.
40. Tun Lin W, Myo Paing, Zaw Myint. A modification of the WHO dipping procedure for well-breeding anophelines in Burma. *Trop. Biomed.* 1988; 5:51-55.
  41. Vythilingam I, Foo LC, Chiang GL, Inder S, Loong KP. The impact of permethrin impregnated bednet on the malaria vector *Anopheles maculatus* (Diptera: Culicidae) in aboriginal villages of Pos Betau Pahang, Malaysia. *Southeast Asian J Trop Med Public Health*. 1995; 26:354-7.