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SHORT COMMUNICATION

Factors influencing *Anopheles* larval habitats in Kudat District, Sabah, Malaysia

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ABSTRACT

A longitudinal survey of *Anopheles* larval habitats was conducted in adjoining areas of Kampung Marabahai, Nangka, Paradason and Tuboh in Kudat District, Sabah from May 2015 to April 2016. Ninety-five out of 368 breeding habitats sampled were positive for *Anopheles* larvae. The significant physicochemical factors that were associated with the presence of *Anopheles* larvae were: turbidity, shadiness, presence of water vegetation, surface area, temperature, pH (negative log of the hydrogen ion concentration), and Electrical Conductivity (EC). Thus, this paper highlighted the physicochemical characteristic of larval habitats of *Anopheles* mosquito with emphasis on *An. balabacensis*, the vector of *Plasmodium knowlesi* malaria, could be targeted for surveillance studies and control interventions.

INTRODUCTION

Understanding the physicochemical characteristics of larval habitats is an important consideration to be targeted for the control of mosquito larvae. Immature stages are more vulnerable to human intervention since their location, growth and development are more restricted in time and space, thus, more vulnerable to control.

In an earlier study on the larval ecology of *Anopheles* mosquitoes following land-use changes in Sabah (Aure et al., 2021), six land-use types, within an area measuring 2 × 3 sq. km, were identified using a drone and verified by ground-truthing (Ground truthing protocol. http://www.missiongroundtruth.com/groundtruth.html). From May 2015 to April 2016, 368 larval habitats located in adjoining areas of Kampung Marabahai, Nangka, Paradason and Tuboh in Kudat District in Sabah, were observed for the presence or absence of mosquito larvae. Larval habitats were assessed for their relative importance as sources of potential vectors of malaria. Kruskal-Wallis H Test by ranks was used to determine if there were significant differences between each aquatic habitat (Kruskal & Wallis, 1952). The physicochemical of larval habitats examined were: water stability, water movement, turbidity, presence of vegetation, shadiness, pH, TDS (Total Dissolved Solids), EC (Electrical Conductivity), temperature, depth, and surface area. The physical and chemical characteristics of the water in each larval habitat sampled were measured with hand-held instruments (Hi9812-5, portable pH/EC/TDS/OC Hanna® Instruments) or noted visually. The association between mean larval density and qualities of the water was analysed by non-parametric methods i.e., Kruskal-Wallis (1952) and Mann-Whitney (Wilcoxon Rank Sum) Tests (Mann & Whitney, 1947).

The distribution of *Anopheles* larvae was not normally distributed and the larvae collected were relatively small. This study revealed significant differences among larval habitats were detected ($\chi 2 = 20.208$, df = 11, p = 0.043). Ninety-five among 368 habitats were found positive for *Anopheles* larvae distributed as follows: artificial container, 11/42 or 26%; ditch, 10/27 or 37%; intermittent stream, 46/127 or 36%; pond, 9/35 or 26%; puddle, 5/28 or 18%; river, 9/56 or 16%; and slow-flowing stream, 5/32 or 16% (Table 1).

Table 1 Distribution of anopheline larvae among different aquatic habitat types, Kudat, Sabah, Malaysia

Habitat	No. of habitats sampled	*Anopheles*-positive habitats (%)
Artificial container	42	11 (26)
Borrow pit	1	0 (0)
Ditch	27	10 (37)
Irrigation canal	2	0 (0)
Intermittent stream	127	46 (36)
Leaf axil	3	0 (0)
Pond	35	9 (26)
Puddle	28	5 (18)
Rockpool	1	0 (0)
River	56	9 (16)
Slow flowing stream	32	5 (16)
Tree hole	14	0 (0)
Total (N)	368	95 (26)

Seven habitats with *Anopheles* larvae by rank were, in descending order of frequency: intermittent stream > ditch > pond > artificial container > puddle > river > slow-flowing stream. Five habitats (borrow pit, irrigation canal, leaf axil, rock pool, and tree hole) were negative for Anopheline larvae. Although the numbers were low, a total of 19 habitats were found positive for *An. balabacensis* and collected from an intermittent stream, artificial containers and puddles (Figure 1) (Aure et al. 2021).

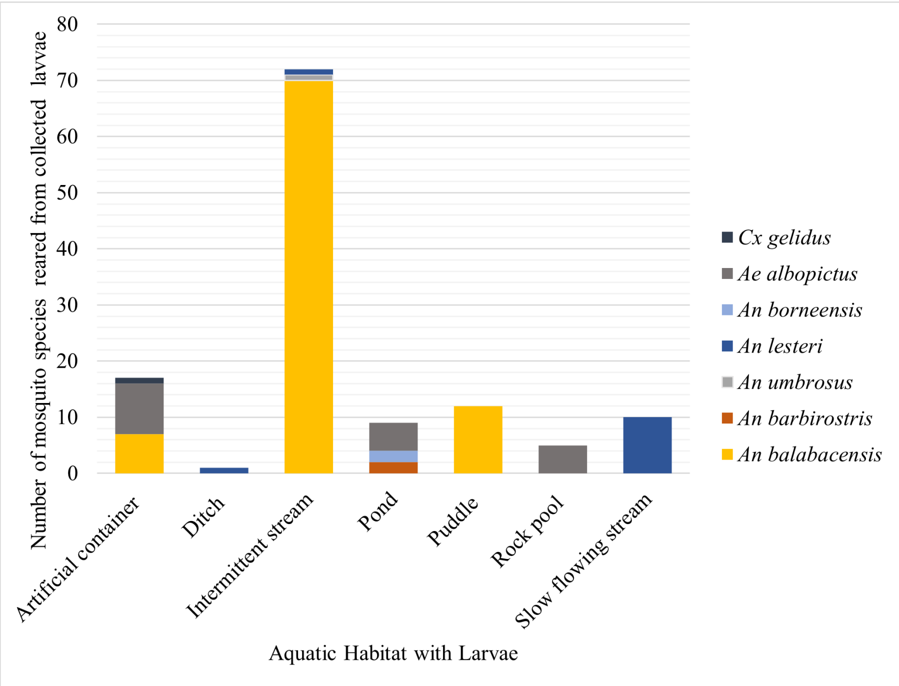

Figure 1 The number of mosquito species from reared larvae collected from aquatic habitats

The result of this study found that the physical characteristics which were significantly associated with *Anopheles*-positive habitats were: partial to full shadiness ($p = 0.04$) and turbidity ($p = 0.03$); pond and the slow-flowing stream had either none or emergent water vegetation ($p = 0.03$); the rest had none. Depth and water stability were not applicable. The surface area (< or >10 sq cm) was significant in a puddle only (Table 2).

Table 2 The Physical parameters (mean + SD) of *Anopheles* breeding habitats in Kudat

Physical characteristics	Larval habitat	Artificial Container	Ditch	Intermittent stream	Pond	Puddle	River	Slow flowing stream	P-values
	Variables	(Mean ± SD)	(Mean ± SD)	(Mean ± SD)	(Mean ± SD)	(Mean ± SD)	(Mean ± SD)	(Mean ± SD)	
Water Stability	Permanent	0	(NA) not applicable	0.7143 ± 1.11	1.6191 ± 3.11	(NA) not applicable	0.6727 ± 1.86	0.1111 ± 0.33	0.28
	Temporary	1.2439 ± 2.62	1.1111 ± 2.26	1.8917 ± 4.22	1.6429 ± 4.7	2.0357 ± 6.01	0	0.7483 ± 1.16	0.56
Water Movement	Standing/Still	1.2143 ± 2.6	1.3044 ± 2.4	1.961 ± 3.74	1.7273 ± 3.85	2.2 ± 6.34	2.75 ± 3.81	0	0.14
	Fast/Slow moving	NA	0	1.62 ± 4.67	0	0.6667 ± 1.54	0.3125 ± 0.97	0.4286 ± 1.07	0.14
Turbidity	Clear	1.4706 ± 2.83	1.7143 ± 3.68	1.2727 ± 2.89	NA	1 ± 3.16	1.1818 ± 2.59	0.0769 ± 0.28	0.63
	Turbid	0.125 ± 0.35	0.9 ± 1.59	2.25 ± 4.83	1.6286 ± 3.76	2.6111 ± 7.15	0.3235 ± 1.04	0.5789 ± 1.26	**0.03**
Water Vegetation	None	1.275 ± 2.65	1.1579 ± 2.59	1.7788 ± 4.17	0.7391 ± 2.8 [a]	3 ± 7.15	0.5122 ± 1.74	0.25 ± 0.8 [b]	**0.03**
	Grass/Emergent	0	1 ± 1.31	2.2143 ± 3.77	3.3333 ± 4.81 [a]	0	1.0667 ± 2.63	1.25 ± 1.82 [b]	0.11
Shadiness	No Shade	0.375 ± 0.74	2.2 ± 4.38	0.5 ± 0.84	1.7727 ± 4.52	0	0	NA	0.78
	Partial/Full shade	1.4118 ± 2.84	0.8636 ± 1.52	1.8926 ± 4.21	1.3846 ± 2.02	2.5909 ± 6.7	0.6727 ± 1.86	0.375 ± 1.01	**0.04**
Surface Area (m^2)	≤10	1.2439 ± 2.62	1.16 ± 2.34	1.8478 ± 4.47	0.7143 ± 1.49	1.037 ± 2.92 [c]	0.2 ± 0.45	0.4444 ± 1.33	0.28
	>10	0	0.5 ± 0.71	1.7714 ± 3.07	2.2381 ± 4.65	29 ± [c]	0.7059 ± 1.92	0.3478 ± 0.88	0.05
Depth (cm)	≤10	2 ± 3.46	0	2.1379 ± 4.17	3 ± 4.24	4..5455 ± 9.14	0.4286 ± 1.13	0	0.3
	>10	1.1539 ± 2.57	1.3044 ± 2.4	1.7347 ± 4.12	1.5455 ± 3.78	0.4118 ± 1.28	0.6939 ± 1.93	0.3871 ± 1.02	0.12

The superscript letter indicates the significance
NA - not applicable

The significant chemical factors that were associated with the overall presence of *Anopheles* larvae were: temperature (>30 Celsius) ($p = 0.04$) and pH (<6.5) ($p = 0.04$) in all larval habitats. TDS (<100 ppm) was significant in the intermittent stream ($p = 0.02$) and EC (<100 µS/cm) was significant in all habitats ($p = 0.05$) (Table 3).

Table 3 The chemical parameters (mean + SD) of *Anopheles* breeding habitats in Kudat

Chemical characteristic	Larval habitat	Artificial Container	Ditch	Intermittent stream	Pond	Puddle	River	Slow flowing stream	P-values
	Variables	(Mean ± SD)	(Mean ± SD)	(Mean ± SD)	(Mean ± SD)	(Mean ± SD)	(Mean ± SD)	(Mean ± SD)	
Temperature (°C)	≥30	1.1143 ± 2.48	1.12 ± 2.3509	1.8413 ± 4.13	1.35 ± 3.1	2.1923 ± 6.22	0.5556 ± 1.66	0.375 ± 1.01	**0.04**
	<30	1.7143 ± 3.3	1	0	2 ± 4.58	0	3.5 ± 4.95	(NA)	0.65
pH	≤6.5	0.3636 ± 1.21	1.3333 ± 3.28	2.9032 ± 6.78	3.5 ± 5.09	1.9167 ± 6.19	0	0.1429 ± 0.38	**0.04**
	>6.5	1.2143 ± 2.7	0.5 ± 1.09	1.394 ± 2.64	1.3044 ± 3.66	2.75 ± 5.5	0.5556 ± 1.8	0.44 ± 1.12	0.45
Total Dissolved Solids (TDS) (ppm)	≤100	1.25 ± 2.66	1.923 ± 3.04	2.3833 ± 4.82 [a]	2.4 ± 4.75	2.4783 ± 6.57	0.6207 ± 1.66	0.7462 ± 1.21	0.14
	>100	0	0.25 ± 0.45	1.1148 ± 3.12 [a]	0.6923 ± 1.38	0	0.7308 ± 2.09	0.1818 ± 0.4	0.51
Electrical Conductivity (EC) (μS/cm)	≤100	1.1842 ± 2.6	1.5882 ± 2.72	1.9 ± 4.25	1.9286 ± 4.13	2.0357 ± 6.01	0.6727 ± 1.86	0.375 ± 1.01	0.05
	>100	0	0.125 ± 0.35	0.1818 ± 0.6	0.6 ± 1.34	NA	NA	NA	0.87

The superscript letter indicates the significance
NA = not applicable

A parallel survey of *An. balabacensis* larval habitats in Kudat District showed a preference for this species to breed in muddy ground pools and tire tracks in plantations and forest fringe (Ahmad et al., 2018). *An. balabacensis* larvae were found in 29 out of 97 breeding sites sampled. However, multiple linear regression analysis indicated no associations between environmental factors and the occurrence of *An. balabacensis* larvae were observed (Ahmad et al., 2018).

Based on the larval ecology study the differences among land-use types were not significant ($\chi 2 = 4.15$, df = 5, p = 0.219). The frequency of the number of *Anopheles* larvae collected in descending order was: rubber tree plantation> coconut plantation > clearing site > oil palm plantation > forest > settlement area (Aure et al. 2021). Mosquito biodiversity by genera was high in all land use types based on Simpson's diversity index, favourable and competitive conditions for different taxonomic groups that could impact their relative abundance. *An. balabacensis*, though mainly a forest dweller, was present in all land use types and was found co-existing with other *Anopheles* species e.g., *An. barbirostris*, *An. lesteri*, *An. borneensis* and *An. umbrosus* in clearing sites and coconut plantations only (Aure et al., 2021).

The composition and abundance of anopheline species according to habitat diversity in Mexico found no significant differences between ALI (absolute larval index) and hydrological types (Villarreal-Treviño et al., 2020). More accurate vector–habitat association could be obtained using aerial remote sensing data that could analyse environmental and spatial risk factors (Byrne et al. 2021). The mentioned vector-habitat association study share the same study population as Aure and colleague (2021). It was confirmed the benefit of remote sensing and Geographic Information System (GIS) mapping in determining the influence of environmental factors on the distribution of malaria vectors in Sudan (Ageep et al., 2009).

In northern Iran, it was noted that interspecific associations between species affect the ecology and development of mosquito larvae because of competition for food, exposure to predators and susceptibility to pesticides (Nikookar et al, 2017). It was also observed that the presence of *Culex* was a risk factor for *Anopheles* breeding (Byrne et al., 2021). A study In Southwest Ethiopia found that anopheline larvae were more abundant in shallow, temporary habitats and the absence of competitors and predators (Mereta et al., 2013). In characterising mosquito larval habitats, claimed that human behaviour and activities accounted for most of the mosquito breeding habitats in Qatar (Alkhayat et al., 2020).

CONCLUSION

The main limitations of this study were the non-parametric distribution of larval habitats and relatively low to nil larval counts. The most prominent breeding sites of *An. balabacensis* were intermittent streams, puddles and artificial containers found in rubber tree plantations and forests. Larval habitats, except large artificial (water storage) containers, were subject to inundation following heavy downpours. The present study indicates a significant relationship between physicochemical parameters such as shadiness, absence of water vegetation, temperature, pH, EC and *Anopheles* larvae distribution and abundance. Some other factors which have not been obtained such as distance to the nearest house, presence of predators, and organic content of the larval habitats may also play a role in defining the associations between the environment and vector populations. Large scale and more systematic methods are needed to fully understand the factors influencing mosquito larval breeding sites for effective planning and implementation of the vector control strategy.

CONFLICT OF INTEREST

The authors declare that they have no competing interests in publishing this article.

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