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Characterization and correlation studies of species composition of mosquitoes and its habitats in Mizoram, North East India

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Abstract
During a systematic survey, mosquito species were collected from various habitats, including domestic cattle sheds, rock holes, permanent ponds, river edges, semi-permanent tanks, temporary ditches, slow streams, tree holes, and domestic containers. Twenty mosquito species from five genera (*Anopheles*, *Aedes*, *Culex*, *Culiseta*, and *Toxorhynchites*) were identified. Permanent ponds harbor the richest breeding habitats, hosting 15 different species of mosquito, while semi-permanent tanks exhibited the lowest species diversity, with only three species recorded. Overall, most species were collected in natural habitats of quiet or stagnant water conditions at depths ranging from 0.1 to 0.5 meters. The majority of species favored partially shaded and temporary water habitats, while moderate vegetation and stagnant clear water habitats also supported a diverse range of mosquito species. Similarity matrix based on coefficient of association showed that *Culex tarsalis* and *Culex peus* have the highest association coefficient (0.97), 0.95 similarity index by *Culiseta melanura* and *Culex tritaeniorhynchus*, while the lowest coefficient of association based on similarity index was observed in *Culex mimeticus* and *Aedes* aegypti (0.25), which fall under different genera. Additionally, statistical analysis using forward multiple regression of habitats and physio-chemical parameters of breeding habitats observed that pH, temperature, dissolved oxygen, total dissolved solids (TDS), total alkalinity and water hardness as key factor in environmental parameters that influenced the relative abundance of collected immature stages mosquitoes.

Keywords: Mosquito species, Species composition, Mosquito habitats, Mizoram, North East India, Anopheles, Aedes

Introduction

Species do not exist alone; instead, they come together with other organisms to form a community within a specific area or habitat. The presence of multiple species in a habitat at the same time suggests that they share similar environmental needs (Devi and Jauhari, 2007) [13]. Mosquito larval habitats are crucial environments where several key life cycle processes occur, including egg laying (oviposition), larval growth and development, emergence of adults, resting, swarming, and mating (Gimnig *et al*., 2002, Mbanzulu *et al*., 2024) [15]. Mosquitoes utilize a wide range of lentic aquatic habitats for breeding, where their immature stages develop. These aquatic environments host not only the larvae of various mosquito species but also encourage the formation of diverse larval mosquito communities, comprising both conspecifics and heterospecifics (Alkhayat *et al*., 2020) [5]. The availability of resources, such as food, as well as the presence of predators and competitors within the habitat, significantly influence the population dynamics of larval mosquitoes, both in qualitative and quantitative aspects. A thorough assessment of larval habitats, focusing on species composition and resource availability, is essential for gaining insights into the bio-ecology of mosquitoes and developing effective pest and vector control strategies (Adityaa *et al*., 2006) [1]. The survival rates of immature stages and the emergence of pupae into adults demonstrate a significant correlation with the altitude and temperature of their breeding environments (Devi and Jauhari, 2007; Kermelita *et al*., 2024) [13, 20]. In the north-east region of India, comprehensive mosquito surveys have been conducted across various states to investigate the occurrences, distributions, species composition, and identification of vector species (Sen *et al*., 1973; Rajagopal, 1979; Malhotra *et al*., 1982, 1984) [24, 25, 38].

Numerous studies have been conducted regarding the distribution and categorization of larval breeding habitats. Ishwara *et al.* (2021) [17] categorize seven distinct types of primary larval habitats based on parameters such as size, persistence of breeding water (permanent versus temporary), and water type (WHO, 1975). The predominant types of breeding sites include standing or stagnant water (both permanent and temporary), slow flowing water, human habitats, livestock enclosures, mixed-dwelling environments and various outdoor resting locations, which encompass vegetation such as bushes, tree cavities, rock crevices, and discarded containers like used tires and plastic containers (Devi and Jauhari, 2008) [14]. A series of investigations have focused on the breeding ecology of Anopheline mosquitoes across diverse habitats (Rajagopalan *et al*., 1979; Sahu *et al*., 1990; Bhatt *et al*., 1993; Kermelita *et al*., 2024) [9, 20, 33], emphasizing the importance of vegetation in providing suitable conditions for larval development and contributing to higher adult mosquito densities

Mosquitoes exhibit variability in their preferences for aquatic habitats for oviposition, which is influenced by various factors, including geographic location, the physicochemical characteristics of the water body (Isaac *et al*., 2021) [18], and the presence of predators (Shililu *et al*., 2003; Kendie *et al*., 2023) [21, 40]. Physicochemical factors in the breeding habitats impacting oviposition, survival, and the spatiotemporal distribution of significant disease vector species comprise water salinity, dissolved organic and inorganic matter, levels of eutrophication, turbidity of water, the presence of suspended particulates, vegetation, temperature and light conditions (Amerasinghe *et al*., 1995) [3]. Water with a nearly neutral pH, ranging from 6.5 to 7.8, is typically ideal for the reproduction of many mosquito species. However, only a few species are known to thrive in specific environments like tree holes or the leaf axils of certain plants, which may affect the strength of their egg shells (Aditya *et al*., 2006; Mbanzulu *et al*., 2024) [1]. Understanding how these environmental conditions affect the distribution of particular vector species and their larval populations is vital for enhancing our knowledge of larval biology. This understanding plays a key role in developing and implementing effective integrated vector management strategies, as emphasized by Sarkar *et al*. (2024) [39].

The existing knowledge regarding mosquito larval ecology in Mizoram remains limited, which hampers effective vector control efforts. The factors contributing to the heterogeneity in vector distribution and abundance, as well as the regulation of mosquito larval populations in diverse aquatic habitats, are poorly understood. Understanding of the aquatic life stages of malaria vectors is essential for the implementation of effective anti-malarial control strategies (Adityaa *et al*., 2006) [1]. Recognizing that a detailed examination of the physicochemical and biological characteristics of breeding habitats is vital, as each habitat supports specific mosquito species and exhibits seasonal variations, A survey was carried out in six districts of Mizoram to enumerate mosquito ovipositional sites and species were classified according to shared habitat characteristics.

This research marks the initial effort to examine mosquito associations within a range of breeding habitats. It aims to characterize the physical, chemical, and biological factors of these larval habitats to identify the influences on mosquito abundance and distribution across different aquatic ecosystems in Mizoram, India.

Materials and methods

The study was conducted from June 2022 to December 2024, involving random sampling across various habitats. It encompassed a significant portion of six districts in Mizoram: Aizawl, Serchhip, Mamit, Lunglei, Lawngtlai, and Kolasib. These districts are situated between 23°43’ N latitude and 92°59’ E longitude, with altitudinal variations ranging from 60 to 1,155 meters. In this study, several habitats were identified as probable sites for mosquito larval development, including domestic cattle sheds, rock holes, permanent ponds, river edges, semi-permanent tanks, temporary ditches, slow streams, tree holes, and domestic containers.

Sample collection: Mosquitoes were collected utilizing the dipping method (WHO, 1975) within a temperature ranging from 19 to 38°C and a relative humidity range of 30 to 85%. Human landing manual collection using an aspirator and CDC light traps from both indoor and outdoor resting habitats during 4:00 – 10:00 pm by aspirator and 12:00 - 6:00 am by CDC light trap. Identification of mosquito species level was conducted by examining adult morphological characteristics utilizing standard taxonomic keys by Nagpal and Sharma (1995) and Oo *et al*. (2005), Gunathilaka (2017). Identification of the species was done through laboratory emergence of adults from collected immature stages. In each study area, collection sites were established in various directions, and random sampling was performed across all available habitats. At the time of mosquito collection, data regarding the accompanying biotic community and breeding behaviors were also documented.

Water quality testing: Eight abiotic factors were assessed in our study: water temperature (mercury-glass thermometer), total suspended solids, total alkalinity, chloride, water hardness, total dissolved oxygen, phosphate content and pH of water. Water samples ranging from 200 to 1000 ml were collected from each positive habitat. These samples were promptly transported to the laboratory for analysis of their physico-chemical properties.

Data analysis: Data regarding both mature and larval forms of mosquitoes were analyzed following a systematic approach consisting of three primary steps: (i) identification of operative taxonomic units (OTUs) (ii) construction of a matrix representing the basic data and (iii) calculation of similarity indices for each pair of mosquito species. The primary objective of this study was to classify mosquito species based on shared breeding characteristics, which guided the selection of OTUs as the species collected during the survey. The breeding habitats and physical characteristics of the breeding sites were evaluated to detect common patterns associated with the immature stages of various mosquito species. A basic data matrix was constructed in tabular format, consisting of rows corresponding to mosquito species and columns denoting positive breeding habitat characteristics along with water quality parameters. The values within this matrix were recorded as either 1 or 0, indicating the positive or negative breeding of each characteristic for the respective species. These data served as the foundation for analyzing and calculating the similarity among all possible pairs of OTUs. The similarity indices for all combinations of mosquito species pairs were computed using the coefficient of association, resulting in the development of a similarity matrix). Linear regression, specifically Pearson’s correlation, was employed to identify the key predictor variables that influence the abundance of selected mosquito species (SPSS Statistics version 20).

Result and Discussion

A total of 8,328 mosquito species representing five genera—*Anopheles, Aedes, Culex, Culiseta,* and *Toxorhynchites*—were collected, comprising 20 species overall. These mosquitoes were found across a diverse range of habitats, with altitudinal variations ranging from 60 to 1,155 meters. The analysis indicated that lower altitudinal zones exhibited greater species abundance compared to higher elevations. The most dominant genus was *Anopheles*, followed by *Culex, Culiseta, Aedes* and the least was *Toxorhynchites*.

During the survey period, the dominant species was most commonly identified as Cx. quinquefasciatus, making up 39.78% of the total collection. This was followed by *An. barbirostris* (30.50%), *An. vagus* (13.51%), *Ae. albopictus* (4.62%), *Cx. tritaeniorhynchus* (3.01%), *Cx. mimeticus* (2.18%), *Cx. bitaeniorhynchus* (1.85%), *Cx. tarsalis* (1.21%), *Cu. melanura* (0.78%), *Cx. peus* (0.81%), *Tx. splendens* (0.62%), *An. philipinensis* (0.46%), *Cu. inornata*

(0.34%), *An. jamesi* (0.28%), *An. nivipes* (0.20%), *An. willmori* (0.15%), *An. jeyporiensis* (0.10%), *An. minimus* (0.08%), *An. dirus* (0.04%), and *Ae. aegypti* (0.03%). Among these species, 11 were collected in their immature forms, while eight were found both as adults and in immature stages.

Table 1. Characterization of mosquito larval breeding habitats across various mosquito groups in six districts of Mizoram, India, during the period from 2022 to 2024.

Group	Category-I						Category -II					Category -III				Category -IV			
Habitats types	Cxr	Cxb	Cxt	Cxu	Cum	Cui	Txr	Anb	Cxm	Ani	Cxq	Anp	Anm	Ani	Anp	Anw	Anj	Aey	Aea
Tem. pond	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
River edges	-	-	+	-	+	-	-	-		+	+	+	+	+	+	+	+	-	-
Cem. tanks	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	+
Slow stream	-	-	-	-	-	-	-	+	-	+	+	+	-	-	+	-	+	-	-
Tem. ditches	+	-	+	+	+	+	-	+	+	+	+	-	-	-	-	-	-	-	-
P. shady	-	-	+	+	-	-	+	-	+	+	+	-	-	+	+	+	-	+	+
Sunlight	+	+	+	+	-	+	-	+	-	-	+	-	+	+	+	+	-	-	-
Stagnant	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	+
Slow flowing	+	-	-	-	-	-	-	-	+	-	-	+	+	+	+	+	+	+	+
Clear water	-	-	-	-	-	-	+	+	+	-	-	+	+	+	-	+	+	+	-
Low turbid	+	-	+	+	+	+	+	+	-	+	+	-	-	-	+	-	-	-	+
High Turbid	-	-	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+
Veg - Moderate	+	+	-	-	+	+	+	+	+	+	-	-	+	+	+	+	-	-	-
Medium	-	-	+	+	-	-	+	-	-	-	+	+	-	-	-	-	-	+	+
Less	-	-	-	-	-	-	+	-	+	+	+	-	+	+	-	+	-	+	+
Depth: 0 - 1 m	-	-	+	+	-	-	-		-	+	+	+	-	+	+	+	+	-	-
>1 m	-	+	-	-	+	+	+	-	+	+	+	-	-		-	-	-	+	+

The study categorizes immature mosquito species into four groups based on their breeding characteristics and occurrence. Category I includes six species: *Cu. melanura, Cx. tarsalis, Cx. peus, Tx. splenden, Cx. tritaeniorhynchus, and Cx. bitaeniorhynchus.* Category II has eight species*: Cx. mimeticus, Cu. inornata, An. barbirostris, An. vagus, Cx. quinquefasciatus, An. jamesii, An. philipinensis,* and *An. nivipes*. Category III consists of three species: *An. jeyporiensi, An. mimimus,* and *An. willmori.* Category IV includes two species: *Ae. aegypti* and *Ae. albopictus*. The highest association coefficient of 0.97 was noted between *Cx. tarsalis* and *Cx. peus,* while the lowest (0.25) was between *Cx. mimeticus* and *Ae. Aegypti.*

The study examined the breeding habitats of various mosquito species, focusing on both quantitative factors (such as water depth) and qualitative factors (including the nature of the habitat—permanent versus temporary, shaded versus exposed, water movement, vegetation condition, and turbidity). The identified breeding habitats included seepage pools, river edges, permanent ponds, cemented tanks, temporary ditches, slow streams, tree holes and domestic containers. Notably, ponds and temporary ditches supported the highest diversity, harboring fifteen mosquito species, while cemented tanks showed the lowest diversity with only three species recorded. Overall, the majority of species thrived in partially shaded and temporary water habitats. Locations with moderate vegetation and slightly turbid water also tended to harbor a greater variety of mosquito species.

Category-I species were predominantly found in ponds, seepage pools, rice fields, tanks, and ditches, favoring habitats characterized by sunlight to partial shade, stagnant or slow-moving water, and water depths ranging from 0 to 2 meters. For category-II species, common breeding sites included riverbeds, streams, ponds, rock holes, and pools. The main characteristics of these habitats were slow-flowing or stagnant waters that were partially shaded and ranged from clear to slightly turbid. Category-III species preferred ponds, riverbeds, and streams, thriving in habitats that were partially shaded to sunlit, with slow-moving water, moderate vegetation, and a depth of 0 to 1 meter.

Interestingly, Category IV species displayed a unique preference for tanks as breeding sites, favoring conditions that were partially shaded, stagnant, clear, and had minimal vegetation. Primary habitats favored by all collected mosquito species were temporary with a mix of shadow to partially shadowy conditions and moderate vegetation.

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Mosquito larval abundance and environmental variables

The findings from the forward multiple regression analysis elucidated the influence of environmental parameters on the relative abundance of immature mosquito stages. *An. barbirostris* exhibited a positive correlation with pH ($P < 0.05$). Additionally, high alkalinity in the habitat was positively correlated with the abundance of *An. vagus* ($P < 0.05$), while a significant negative association was observed against dissolved oxygen levels ($P < 0.01$). Habitats characterized by alkaline water bodies and slight turbidity, coupled with an increased presence of detritus, demonstrated a positive correlation with *Cx. quinquefasciatus* abundance ($P < 0.05$). Conversely, *An. phillipinensis* larval abundance exhibited a negative correlation with temperature ($P < 0.05$) and was predominantly found in small, open, clear water environments, thereby showing a positive association with both water hardness and dissolved oxygen levels ($P < 0.05$). *Cx. tritaeniorhynchus* also displayed a positive association with water alkalinity ($P < 0.05$). Other environmental variables were excluded due to their weaker associations with mosquito larval abundance.

Table 2: Physiochemical parameters of different collection sites (mean ± SE).

Collection site	pH	Alkalinity	Hardness	Temp.	DO	TDS	Phosphate	Chloride
Mamit	7.2 ± 0.3	92.5 ± 10.5	159.8 ± 2.3	25.1 ± 2.1	4.90 ± 0.5	312.8 ± 18.1	1.2 ± 0.3	6.7 ± 0.8
Lunglei	7.4 ± 0.2	88.0 ± 8.21	166.1 ± 4.2	28.4 ± 1.8	5.55 ± 0.6	189.3 ± 5.1	0.9 ± 0.05	11.6 ± 1.2
Lawngtlai	7.1 ± 0.1	58.8 ± 1.7	125.5 ± 9.0	26.7 ± 0.8	7.55 ± 0.8	127 ± 19.1	3.5 ± 0.45	3.15 ± 0.9
Siaha	7.3 ± 0.2	62.3 ± 3.5	102 ± 4.0	27.3 ± 1.9	3.9 ± 0.8	362.0 ± 15.6	3.1 ± 1.2	28.8 ± 1.4
Kolasib	6.9 ± 0.5	69.4 ± 4.2	108.5 ± 13.9	31.2 ± 0.3	5.9 ± 0.8	221.0 ± 12.5	3.98 ± 0.2	15.7 ± 0.6

Almiron and Brewer (1996) [2] highlighted that variations among mosquito species can be primarily attributed to factors such as habitat types, vegetation characteristics, flowing movement of water and depth of water. Their cluster analysis revealed four unique categories of species, a finding that is consistent with the current study, which also identifies four distinct groups.
Numerous studies have examined the relationship between the larval stages of mosquitoes and vegetation, with several authors (Rajnikant *et al*., 1996; Poonja, 2023) [30] indicating that the abundance of larvae depends on the presence of certain types of vegetation. Aditya *et al*. (2006) [1] noted that cemented temporary pools, which are rich in detritus and algae, facilitate the coexistence of various species.

In the present study, immature mosquitoes collected from turbid water were predominantly identified as Culicines, corroborating the results of Sattler *et al*. (2005) [41]. Furthermore, the preference of Anophelines for breeding in clear to slightly turbid water aligns with findings from Bates (1994), Robert *et al*. (1998) [36], and Pawar (2024) [31]. However, in contrast to these conclusions, Rodriguez *et al*. (1993) [35] reported an increase in *An. gambiae* larval densities with greater turbidity. Additionally, the present findings contrast to those reported by Bhat *et al*. (1990) and Sen *et al*. (1973), which indicated differing mosquito species and fluctuating ecological conditions within the study area.

The current study has found that the positive associations between different mosquito species may stem from their shared preference for specific habitats, whereas negative associations might lead to variations in habitat preferences. The highest levels of immature associations in habitats like ponds, ditches, and riverbeds indicate favorable conditions for survival, ovipositional preferences, and beneficial physicochemical characteristics. It was also observed that continuous waterlogging, combined with rapidly changing ecological conditions and the large surface area of these habitats, creates optimal breeding environments for various mosquito species, including those that transmit diseases. The presence of multiple species in a habitat at the same time suggests interactions among mosquito species with similar preferences (Pawar *et al*., 2024) [31].

Table 3: Correlation coefficient of mosquito abundance and physico-chemical properties of habitats.

Mosquito	Water quality	Co-efficient correlation	Significant value
An. phillipinensis	Hardness	1.76	<0.02
An. barbirostris	pH	0.38	<0.02
Cx tritaeniorhynchus	Alkalinity	0.09	<0.05
	pH	0.26	<0.02
	Alkalinity	0.01	<0.02
An. vagus	DO	-0.37	<0.05
	Alkalinity	0.07	<0.02
	TDS	0.003	<0.05
	DO	0.65	<0.02
Cx. quinquefasciatus	Temperature	-0.45	<0.05

Numerous studies have examined water parameter like dissolved oxygen concentration affect the abundance of *Anopheles* and *Culex* species, with varying findings. Grillet (2000) noted a positive correlation of dissolved oxygen and the abundance of *An. oswaldoi*, while Salome (2023) [37] argued that increased algal productivity and photosynthesis lead to increased dissolved oxygen levels in aquatic habitats, boosting mosquito larval survival. In this study, dissolved oxygen was a significant factor influencing the productivity of *An. phillipinensis* ($p < 0.05$) but showed a negative association with *An. vagus* ($p < 0.05$). Total Dissolved Solids (TDS)—representing all dissolved organic, inorganic, and suspended solids in water—was also significant in the productivity of *Cx. quinquefasciatus* ($p < 0.05$). Typically, *Cx. quinquefasciatus* thrive in habitats rich in dissolved matter (Roy, 2024) [32], which are often characterized by high TDS. Species like *An. barbirostris*, *An. vagus* and *Cx. tritaerniorhynchus*, primarily found in ponds with alkaline waters indicating their ability to tolerate alkaline environments.

This study explored the aquatic habitats, inhabited by mosquito larvae. While this approach allowed for a detailed examination of the distribution of various mosquito larvae species within their ecological niches, it also imposed certain limitations. Notably, we focused exclusively on habitats that supported the presence of mosquito larvae, leaving those devoid of larvae unexamined.

The finding highlighted is crucial for developing effective mosquito control strategies. this foundational research provides the necessary data to inform policymakers and implement efficient, sustainable mosquito control strategies that protect public health.

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Conflict of Interest
The authors declare no conflict of interest.

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