

Seasonal species composition, abundance and public health importance of mosquito vectors (Diptera: Culicidae) in Huai Tha Khoei Reservoir, Ratchaburi, Thailand

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Abstract. Chaiphongpachara T, Laojuna S. 2024. Seasonal species composition, abundance and public health importance of mosquito vectors (Diptera: Culicidae) in Huai Tha Khoei Reservoir, Ratchaburi, Thailand. *Biodiversitas* 25: 1264-1275. Mosquitoes are vectors for pathogens that cause life-threatening diseases in humans and animals. Significant mosquito-borne diseases include malaria, dengue, chikungunya, Zika, Japanese encephalitis, and filariasis. Anthropogenic environmental changes can significantly impact the dynamics of disease vector populations, which can lead to outbreaks of mosquito-borne diseases. This study investigated the seasonal variation in species composition and abundance of mosquito vectors around the Huai Tha Khoei Reservoir in Ratchaburi Province, Thailand, across cool, hot, and rainy seasons. A total of 1363 mosquitoes, representing 30 species from five genera, were collected. In particular, 25 of these species, accounting for 83.33% of the total, were identified as vectors of human pathogens. The most dominant species across all seasons was *Culex tritaeniorhynchus* Giles 1901, representing 39.47% (n=538) of the total, followed by *Anopheles barbirostris* Wulp 1884 s.l. (14.38%, n = 196) and *Culex vishnui* Theobald 1901 (9.02%, n=123). The Shannon diversity index was highest during the rainy season ($H' = 2.144$) and lowest in the hot season ($H' = 1.788$). This is consistent with the results of the UPGMA tree based on beta diversity, indicating that mosquito diversity in the rainy season differs significantly from that in the cool and hot seasons. The findings enhance our understanding of the impact of seasonality on mosquito vectors in this region, which is crucial for developing effective disease control strategies. Additionally, this study serves as a valuable reference for understanding the diversity of mosquito vectors in other areas.

Keywords: Mosquito-borne diseases, mosquito vectors, season, species diversity, Thailand

INTRODUCTION

Mosquitoes are considered the most lethal vectors in the world due to their role in transmitting deadly diseases to humans and animals (Agboli et al. 2021; Breedlove 2022; Wang et al. 2022). They are responsible for spreading important mosquito-borne diseases in humans, such as malaria, dengue, chikungunya, Zika, Japanese encephalitis, and filariasis (Chala and Hamde 2021). Malaria, in particular, is a mosquito-borne disease caused by *Plasmodium* parasites and represents one of the most significant global health challenges. In 2022, there were approximately 249 million reported cases of malaria worldwide, with 608,000 deaths (World Health Organization 2023). Dengue, Zika and Japanese encephalitis (JE) are diseases caused by viruses belonging to the genus *Flavivirus* within the family *Flaviviridae* (Amicizia et al. 2018). *Aedes* mosquitoes, especially *Aedes aegypti* Linnaeus, 1762 and *Aedes albopictus* Skuse, 1894, are responsible for transmitting dengue and Zika (Ahebwa et al. 2023), while *Culex* mosquitoes are the primary vectors for JE (van den Eynde et al. 2022). Chikungunya, caused by the chikungunya virus of the genus *Alphavirus* and family *Togaviridae*, is prevalent in over 110 countries, and there were more than 2 million reported cases in 2005 (World Health Organization 2022). Lymphatic filariasis,

caused by filarial parasites transmitted through mosquito bites, continues to pose a risk to 882 million people in 44 countries worldwide (Bizhani et al. 2021).

Currently, there are 3723 officially reported mosquito species (Harbach 2023). Mosquitoes exhibit a broad geographical distribution, inhabiting temperate, subtropical and tropical climates worldwide, and their range even extends to areas beyond the Arctic Circle (Harbach 2023). Thailand, located in the central part of mainland Southeast Asia, is characterized by a consistently warm and humid tropical climate. This tropical climate contributes to the diversity of several mosquito vectors, making Thailand a high-risk area for mosquito-borne diseases (Chaiphongpachara et al. 2022b). Surveying the diversity of mosquitoes in different regions is crucial for determining the risk level of mosquito-borne diseases (Kabore et al. 2023; Rakotonirina et al. 2023; Laojun et al. 2024). Furthermore, this information is essential for establishing effective guidelines to control mosquito vector populations, considering the distinct habitat and biology of each species.

Anthropogenic environmental changes should be closely monitored for their impact on disease vector mosquitoes, which may contribute to outbreaks of mosquito-borne infectious diseases (Caminade et al. 2019). Previous studies have reported that the expansion of urban areas can lead to the emergence of outbreaks of dengue

fever, primarily due to an increase in the number of *A. aegypti* mosquitoes (Kolimenakis et al. 2021; Wilke et al. 2021). A reservoir, typically a medium-sized man-made water storage facility, is primarily used for agricultural purposes. Constructing a reservoir necessitates significant alterations to the surrounding environment, leading to modifications in the local ecosystem (Eekhout et al. 2020; Zhao et al. 2021). The Tha Khoei Reservoir, also known as the Pong Krating Reservoir, is situated in the village of Phu Nam Ron within the Ban Bueng Sub-district of Ban Kha District, Ratchaburi Province, Thailand. The creation of the reservoir has not only altered the local environment but also attracted more tourists to the area. Reports of mosquito-borne diseases in Thailand indicate occurrences of malaria and dengue fever in this region (Bureau of Epidemiology 2023). A recent study also identified *Anopheles* mosquitoes in the area infected with the goat malaria parasite *Plasmodium caprae* (Nguyen et al. 2023). Despite these reports, comprehensive data on mosquito diversity in this area remains limited. This lack of detailed information could lead to ineffective disease control measures and potentially contribute to disease outbreaks in other areas, particularly when infected tourists return from this area.

Understanding the seasonal dynamics of mosquito vectors is crucial because it leads to variations in species composition and abundance (Foley et al. 2017). Meteorological factors, which vary with each season, significantly influence adult mosquito abundance by altering the quality and quantity of breeding habitats (Foley et al. 2017; Abdulloh et al. 2024). A recent survey on the environmental dynamics of coastal mosquito vectors in Central Thailand revealed that seasonal fluctuations significantly affect the larval densities of these mosquito species (Abdulloh et al. 2024). The study also found that specific water quality factors and meteorological parameters in coastal areas have a considerable influence on mosquito vector populations. Consequently, conducting surveillance of disease vectors each season is crucial for effective public health disease control.

To address these issues, this study investigated the composition of seasonal species and the abundance of mosquito vectors in the area surrounding the Huai Tha Khoei Reservoir in Ratchaburi Province, Thailand. The results may provide essential public health information for understanding the seasonal patterns of disease vector mosquitoes in this region. Such understanding may allow for the identification of target mosquito vectors for effective control, which may lead to a reduction in the cases of mosquito-borne infectious diseases in the area. Additionally, the study may serve as a valuable case study for understanding the seasonal diversity of mosquito vectors in other regions.

MATERIALS AND METHODS

Study area

The study site for this research was the area surrounding the Huai Tha Khoei Reservoir (13°17'15.7"N,

99°24'57.7"E) in the village of Phu Nam Ron, Ban Bueng Sub-district, Ban Kha District, Ratchaburi Province, western Thailand (Figure 1). The reservoir is located adjacent to the village. The surrounding region comprises a mixed deciduous forest and a plain at the foot of a mountain. Additionally, this location serves as a camping spot featuring beautiful natural scenery and hot springs within the Chaloem Phra Kiat Thai Prachan National Park, which is part of the national reserved forest on the left bank of the Phachi River, attracting an increasing number of tourists. In Western Thailand, the climatic year is traditionally segmented into three distinct seasons, each reflecting the region's unique weather patterns within a tropical setting. The seasons are as follows: the wet or rainy season (June to October), known for its high rainfall and hot temperatures; the cool season (November to February), characterized by lower temperatures and minimal rainfall; and the hot season (March to May), where temperatures peak and the climate remains dry. This classification caters to the nuanced variations observed in the area's climate. Meteorological data, including rainfall, relative humidity, temperature, and wind speed for these seasons, were collected from Thailand's meteorological stations and are detailed in Table 1.

Mosquito sample collection

Mosquito samples were collected around the Huai Tha Khoei Reservoir using Centers for Disease Control and Prevention (CDC) light traps (John W. Hock Co., Gainesville, FL, USA) during three distinct seasons: the rainy season in October 2021, the cool season in January 2022, and the hot season again in April 2022. Six CDC light traps were installed approximately 1.5 m above ground at different locations around the reservoir: two in the upper area (13°17'15.7"N, 99°24'57.7"E), two in the middle area (13°16'29.2"N, 99°25'38.9"E), and two in the lower area (13°14'48.4"N, 99°25'34.6"E; Figure 1B). These traps operated overnight, from 6:00 P.M. to 6:00 A.M., for five consecutive days during each season. In the morning (6:00-7:00 A.M.), the catch bags were removed from the CDC light traps and placed in a box with dry ice for 20 min to euthanize the captured mosquitoes. The catch bags containing the mosquito samples were then stored in a freezer at -20°C. After the five-day collection period, all samples were transported by vehicle to the College of Allied Health Sciences Laboratory at Suan Sunandha Rajabhat University, Samut Songkhram Campus. This study received ethical approval from the Institutional Animal Care and Use Committee of Suan Sunandha Rajabhat University (Ref. No. IACUC 64-002/2021).

Mosquito identification

Morphological identification

The collected mosquitoes were identified at the species level using a Nikon SMZ 800 N stereomicroscope (Nikon Corp., Tokyo, Japan), based on the illustrated keys for the mosquitoes of Thailand (Rattanaarithikul et al. 2005; Rattanaarithikul et al. 2006a; Rattanaarithikul et al. 2006b; Rattanaarithikul et al. 2007; Rattanaarithikul et al. 2010).

Molecular identification

For identifying cryptic *Anopheles* species within complexes or groups, known as primary malaria vectors, multiplex allele-specific polymerase chain reaction (AS-PCR) assays based on the internal transcribed spacer 2 (ITS2) region were used. The *Anopheles dirus* Peyton and Harrison 1979 complex was identified following the procedure of Walton et al. (1999), using primers specific to *A. dirus* sensu stricto (s.s.), *Anopheles baimaii* Sallum and Peyton 2005, *Anopheles cracens* Sallum and Peyton 2005, *Anopheles nemophilous* Peyton and Ramalingam 1988, and *Anopheles scanloni* Sallum and Peyton 2005. The *Anopheles minimus* Theobald, 1901 complex was identified according to Garros et al. (2004), using primers specific to *A. minimus* s.s. and *Anopheles harrisoni* Harbach and Manguin 2007. Members of the *Anopheles maculatus* Theobald 1901 group were identified using the AS-PCR assay as outlined by Walton et al. (2007), with primers

specific to *A. maculatus* s.s., *Anopheles dravidicus* Christophers 1924, *Anopheles pseudowillmori* Theobald 1910, *Anopheles rampae* Harbach and Somboon 2011, and *Anopheles sawadwongporni* Rattanakrithikul and Green 1987. All primer sets used are shown in Table 2. Genomic DNA was extracted from two to six legs of primary malaria vector samples using the FavorPrep™ Mini Kit (Favorgen Biotech, Ping-Tung, Taiwan), following the manufacturer's instructions. Platinum Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA) was used in all multiplex PCR assays. The amplified PCR products were subjected to electrophoresis using 2% agarose gels, which were stained with Midori Green DNA stain (Nippon Gene, Tokyo, Japan). This staining facilitated the identification of species-specific DNA bands, which were then imaged using an ImageQuant LAS 500 imager (GE Healthcare Japan Corp., Tokyo, Japan).

Table 1. Meteorological data for three seasons at the study site

| | Rainy season | | Cool season | | Hot season | |
|-----------------------|--------------|-------|-------------|-------|------------|-------|
| | Mean | SD | Mean | SD | Mean | SD |
| Rainfall (mm) | 143.22 | 40.01 | 26.15 | 24.09 | 24.80 | 34.25 |
| Relative humidity (%) | 81.80 | 2.17 | 72.25 | 7.14 | 72.67 | 1.53 |
| Temperature (°C) | 27.98 | 0.73 | 26.05 | 1.10 | 29.93 | 1.33 |
| Wind speed (knots) | 1.46 | 1.20 | 3.55 | 0.69 | 3.07 | 0.06 |

Note: SD: standard deviation

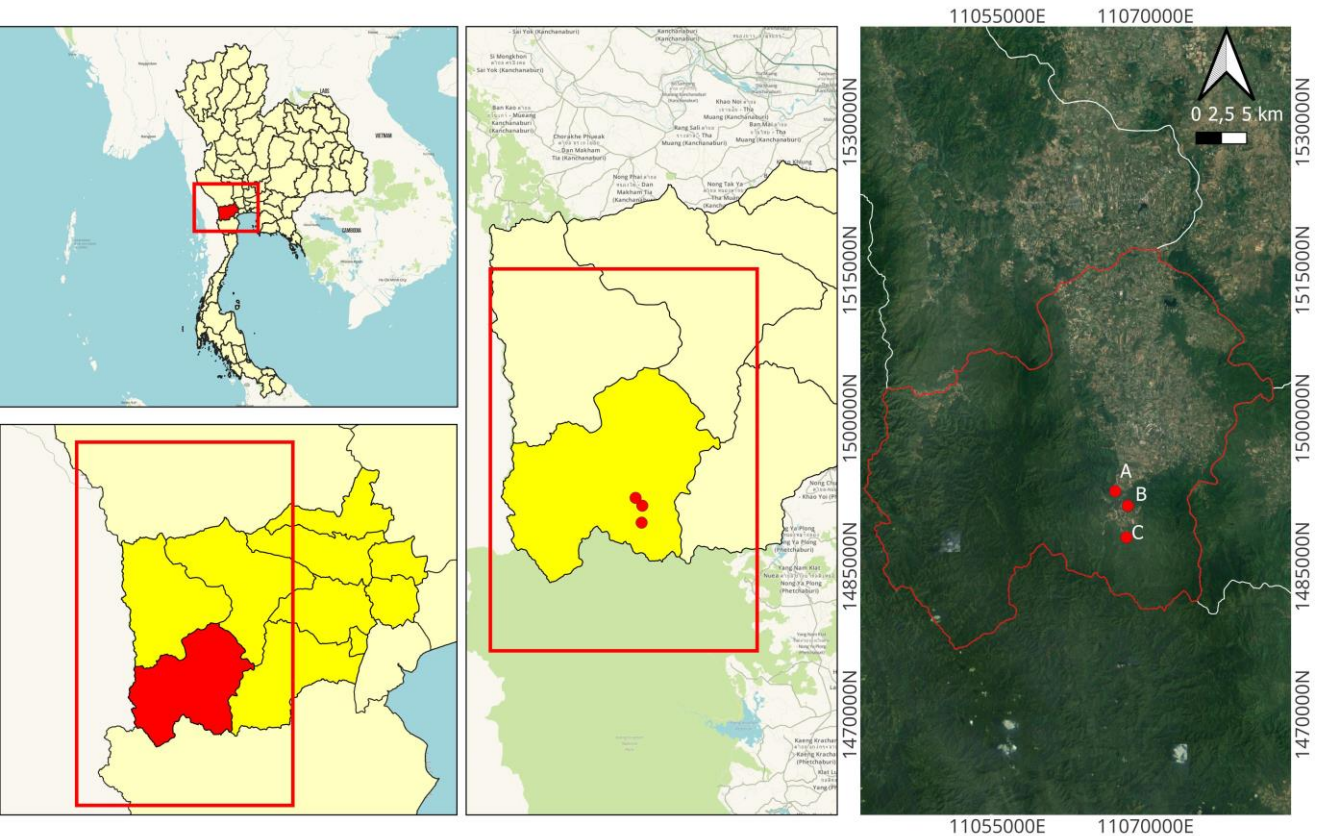


Figure 1. Map of the Huai Tha Khoei Reservoir area in Thailand, showing the designated study site within Ban Kha District, Ratchaburi, Thailand. A. Upper area; B. Middle area; C. Lower area

Statistical analysis

The seasonal composition, species richness, and diversity of mosquitoes collected from the study site were analyzed using the Paleontological Statistics (PAST) software package, Version 4.01 (Hammer et al. 2001). Relative abundance was calculated as the number of specimens of a particular species divided by the total number of specimens collected and then multiplied by 100 (Attallah et al. 2023). Species richness is defined as the number of mosquito species identified in each season and across all seasons. Several diversity indices were calculated, including the Shannon-Wiener index, Simpson's dominance index, Margalef's species richness index, and species evenness.

The Shannon-Wiener index, also known as the Shannon diversity index, is calculated using the formula $H' = -\sum [p_i(\ln p_i)]$, where p_i is the proportion of all species i and \ln denotes the natural logarithm; $p_i = n_i/N$, with n_i representing the number of individuals of taxon i and N the total number of individuals (Magurran 1988). Simpson's dominance index (1-D) is estimated by $1 - \sum (p_i^2)$, where $p_i = n_i/N$. In this index, a value of 0 signifies infinite diversity, whereas a value of 1 indicates no diversity. As such, the larger the value of D , the lower the diversity (Magurran 1988). Margalef's species richness index (d) measures the total number of species in a community and is calculated using the formula: $S-1/\ln N$, where S is the total number of species and N is the total number of individuals. Higher values of d indicate greater species richness (Magurran 1988). Species evenness, denoted as $J = H'/H_{\max}$, ranges from 0 to 1. A value of 0 suggests minimum evenness, reflecting a complete lack of equity among species, whereas a value of 1 denotes perfect evenness, indicating an equal distribution of organisms across all species (Magurran 1988).

The rarefaction curve was employed to estimate species richness and diversity from sampling results across different seasons (Shimadzu 2018). The analysis was performed using PAST software. Additionally, to determine the similarity in mosquito species diversity among different seasons, a UPGMA (Unweighted Pair Group Method with Arithmetic Mean) tree was constructed. This was based on

pairwise beta diversity calculations, which were also performed using PAST software.

RESULTS AND DISCUSSION

Seasonal mosquito species composition and abundance

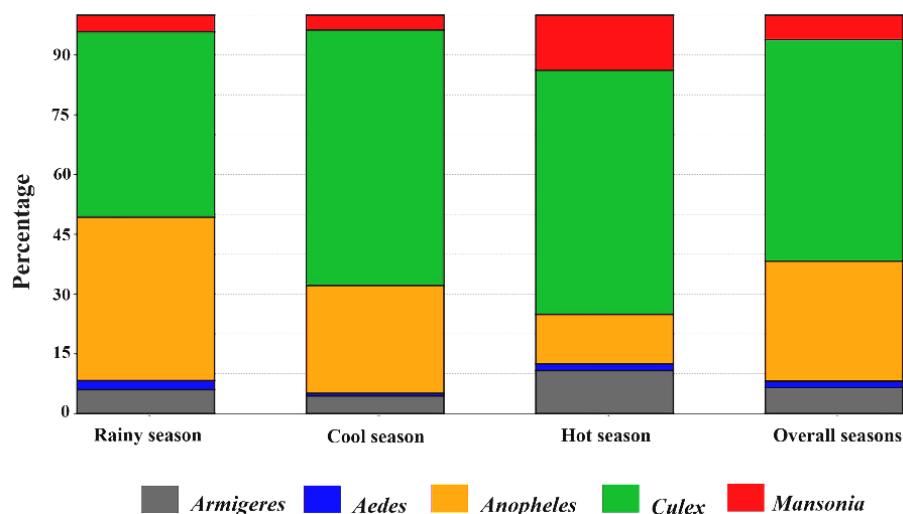
A total of 1363 mosquitoes were collected around the Huai Tha Khoei Reservoir, representing 30 species from five genera (Figure 2, Table 3). The five genera included in the collection were: *Armigeres* with two species (*Armigeres flavus* Leicester 1908 and *Armigeres subalbatus* Coquillett 1898), *Aedes* with four species (*Aedes albopictus* Skuse 1894, *Aedes lineatopennis* Ludlow 1905, *Aedes niveus* Eichwald 1837 and *Aedes vexans* Meigen 1830), *Anopheles* with fourteen species (*Anopheles aconitus* Dönitz 1902, *Anopheles barbirostris* Wulp 1884 s.l., *Anopheles crawfordi* Reid 1953, *Anopheles dirus* Peyton and Harrison 1979 s.s., *Anopheles hyrcanus* Pallas, 1771, *Anopheles indefinitus* Ludlow 1904, *Anopheles jeyporiensis* James 1902, *Anopheles karwari* James 1903, *Anopheles maculatus* Theobald 1901 s.s., *Anopheles minimus* Theobald 1901 s.s., *Anopheles nivipes* Theobald 1903, *Anopheles pseudojamesii* Strickland and Chowdhury 1927, *Anopheles sawadwongporni* Rattanakul and Green 1987 and *Anopheles varuna* Iyengar 1924), *Culex* with seven species (*Culex bitaeniorhynchus* Giles 1901, *Culex fuscocephala* Theobald 1907, *Culex gelidus* Theobald 1901, *Culex pseudovishnui* Colless 1957, *Culex tritaeniorhynchus* Giles 1901, *Culex vishnui* Theobald 1901 and *Culex whitei* Barraud 1923) and *Mansonia* with three species (*Mansonia annulifera* Theobald 1901, *Mansonia indiana* Edwards 1930 and *Mansonia uniformis* Theobald 1901) (Table 2). The highest proportion of mosquitoes caught belonged to the *Culex* genus in all seasons, accounting for 46.50% in the rainy season, 64.08% in the cool season, 61.28% in the hot season, and 55.54% across all seasons. In contrast, the *Aedes* genus had the lowest proportion in all seasons, accounting for 2.28% in the rainy season, 0.67% in the cool season, 1.68% in the hot season, and 1.61% across all seasons (Figure 2).

Table 2. Primers for *Anopheles* species identification

| Mosquitoes | Primer name | Sequence (5' to 3') | Size of the product (bp) |
|--|-------------|----------------------------|--------------------------|
| <i>Anopheles dirus</i> complex | | | |
| <i>A. dirus</i> s.s./ <i>A. scanloni</i> | D-AC | CAC AGC GAC TCC ACA CG | 562/353 |
| <i>A. baimai</i> | D-D | GCG CGG GAC CGT CCG TT | 306 |
| <i>A. cracens</i> | D-B | CGG GAT ATG GGT CGG CC | 514 |
| <i>A. nemophilous</i> | D-F | AAC GGC GGT CCC CTT TG | 223 |
| Forward primer | D-U | CGC CGG GGC CGA GGT GG | |
| <i>Anopheles minimus</i> complex | | | |
| <i>A. minimus</i> s.s. | MIA | CCC GTG CGA CTT GAC GA | 310 |
| <i>A. harrisoni</i> | MIC | GTT CAT TCA GCA ACA TCA GT | 180 |
| Forward primer | ITS2A | TGT GAA CTG CAG GAC ACA T | |
| <i>Anopheles maculatus</i> group | | | |
| <i>A. maculatus</i> s.s. | MAC | GAC GGT CAG TCT GGT AAA GT | 180 |
| <i>A. dravidicus</i> | DRAV | GCC TAC TTT GAG CGA GAC CA | 477 |
| <i>A. pseudowillmori</i> | PSEU | GCC CCC GGG TGT CAA ACA G | 203 |
| <i>A. rampae</i> | K | TTC ATC GCT CGC CCT TAC AA | 301 |
| <i>A. sawadwongporni</i> | SAW | ACG GTC CCG CAT CAG GTG C | 242 |
| Forward primer | 5.8F | ATC ACT CGG CTC GTG GAT CG | |

Table 3. Species composition and seasonal abundance of mosquitoes collected around the Huai Tha Khoei Reservoir, Thailand

| Mosquito species | Rainy season | | Cool season | | Hot season | | Overall seasons | |
|-----------------------------|-----------------|------------------------|-----------------|------------------------|-----------------|------------------------|-----------------|------------------------|
| | Total collected | Relative abundance (%) | Total collected | Relative abundance (%) | Total collected | Relative abundance (%) | Total collected | Relative abundance (%) |
| <i>Armigeres</i> (n = 89) | | | | | | | | |
| <i>A. flavus</i> | 1 | 0.16 | 0 | 0.00 | 0 | 0.00 | 1 | 0.07 |
| <i>A. subalbatus</i> | 36 | 5.85 | 20 | 4.43 | 32 | 10.77 | 88 | 6.46 |
| <i>Aedes</i> (n = 22) | | | | | | | | |
| <i>A. albopictus</i> | 10 | 1.63 | 1 | 0.22 | 3 | 1.01 | 14 | 1.03 |
| <i>A. lineatopennis</i> | 0 | 0.00 | 1 | 0.22 | 0 | 0.00 | 1 | 0.07 |
| <i>A. niveus</i> | 0 | 0.00 | 0 | 0.00 | 2 | 0.67 | 2 | 0.15 |
| <i>A. vexans</i> | 4 | 0.65 | 1 | 0.22 | 0 | 0.00 | 5 | 0.37 |
| <i>Anopheles</i> (n = 411) | | | | | | | | |
| <i>A. aconitus</i> | 0 | 0.00 | 2 | 0.44 | 0 | 0.00 | 2 | 0.15 |
| <i>A. barbirostris</i> s.l. | 163 | 26.50 | 25 | 5.54 | 8 | 2.69 | 196 | 14.38 |
| <i>A. crawfordi</i> | 1 | 0.16 | 0 | 0.00 | 0 | 0.00 | 1 | 0.07 |
| <i>A. dirus</i> s.s. | 31 | 5.04 | 2 | 0.44 | 1 | 0.34 | 34 | 2.49 |
| <i>A. hyrcanus</i> group | 25 | 4.07 | 11 | 2.44 | 1 | 0.34 | 37 | 2.71 |
| <i>A. indefinitus</i> | 0 | 0.00 | 1 | 0.22 | 0 | 0.00 | 1 | 0.07 |
| <i>A. jeyporiensis</i> | 0 | 0.00 | 1 | 0.22 | 0 | 0.00 | 1 | 0.07 |
| <i>A. karwari</i> | 3 | 0.49 | 0 | 0.00 | 0 | 0.00 | 3 | 0.22 |
| <i>A. maculatus</i> s.s. | 2 | 0.33 | 4 | 0.89 | 1 | 0.34 | 7 | 0.51 |
| <i>A. minimus</i> s.s. | 13 | 2.11 | 34 | 7.54 | 17 | 5.72 | 64 | 4.70 |
| <i>A. nivipes</i> | 1 | 0.16 | 0 | 0.00 | 0 | 0.00 | 1 | 0.07 |
| <i>A. pseudojamesii</i> | 0 | 0.00 | 1 | 0.22 | 0 | 0.00 | 1 | 0.07 |
| <i>A. sawadwongporni</i> | 13 | 2.11 | 35 | 7.76 | 6 | 2.02 | 54 | 3.96 |
| <i>A. varuna</i> | 0 | 0.00 | 6 | 1.33 | 3 | 1.01 | 9 | 0.66 |
| <i>Culex</i> (n = 757) | | | | | | | | |
| <i>C. bitaeniorhynchus</i> | 0 | 0.00 | 1 | 0.22 | 1 | 0.34 | 2 | 0.15 |
| <i>C. fuscocephala</i> | 0 | 0.00 | 1 | 0.22 | 0 | 0.00 | 1 | 0.07 |
| <i>C. gelidus</i> | 40 | 6.50 | 0 | 0.00 | 0 | 0.00 | 40 | 2.93 |
| <i>C. pseudovishnui</i> | 17 | 2.76 | 18 | 3.99 | 12 | 4.04 | 47 | 3.45 |
| <i>C. tritaeniorhynchus</i> | 180 | 29.27 | 222 | 49.22 | 136 | 45.79 | 538 | 39.47 |
| <i>C. vishnui</i> | 49 | 7.97 | 41 | 9.09 | 33 | 11.11 | 123 | 9.02 |
| <i>C. whitei</i> | 0 | 0.00 | 6 | 1.33 | 0 | 0.00 | 6 | 0.44 |
| <i>Mansonia</i> (n = 84) | | | | | | | | |
| <i>M. annulifera</i> | 12 | 1.95 | 16 | 3.55 | 41 | 13.80 | 69 | 5.06 |
| <i>M. indiana</i> | 13 | 2.11 | 1 | 0.22 | 0 | 0.00 | 14 | 1.03 |
| <i>M. uniformis</i> | 1 | 0.16 | 0 | 0.00 | 0 | 0.00 | 1 | 0.07 |
| Total | 615 | 100.00 | 451 | 100.00 | 297 | 100.00 | 1363 | 100.00 |

**Figure 2.** Proportions of mosquito genera captured in each season and across all seasons, expressed as percentages. Each color represents the number of mosquitoes from a particular genus collected during each season: grey, *Armigeres*; blue, *Aedes*; orange, *Anopheles*; green, *Culex*; and red, *Mansonia*

Across all seasons, *C. tritaeniorhynchus* was the most dominant species, accounting for 39.47% ($n = 538$) of the mosquitoes, followed by *A. barbirostris* s.l. ($n = 196$; 14.38%) and *C. vishnui* ($n = 123$; 9.02%; see Table 2). When examining each season individually, the findings were as follows: during the rainy season, *C. tritaeniorhynchus* remained the most dominant at 29.27% ($n=180$) of the mosquitoes, followed by *A. barbirostris* s.l. ($n=163$; 26.50%) and *C. vishnui* ($n=49$; 7.97%); in the cool season, the dominance of *C. tritaeniorhynchus* increased to 49.22% ($n=222$), followed by *C. vishnui* ($n=41$; 9.09%) and *An. sawadwongporni* ($n=35$; 7.76%); and in the hot season, *C. tritaeniorhynchus* again topped the list, representing 45.79% ($n = 136$) of mosquitoes, followed by *M. annulifera* ($n=41$; 13.80%) and *C. vishnui* ($n=33$; 11.11%). Of the mosquitoes found in the study area, 25 of the 30 species were reported as vectors of human pathogens, which constitutes 83.33%, as indicated in Table 4.

Mosquito diversity

Diversity indices of mosquitoes around the Huai Tha Khoei Reservoir for each season, as well as across all seasons, are presented in Table 5. Species richness was highest in the cool season with 23 species, followed by the rainy season with 20 species and the hot season with 15 species. The Shannon diversity index (H') peaked during the rainy season at 2.144, whereas the lowest value was observed in the hot season at 1.788. Conversely, Simpson's dominance index ($1-D$) was highest in the cool season at 0.271 and lowest in the rainy season at 0.177. Margalef's species richness index (d) also reached its peak in the cool season at 3.6 and was lowest in the hot season at 2.459. In terms of species evenness, the highest value was found in the rainy season at 0.716, with the lowest in the cool season at 0.613.

Table 4. List of medically important mosquito species collected around the Huai Tha Khoei Reservoir, Thailand

| Species | Potential vectors |
|-----------------------------|---|
| <i>Armigeres</i> | |
| <i>A. flavus</i> | No report |
| <i>A. subalbatus</i> | Viruses: Japanese encephalitis (JE) virus (Liu et al. 2023) and Zika virus (Yang et al. 2022); Parasites: zoonotic filarial nematodes and <i>Dirofilaria immitis</i> (Intarapuk and Bhumiratana 2021) |
| <i>Aedes</i> | |
| <i>A. albopictus</i> | Viruses: dengue virus (Ahebwa et al. 2023), chikungunya virus (Vega-Rúa et al. 2020), JE virus (Hernández-Triana et al. 2022), Rift Valley fever (RVF) virus (Brustolin et al. 2017), West Nile virus (Rothman et al. 2021), yellow fever virus (Damasceno-Caldeira et al. 2023) and Zika virus (Gomes et al. 2023) |
| <i>A. lineatopennis</i> | Viruses: JE virus, RVF virus and West Nile virus (Jitpakdi et al. 2005) |
| <i>A. niveus</i> | Virus: dengue virus (Young et al. 2017); Parasites: lymphatic filariae <i>Wuchereria bancrofti</i> (Premkumar et al. 2022) |
| <i>A. vexans</i> | Viruses: dengue virus (Hasty et al. 2020), chikungunya virus (Karliuk et al. 2021), RVF virus (Ndiaye et al. 2016), West Nile virus (Birnberg et al. 2019) and Zika virus (Gendernalik et al. 2017) |
| <i>Anopheles</i> | |
| <i>A. aconitus</i> | Parasites: malaria parasite <i>Plasmodium</i> spp. (Sukkanon et al. 2022) |
| <i>A. barbirostris</i> s.l. | Parasites: <i>Plasmodium</i> spp. (Sriwichai et al. 2016) and <i>W. bancrofti</i> (Muturi et al. 2008) |
| <i>A. crawfordi</i> | No report |
| <i>A. dirus</i> s.s. | Parasites: <i>Plasmodium</i> spp. (Sukkanon et al. 2022), <i>W. bancrofti</i> (Pothikasikorn et al. 2008) and zoonotic filarial nematodes (Siriyaasatien et al. 2023) |
| <i>A. hyrcanus</i> group | Parasites: <i>Plasmodium</i> spp. (Sukkanon et al. 2022) and zoonotic filarial nematodes (Siriyaasatien et al. 2023) |
| <i>A. indefinitus</i> | No report |
| <i>A. jeyporiensis</i> | Parasite: <i>Plasmodium</i> spp. (Chinh et al. 2019) |
| <i>A. karwari</i> | Parasite: <i>Plasmodium</i> spp. (Sukkanon et al. 2022) |
| <i>A. maculatus</i> s.s. | Parasites: <i>W. bancrofti</i> (Pothikasikorn et al. 2008) and <i>Plasmodium</i> spp. (Sriwichai et al. 2016) |
| <i>A. minimus</i> s.s. | Parasites: <i>W. bancrofti</i> (Pothikasikorn et al. 2008) and <i>Plasmodium</i> spp. (Sriwichai et al. 2016) |
| <i>A. nivipes</i> | Parasite: <i>Plasmodium</i> spp. (Sukkanon et al. 2022) |
| <i>A. pseudojamesii</i> | No report |
| <i>A. sawadwongporni</i> | Parasite: <i>Plasmodium</i> spp. (Sukkanon et al. 2022) |
| <i>A. varuna</i> | Parasite: <i>Plasmodium</i> spp. (Sukkanon et al. 2022) |
| <i>Culex</i> | |
| <i>C. bitaeniorhynchus</i> | Virus: JE virus (Auerswald et al. 2021) |
| <i>C. fuscicephala</i> | Viruses: JE virus (Auerswald et al. 2021) |
| <i>C. gelidus</i> | Virus: JE virus (Auerswald et al. 2021) |
| <i>C. pseudovishnui</i> | Viruses: JE virus (van den Eynde et al. 2022) and West Nile virus (Khan et al. 2017) |
| <i>C. tritaeniorhynchus</i> | Viruses: JE virus (Auerswald et al. 2021) and West Nile virus (Khan et al. 2017); Parasite: zoonotic filarial nematodes (Siriyaasatien et al. 2023) |
| <i>C. vishnui</i> | Viruses: JE virus (Faizah et al. 2021) and West Nile virus (Khan et al. 2017) |
| <i>C. whitei</i> | No report |
| <i>Mansonia</i> | |
| <i>M. annulifera</i> | Parasite: lymphatic filariae <i>Brugia malayi</i> (Rojanapanus et al. 2019) |
| <i>M. indiana</i> | Parasite: <i>B. malayi</i> (Rojanapanus et al. 2019) |
| <i>M. uniformis</i> | Parasites: <i>B. malayi</i> (Rojanapanus et al. 2019) and <i>W. bancrofti</i> (Pothikasikorn et al. 2008) |

Rarefaction curve

To assess the species richness and diversity of mosquitoes from the sampling results across different seasons, rarefaction curves were used, as illustrated in Figure 3. These curves demonstrate that species richness (the total number of species) in the three seasons tended to increase with the number of samples collected but without reaching a detectable saturation point (Figure 3.A). This pattern suggests that across all three seasons, the higher the number of samples collected, the greater the number of species discovered. Conversely, the rarefaction curves for the Shannon diversity index (H') tended to stabilize within specific ranges: 1.6-1.7 in the hot season, 1.7-1.8 in the cool season, and 2.0-2.1 in the rainy season. This stabilization reflects the actual species diversity present in this habitat (Figure 3.B).

Table 5. Diversity indices of mosquitoes in each season and across all seasons.

| Diversity indices | Rainy season | Cool season | Hot season | Overall seasons |
|---------------------------------------|--------------|-------------|------------|-----------------|
| Individual count | 615 | 451 | 297 | 1363 |
| Species richness | 20 | 23 | 15 | 30 |
| Shannon diversity index (H') | 2.144 | 1.923 | 1.788 | 2.161 |
| Simpson's dominance index (1-D) | 0.177 | 0.271 | 0.259 | 0.199 |
| Margalef's species richness index (d) | 2.959 | 3.6 | 2.459 | 4.018 |
| Species evenness | 0.716 | 0.613 | 0.660 | 0.635 |

UPGMA tree

To quantify the differences in mosquito species diversity among the seasons, beta diversity was calculated, as presented in Table 6. The UPGMA tree, based on beta diversity analysis, indicates that the species diversity in the cool season was similar to that in the hot season. Conversely, the rainy season exhibited a diversity profile that differs from both the cool and hot seasons (Figure 4).

Table 6. Pairwise beta diversity of mosquitoes around the Huai Tha Khoei Reservoir in each season

| | Rainy season | Cool season | Hot season |
|--------------|--------------|-------------|------------|
| Rainy season | 0 | | |
| Cool season | 0.349 | 0 | |
| Hot season | 0.314 | 0.263 | 0 |

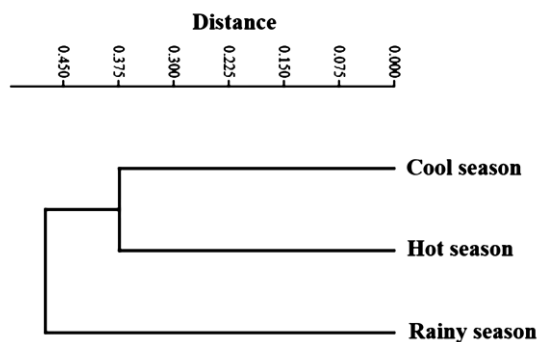


Figure 4. UPGMA tree based on beta diversity analysis

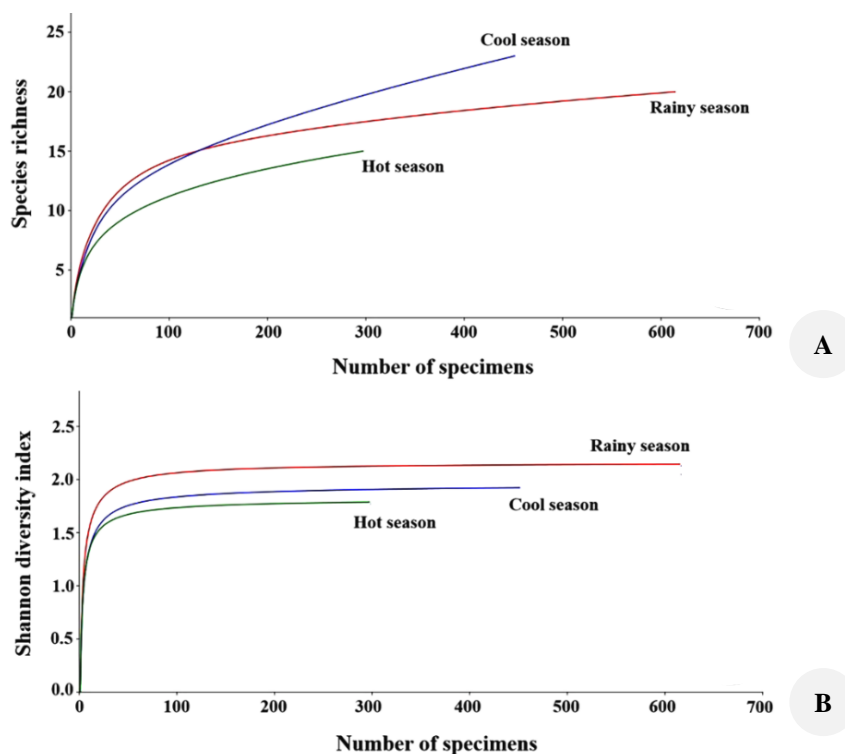


Figure 3. Rarefaction curves showing A. The species richness and; B. Shannon diversity index in samples collected from the Huai Tha Khoei Reservoir across different seasons

Discussion

Surveying the seasonal diversity of mosquitoes in various areas is essential for the surveillance of mosquito-borne diseases. Additionally, the information obtained from such surveys helps to establish tailored guidelines for mosquito vector population control specific to each area. Thus, this study focused on examining the composition of seasonal species and the abundance of mosquito vectors in the Huai Tha Khoei Reservoir, Ratchaburi, Thailand. The study area is unique, characterized by an environment that is undergoing changes influenced by human activities.

Species composition analysis in the Huai Tha Khoei Reservoir revealed several important mosquito vectors, particularly those for malaria and JE. Important malaria vectors identified include *A. dirus* s.s., *A. maculatus* s.s., *A. minimus* s.s., *A. pseudowillmori* and *A. sawadwongporni*, as confirmed by molecular methods (Tainchum et al. 2015; Tananchai et al. 2019). These *Anopheles* species are predominantly found in forest areas or forest terraces, reflecting the abundance of natural habitats around the reservoir and the potential malaria risk in the area. Their breeding sites are typically located in natural water sources, which complicates the control of their immature stages (Tainchum et al. 2015; Tananchai et al. 2019). As a result, effective control measures often focus on adult mosquitoes, employing strategies such as indoor residual spraying and the use of insecticide-treated nets (Parker et al. 2015). Additionally, it is crucial to inform tourists about the risk of mosquito-borne diseases in the area. Tourists should be advised to consistently apply insect repellent in order to prevent infections from mosquito bites. For instance, previous studies have reported cases of *Plasmodium knowlesi* infection among foreign tourists in southern Thailand (Froeschl et al. 2018).

Significant vectors of JE, including *C. pseudovishnui*, *C. tritaeniorhynchus*, *C. vishnui*, *C. gelidus*, and *C. fuscocephala*, have been identified in the area, with *C. tritaeniorhynchus* being the most prevalent (Auerswald et al. 2021; Lessard et al. 2021). These *Culex* mosquitoes typically inhabit rural areas with agricultural activities, reflecting the landscape surrounding the reservoir, which includes communities and agricultural land. Previous studies have indicated that an increase in agricultural land leads to a rise in the number of JE vectors, often breeding in rice fields (Ha et al. 2021). Moreover, the *Culex* genus is found more commonly throughout all seasons, possibly because their breeding sites are more varied than those of other mosquito genera, which usually prefer specific habitats (Rattanaarithikul et al. 2005). During the rainy season, it was observed that *Culex* mosquitoes were less abundant compared to other seasons. This reduction in abundance is speculated to result from the impact of rainstorms on their breeding grounds. For instance, excessive rainfall can lead to flooding that washes away the larvae from rice fields, posing a significant obstacle to their survival (Rattanaarithikul et al. 2005). However, there is a vaccine available against JE, and its administration is strongly recommended for individuals in high-risk areas such as this one (Sudjaritruk et al. 2022; Srivastava et al. 2023). Consequently, it is crucial for local health

authorities to initiate comprehensive vaccination campaigns for the community. Additionally, efforts should be made to prevent *Culex* mosquito bites, thereby minimizing the risk of JE transmission.

In addition to JE vectors, filariasis vectors such as *M. annulifera*, *M. indiana*, and *M. uniformis* (Rojanaparnus et al. 2019), along with *A. albopictus*—a known vector for chikungunya and Zika (Vega-Rúa et al. 2022; Gomes et al. 2023) were detected in the area, albeit at relatively low prevalences. Our study found a limited number of *Aedes* mosquitoes, including the absence of *A. aegypti*, the primary vector for dengue. This could be attributed to the fact that the traps were set up at night, whereas *Aedes* mosquitoes are predominantly active during daytime hours (Ndenga et al. 2017). Consequently, our findings predominantly offer insights into nocturnal mosquito species. However, in the event of an outbreak of *Aedes*-borne diseases in this or any other area, local public health agencies should conduct surveys of mosquito vectors during the daytime to align with the activity patterns of the mosquitoes being monitored.

Seasonal abundance patterns of mosquito species exhibit distinct variations, confirming that different environmental conditions in each season significantly affect the dynamics of mosquito populations in an area (Biteye et al. 2018; Baril et al. 2023). These seasonal changes are particularly notable in *Anopheles* mosquitoes, aligning with previous studies that have reported their sensitivity to environmental conditions and seasonal variability (Foley et al. 2017). *C. tritaeniorhynchus* was the most common species in all seasons and its relative abundance varied: 29.27% during the rainy season and 49.22% and 45.79% in the cool and hot seasons, respectively. The lower number of *C. tritaeniorhynchus* in the rainy season is attributed to the significant increase of *A. barbirostris* s.l., which had a relative abundance of 26.50%.

Anopheles barbirostris s.l. has been identified as a species complex, comprising a group of closely related species (Chaiphongpachara et al. 2022a). The *Barbirostris* complex includes six sibling species: *A. barbirostris* s.s., *A. campestris*, *A. dissidens*, *A. saeungae*, *A. vanderwulpi* and *A. wejchoochotei* (Wilai et al. 2020). Certain species within the *Barbirostris* complex have been closely associated with the transmission of malaria, as well as filarial worms, specifically *Brugia timori* and *Brugia malayi* (Townson et al. 2013). Previous studies in Thailand have suggested that the seasonal abundance of *Anopheles* mosquitoes may be influenced by the availability of quality breeding sites (Prakash et al. 1997; Rahman et al. 2002; Dev and Manguin 2016). The prevalence of *A. barbirostris* s.l. in the rainy season is possibly due to their breeding grounds, such as paddies, fishponds, swamps and canals, which are more abundant during this period (Rahman et al. 2002). In contrast, during the cool and hot seasons, these breeding grounds diminish with the lack of rain, leading to a marked decrease in the numbers of *A. barbirostris* s.l. Similarly, *A. dirus* s.s., the primary malaria vector, is more common in the rainy season but rarely found in other seasons. This prevalence is due to its breeding sites, which are typically animal footprints or natural ground depressions that often

contain water during the rainy season (Prakash et al. 1997). On the other hand, *A. minimus*, *A. sawadwongporni* and *A. maculatus* s.s. are less common in the rainy season but more prevalent during the cool season. This pattern is linked to their breeding habitats in small streams with slow-flowing water, which are more typical in the cool season (Dev and Manguin 2016). The fast water flow during the rainy season and significantly reduced water levels in the hot season render these streams unsuitable for breeding.

A diversity index refers to the variety of different species within an ecosystem and the relative abundance of each species (Magurran 1988). A community exhibits high species diversity when it consists of many species, each with similar levels of abundance. In contrast, a community with only a few species demonstrates low species diversity, which can lead to instability within the ecosystem. The diversity indices of mosquitoes in the area around the Huai Tha Khoei Reservoir revealed a species richness of 30, with a Shannon diversity index (H') of 2.161. This value is classified as exhibiting moderate diversity according to the ecological index levels defined by Hussain et al. (2012). When comparing mosquito diversity in the Huai Tha Khoei Reservoir area with other areas in Thailand, it was found that the reservoir area had higher diversity than the coastal areas in Samut Songkhram Province ($H'=0.94$) (Chaiphongpachara and Sumruayphol 2017) and higher than both the suburban ($H'=1.80$) and urban areas ($H'=2.00$) in Nakhon Nayok Province (Thongsripong et al. 2013). However, the diversity index in this study area is lower than in areas with forest environments, such as Doi Inthanon National Park in Chiang Mai Province ($H'=3.81$) and rural areas in Nakhon Nayok Province ($H'=2.30$) (Srisuka et al. 2022). Furthermore, compared to other countries in Asia, our study reveals a higher species diversity index than those reported in India (Singh et al. 2019; Radhakrishnan et al. 2019; Singh et al. 2020) and the Koh Kong mangrove forests in Cambodia (Maquart et al. 2022).

When comparing the diversity indices of mosquitoes across different seasons, it was found that the cool season exhibited the highest species richness and Margalef's species richness index values. This trend is attributed to the characteristics of the rainy season: although rainwater creates more breeding grounds, it is often accompanied by strong natural streams. In contrast, during the cool season, the intensity of stream flows and the impact of storms typically decrease, leading to an increase in some mosquito species. In addition, one previous study reported that flash flooding affects the mosquito population due to the effect of removing mosquito food resources, leading to a significant reduction in mosquito egg laying (Duchet et al. 2017). Meanwhile, the hot season had the lowest species richness, possibly because of the limited breeding sites due to water scarcity. However, rarefaction curve analysis shows that there is no end point for mosquito species found in the area in all three seasons because the area may contain species that we were not able to collect. This is consistent with a previous study that examined zoonotic malaria infection in *Anopheles* mosquitoes and found some mosquito species, including *A. subpictus*, that were not

found in this study (Nguyen et al. 2023). Therefore, relevant agencies should conduct additional surveys to report some mosquito species that have not been reported this time.

Although the highest species richness was observed in the cold season, the greatest species diversity was observed in the rainy season. This difference is due to differences in analytical approaches. Species richness refers to the number of different species present in a study area, whereas the species diversity index considers both the richness and the evenness of mosquito species (Magurran 1988). The highest species evenness was observed in the rainy season compared to the other seasons, leading to greater diversity despite potentially lower richness. While high rainfall and strong currents in streams can negatively affect many stream-dwelling mosquito species, certain species, such as *A. barbirostris* s.l., *A. dirus* s.s. and *A. karwari* derive benefits from the presence of rain. These species have breeding sites in puddles on the surface of the soil. This is consistent with the results of the UPGMA tree based on beta diversity, indicating that the diversity of mosquitoes in the rainy season was different from the other seasons. In addition, rarefaction curve analysis revealed that the mosquito diversity indexes for each season accurately represent the true species diversity of mosquitoes around the Huai Tha Khoei Reservoir. This is evidenced by the fact that the curves show no increase, indicating an end point. The results of this study indicate that rainfall is the most significant factor influencing mosquito diversity. This finding aligns with previous studies that have recognized rainfall as an important determinant of mosquito population diversity across different countries, such as Qatar (Tahir et al. 2023), Costa Rica (Romero-Vega et al. 2023), Estonia (Kirik et al. 2021), India (Prasad et al. 2021) and Spain (Roiz et al. 2014).

In conclusion, surveying the seasonal diversity of mosquitoes in various areas is crucial for monitoring mosquito-borne diseases. The information obtained from this study aids in establishing tailored guidelines for controlling mosquito vector populations in the area. However, the study's scope was limited by the brief data collection period, hindering our ability to explore correlations between mosquito diversity and environmental factors comprehensively. Future research should extend the duration and increase the frequency of monitoring to better assess the effects of environmental changes on mosquito populations in the region.

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