Faculty of Medical and Health Sciences, University of Poonch Rawalakot
Journal of Pharma and Biomedics
ISSN: 3007-1984(online), 3007-1976 (Print)
https://www.jpbsci.com/index.php/jpbs

Biomedical perspectives on the ecology and resting habits of *Culex quinquefasciatus* and *Anopheles gambiae* in district lower Dir, Pakistan

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Received: February 16, 2025; *Revised: March 22, 2025;* *Accepted: March 30, 2025*

A B S T R A C T

This study investigates the abundance and distribution of *Culex quinquefasciatus* and *Anopheles gambiae* sensu lato across different habitats and collection methods, with a focus on urban and rural settings. A total of 853 mosquitoes were collected from three primary habitats-house (C), deep shade (A), and grass scrub (B)- using two collection methods: hand catch and box shelters. Results revealed that *Culex quinquefasciatus* dominated the mosquito population, constituting 98.5% of the total catch, with the majority collected from house habitats (94.8%). Hand catching was more effective, yielding 62.6% of the total catch, while box shelters captured 37.4%. Statistical analysis showed a significant difference in catch rates between methods ($p = 0.0257$). Monthly variations indicated that rainfall positively correlated with mosquito abundance, especially for *Culex quinquefasciatus*, which peaked in May. *Anopheles gambiae* was primarily found in house habitats during August, suggesting seasonal and environmental influences on its distribution. There were no significant variations in mosquito numbers across different sites, emphasizing the importance of habitat type over geographic location. Meteorological conditions, including rainfall and humidity, played a crucial role in shaping mosquito populations. These findings highlight the need for targeted vector control strategies, particularly in indoor environments during peak breeding seasons, and emphasize the importance of adapting interventions based on seasonal and environmental factors. This study provides crucial insights for enhancing vector surveillance and control programs, especially in urban areas.

Keywords: *Culex quinquefasciatus*, *Anopheles gambiae*, Habitats, Breeding seasons.

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INTRODUCTION

Mosquitoes serve as vectors of many serious animal diseases to humans. Mosquito borne disease include malaria, filariasis, dengue and other arboviruses, that cause human suffering, loss of lives and considerably impaired economic development (1, 2). Globally, in 2013, mosquito borne diseases included malaria which caused a total of 584,000 deaths and 198 million cases (3), and dengue causes over 50-100 million new cases per year (4). Various mosquito species including Culex, Aedes and Anopheles spp are known to transmit arboviruses (2). The distribution of the mosquito species is influenced, among other factors, by the physical environment for breeding and resting (4-6) each of which can be altered by human activities and modify the disease transmission dynamics (5,7). Jos, a city in Nigeria, like most African urban centers, has been gradually expanding with growing population.

This expansion could bring forth the rise in contact between humans and wild monkeys that inhabit the surrounding hills. Such situation obviously increases the danger of the spread of zoonoses as well as arboviruses (9). Studies conducted in Jos Plateau showed that there was a significant difference in the abundance of mosquitoes in breeding sites in between the urban, semi-urban and rural areas, with Culex quinquefasciatus and Aedes aegypti being predominant in the larval samples from the containers (10).

Adult mosquitoes are most of the time resting in places of their preference than being in flight (7). Most species rest totally outdoors in natural resting places and only a few species like artificial shelters (7). The only few mosquito species found to rest indoors are known to be the vectors of malaria, filariasis and arboviruses (7). Outdoor resting mosquitoes tend to be dispersed in available habitats (6) and a number of methods have been used to collect them (6, 10, 11) sensitive and accurate sampling of Anopheles mosquitoes is a prerequisite for effective management of malaria vector control programmes.

The most reliable existing means to measure mosquito density is the human landing catch (HLC, 12 sensitive and accurate sampling of Anopheles mosquitoes is a prerequisite for effective management of malaria vector control programmes. The most reliable existing means to measure mosquito density is the human landing catch (HLC] including among others box310 shelters and powered aspirators and sweep nets. The present study was therefore carried out from the month of February to August to determine the resting habitat preferences for adult *Cx quinquefasciatus* and *An. gambiae* s.l along an urban-rural transect in Jos city using box shelters, powered aspirator and sweepnet. The results obtained aims to add to the information available for use in vector and disease control planning and implementation.

MATERIALS AND METHODS

Study Area

The study was conducted in District Dir Lower, Khyber Pakhtunkhwa, Pakistan, covering the following tehsils: Adenzai, Balambot, Timergara, Munda, Samrbagh, Lal Qilla, and Khall. The study aimed to assess the ecological characteristics, resting sites, and distribution of Culex quinquefasciatus and Anopheles gambiae in both urban and rural habitats across these tehsils.

The study area consisted of a transect from the urban areas to the surrounding rural bush areas, with distinct habitat types in each location. Sampling sites were chosen from both urban and rural settings to capture the full range of ecological conditions that might influence mosquito populations.

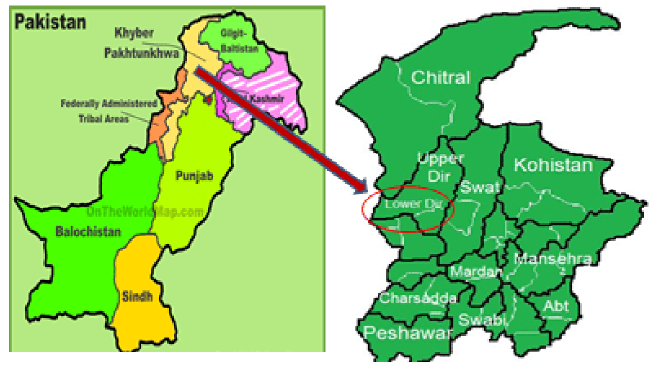


Figure 1: Lower Dir District is located in the Khyber Pakhtunkhwa province of Pakistan

Habitats

Three major habitat types were identified for sampling purposes

Habitat A (Deep Shade Riverbed Habitat)

Seasonal stream areas with pools of water that dry up as the dry season progresses. These sites are often shaded by dense vegetation along the river.

Habitat B (Grass Scrub Habitat)

Areas dominated by grass and low scrub vegetation, prevalent in the rural zones.

Habitat C (House Habitat)

Residential areas, water treatment stations, and other structures, primarily in urban settings, including homes and public buildings.

Sampling Sites

For each of the seven tehsils (Adenzai, Balmbot, Timergara, Munda, Samrbagh, Lal Qilla, and Khall), 20 sites were selected: 10 from urban areas and 10 from rural areas. This included a combination of indoor and outdoor habitats, chosen to cover diverse ecological conditions.

Each site was carefully selected to reflect the unique characteristics of the habitat types, including areas with a mix of vegetation, water sources, and human settlements. The total sampling sites for each tehsil included:

Urban Areas (10 sites)

Including residential homes, commercial buildings, public spaces, and water treatment facilities.

Rural Areas (10 sites)

Including areas with seasonal streams, scrubland, agricultural fields, and distant settlements.

Study Design

Sampling was carried out across various sites in rural and urban habitats, with the main objective of assessing the ecological conditions, mosquito populations, and resting sites in both types of environments. Sampling was conducted twice a week from February to August, with a total of 25 sampling occasions. However, logistical challenges reduced the frequency of sampling during the initial months, and the number of sampling days was adjusted based on transport availability.

Each sampling occasion involved the collection of mosquitoes from indoor and outdoor habitats using the methods outlined below.

Sampling Methods

Artificial Resting Sites: Box Shelters

Modified box shelters, measuring 30 cm high, 40 cm wide, and 20 cm deep, were used to collect mosquitoes. These shelters were equipped with water pots to increase humidity levels, making them more attractive to mosquitoes. Shelters were placed in various locations within each habitat type, including rural and urban areas. The shelters in urban areas were positioned below windows to capture mosquitoes exiting the buildings.

Sweep Net and Power Operated Aspirator

A 35 cm sweep net was used for catching mosquitoes resting in outdoor vegetation, crevices, and cracks. The power-operated aspirator, modified from a car vacuum cleaner, was used in areas with dense vegetation or where sweeping was difficult. The aspirator was particularly useful for sampling in rock holes, scrub areas, and other hard-to-reach locations.

Meteorological Recordings

Meteorological data, including temperature, humidity, and rainfall, were recorded at the time of each sampling session using a whirling hygrometer. Additional rainfall data was collected from the Geography Department of the University of Jos.

The temperature in the study area ranged from 21°C to 24°C, with annual rainfall ranging from 101.6 cm to 152.4 cm, peaking from April to October. The rest of the year remained dry, with less than 2.5 cm of rainfall per month, contributing to a lower humidity during the dry season due to the influence of Harmattan winds.

Identification of Mosquitoes

Mosquitoes collected from the various sampling methods were identified under a Bausch and Lomb dissecting microscope (0.7x – 3x magnification). Identification keys by Edwards, Gillies, and DeMeillon were used for the classification of Culex and Anopheles species.

RESULTS

The study investigated the abundance and distribution of Cx. quinquefasciatus and *An. gambiae* in various habitats and across different collection methods, including hand catch and box shelter. Sampling occurred over several months, from February to August, at various sites across three main habitats: house habitat (C), deep shade habitat (A), and grass scrub habitat (B). A total of 853 mosquitoes were collected, with Cx. quinquefasciatus making up 98.5% (840 mosquitoes) and *An. gambiae* contributing 1.5% (13 mosquitoes), as shown in Table 2.

Table 1: Number of Mosquito Sampling Occasions Achieved.

Collection Method	Feb	Mar	Apr	May	June	July	Aug	Total
Box Shelters	6	15	9	21	6	-	-	57
Hand catch (Sweep net/Aspirator)	6	15	9	21	12	3	9	75

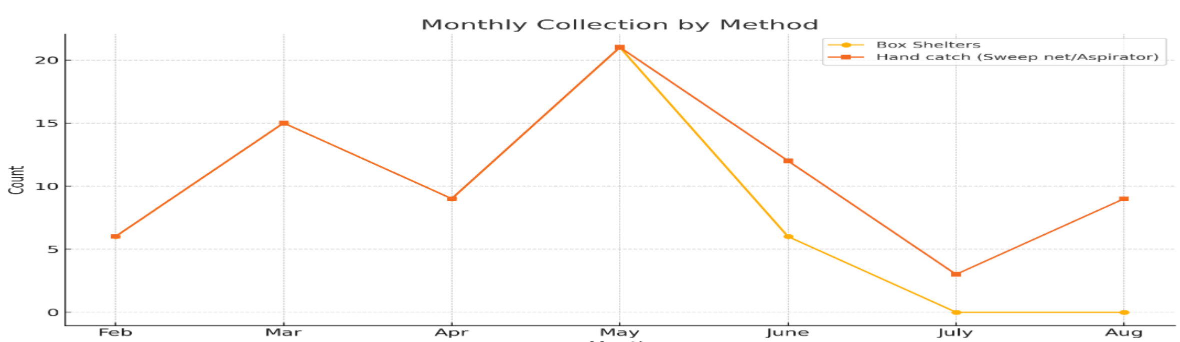
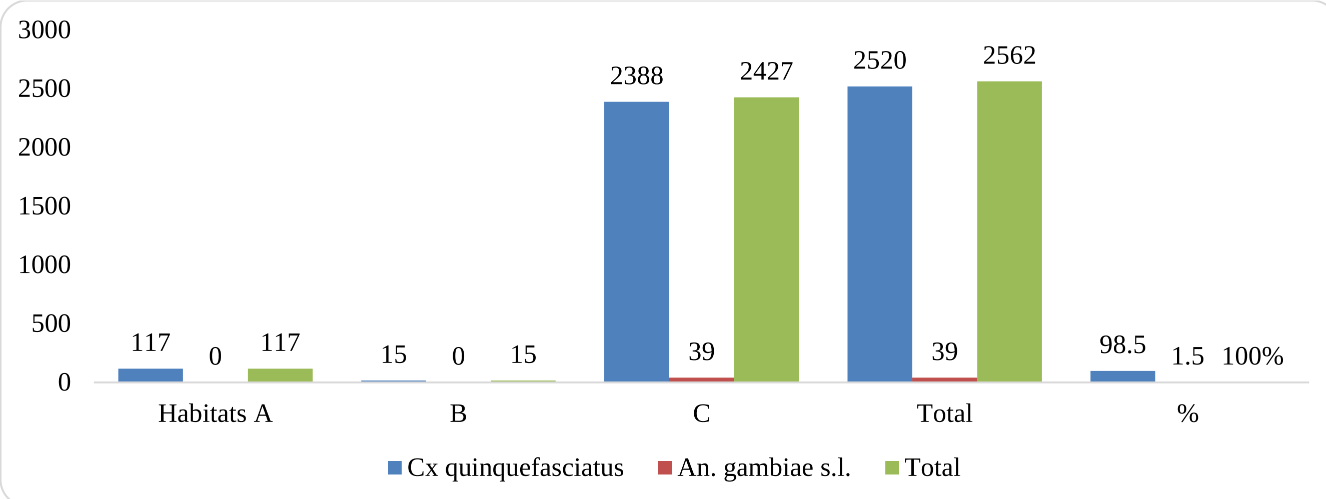


Table 2: Number of *Cx quinquefasciatus* and *An. gambiae* Caught in Each Habitat.

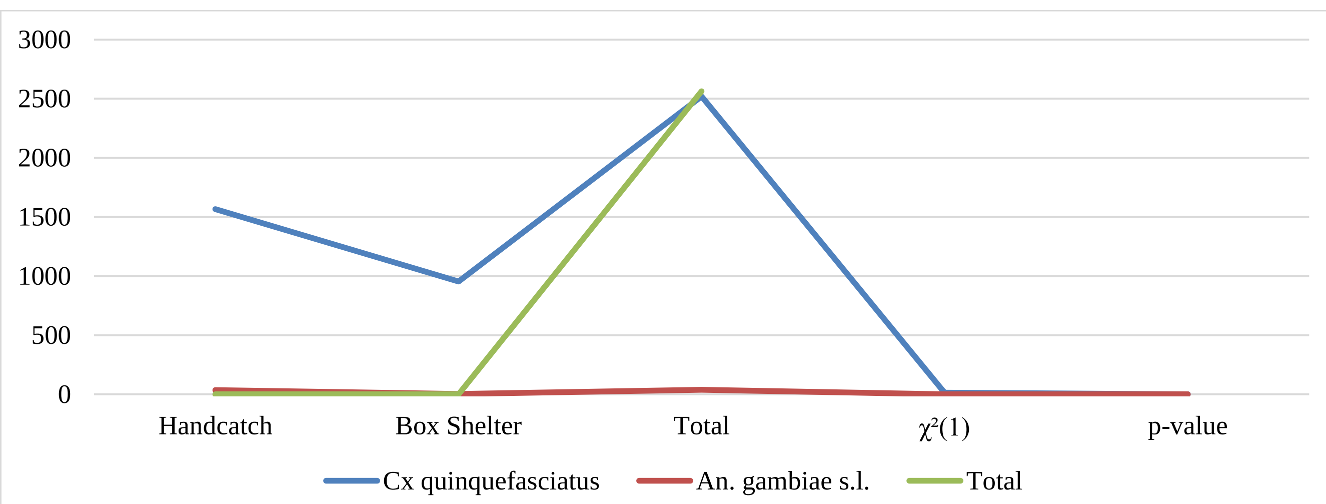
Mosquito Species	Habitats A	B	C	Total	%
Cx quinquefasciatus	117	15	2388	2520	98.5
An. gambiae	0	0	39	39	1.5
Total	117	15	2427	2562	100%



Of these, the majority (94.8%) were caught in the house habitat (C), while only 4.6% were from deep shade habitat (A) and 0.6% from grass scrub habitat (B). The relative abundance of the two mosquito species varied depending on the collection method used. Hand catching resulted in a higher catch rate, yielding 534 mosquitoes (62.6% of total catches), while box shelters collected 319 mosquitoes (37.4%) as seen in Table 3.

Table 3: Relative Abundance of *Cx quinquefasciatus* and *An. gambiae* collected by hand catch and Box Shelter Methods.

Mosquito Species	hand catch	Box Shelter	Total	$\chi^2(1)$	p-value
Cx quinquefasciatus	1566	954	2520	14.928	0.0257
An. gambiae	36	3	39	-	-
Total	1602 (62.6%)	957 (37.4%)	2562		



This was further supported by statistical analysis, which showed that the hand catch method resulted in significantly higher numbers of both species than the box shelter method (p = 0.0257). In terms of monthly variations, the box shelter method yielded the highest abundance of Cx. quinquefasciatus in April and May, with these two months contributing 35.8% and 36.6% of the total catch, respectively, as presented in Table 5. In comparison, the hand catch method showed a more consistent yield, with notable peaks occurring in April, May, and June, while *An. gambiae* was mainly found in the house habitat (C) in August, with a particularly high abundance observed in that month, as shown in Table 7. A comparison of mosquito abundance across sites using the box shelter method revealed variations in mosquito numbers along an urban to rural transect, but there were no significant trends or variations in abundance between sites, with site 6 consistently yielding no mosquitoes, as seen in Table 4. Similarly, the hand catch data showed no significant differences between sites, though differences between habitats were observed, with house habitat (C) yielding the highest abundance of mosquitoes, as demonstrated in Table 6. Meteorological conditions, particularly rainfall and relative humidity, appeared to influence mosquito abundance, with peaks in rainfall correlating with increased mosquito numbers, especially for Cx. quinquefasciatus, which showed a peak in May, as seen in Figure 3. The data demonstrated that Cx. quinquefasciatus was consistently more abundant in indoor house habitats (C) as compared to the outdoor deep shade (A) and grass scrub habitats (B). Further statistical analysis of the hand catch and box shelter data indicated significant differences in mosquito abundance between months, particularly in the house habitat (C), but not between sites, reinforcing the idea that habitat type was a more significant factor influencing mosquito populations than the specific location within the study area. These findings highlight the importance of habitat type and collection method in mosquito surveillance, with house habitats (C) consistently providing the highest yields for both mosquito species. This study provides valuable insights for vector control strategies, emphasizing the need for targeted interventions in urban and rural settings, particularly indoors, where mosquito populations are most abundant.

Table 4: Total Collections of Cx quinquefasciatus and An. gambiae from Different Sites by Box Shelter.

Species	House Habitat C (Urban to Rural)	Deep Shade Habitat A (Urban to Rural)	Total	%
Cx quinquefasciatus	192	39	231	99.7%
An. gambiae	3	0	3	0.3%
Total	195	39	234	100%

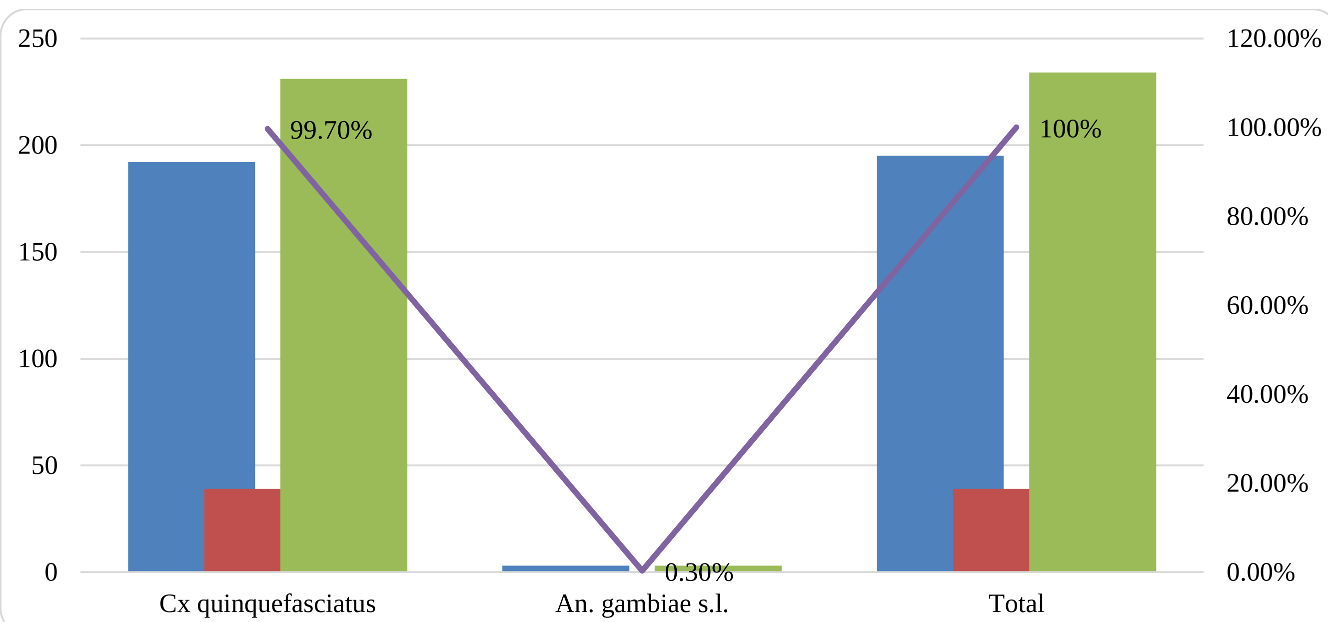


Table 5: Mean Monthly Abundance for Cx quinquefasciatus by Site and Habitat for Box Shelter Catches.

DOI: 10. 39401/jpbm.003.01.0023

Site	February	March	April	May	June	Total
1	0.6	1.7	2.1	6.3	1.5	12.2
2	0.6	3.0	1.9	6.4	1.8	13.7
3	0.0	1.5	2.1	2.1	1.3	7.0
4	0.0	3.5	3.6	3.2	1.5	12.4
5	0.6	6.0	4.2	2.1	1.1	14.0
6	0.0	0.9	1.5	4.0	1.3	7.7
Total	1.8	16.6	15.3	24.1	8.4	66.3

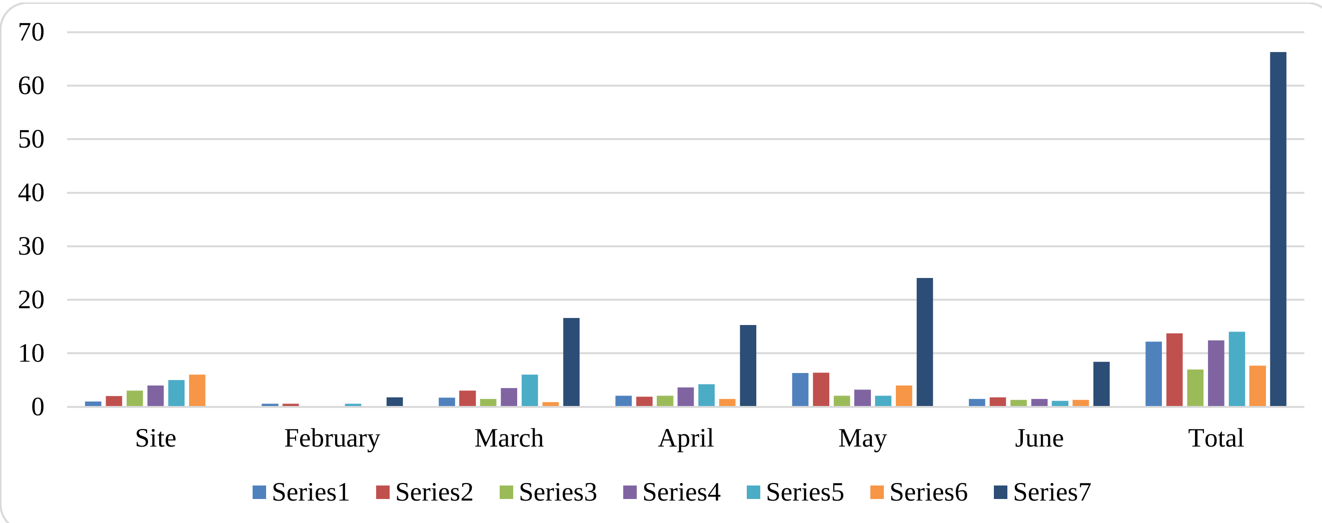


Table 6: Cx quinquefasciatus and An. gambiae Abundance between Sites by hand catch Collection Method.

Species	House Habitat C (Urban to Rural)	Grass-Scrub Habitat B (Urban to Rural)	Deep Shade Habitat A (Urban to Rural)	Total	%
Cx quinquefasciatus	1451	15	78	1544	97.6%
An. gambiae	24	0	15	39	2.4%
Total	1475	15	93	1583	100%

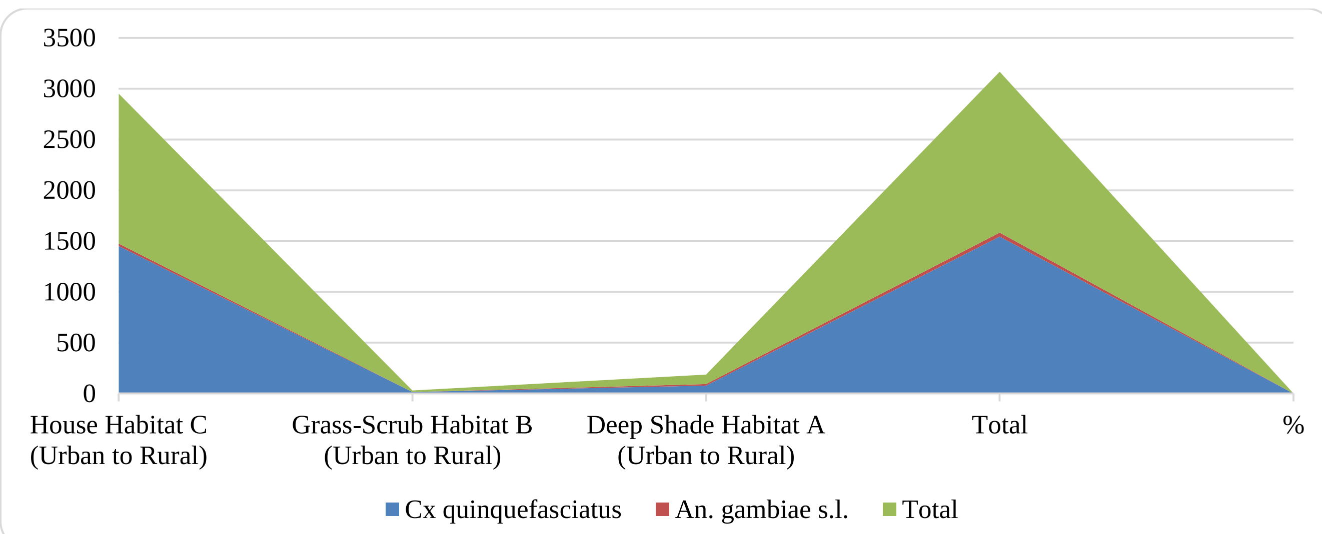


Table 7: Mean Monthly Abundance of Cx quinquefasciatus from the House Habitat C hand catch Method.

Months	Feb	Mar	Apr	May	June	July	Aug	Total
Site 1	0.6	6.8	12.0	5.7	2.0	7.5	10.5	45.1
Site 2	0.9	7.3	13.7	8.6	0.9	9.2	5.6	46.2
Site 3	0.3	8.2	7.8	7.2	1.3	4.5	8.2	37.5
Site 4	0.0	5.5	9.6	5.3	3.0	5.0	8.7	37.1
Site 5	1.0	6.2	8.4	7.8	2.0	4.8	7.9	38.1
Site 6	0.0	1.0	6.1	6.2	0.9	5.2	8.0	27.4
Total	2.8	34.8	57.6	40.8	9.9	36.2	48.9	230.8

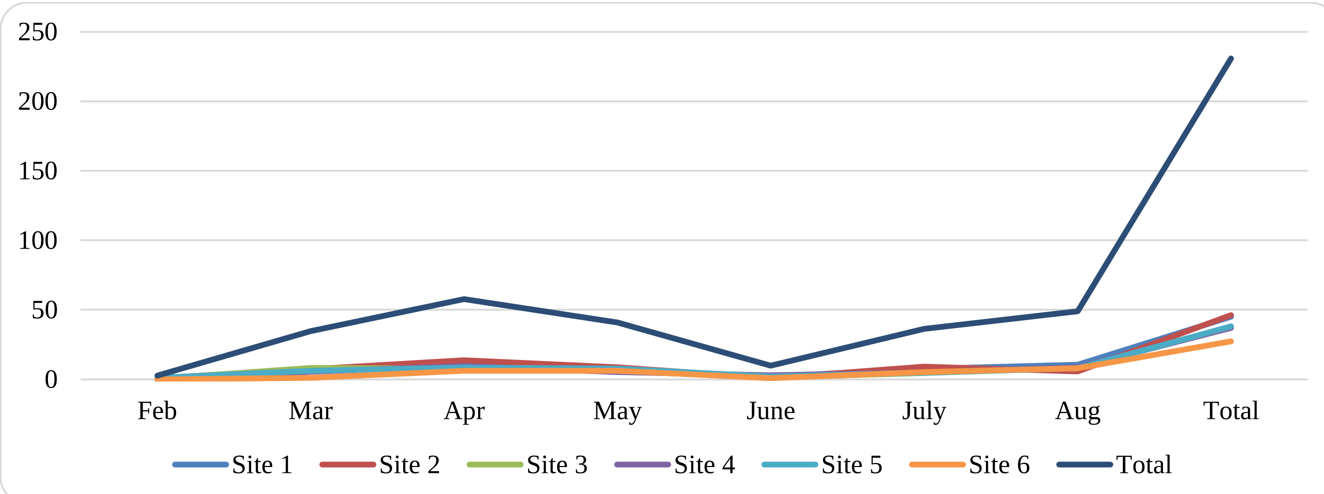
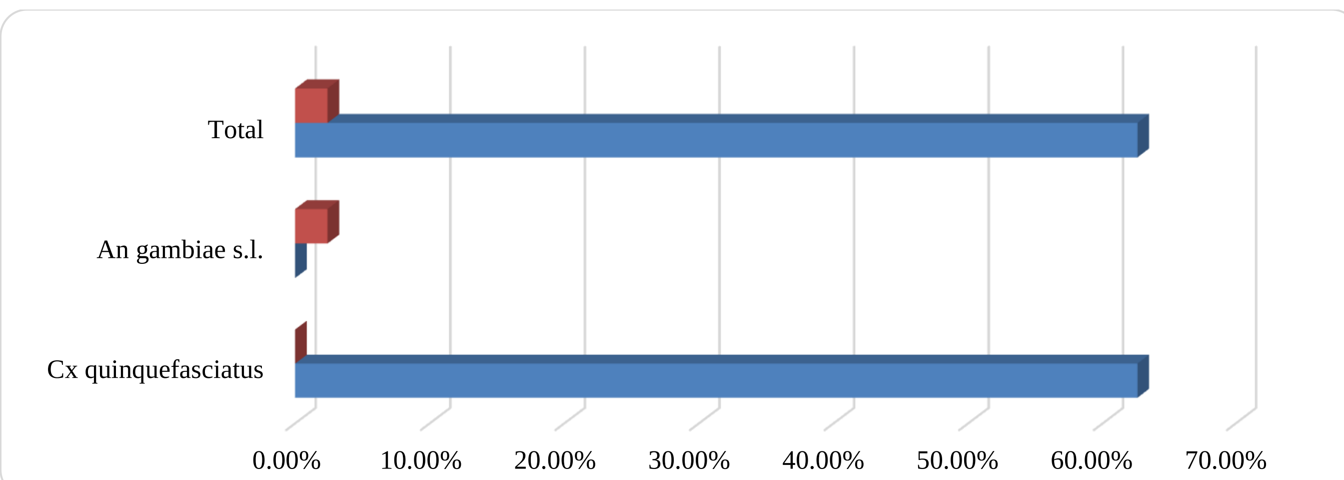


Table 8: Relationship of Species Abundance to Meteorological Conditions.

Species	Monthly Rainfall (cm)	Monthly Mean Temperature (°C)	Monthly Relative Humidity (%)	Cx quinquefasciatus Abundance (%)	An gambiae Abundance (%)
Cx quinquefasciatus	0.0 - 2.0	22 - 28	60% - 85%	62.6%	-
An gambiae.	1.0 - 5.0	22 - 28	65% - 85%	-	2.4%
Total	0.0 - 5.0	22 - 28	60% - 85%	62.6%	2.4%



DOI: 10. 39401/jpbm.003.01.0023

DISCUSSION

This study provides comprehensive insights into the abundance, distribution, and influencing factors on the populations of Culex quinquefasciatus (Cx. quinquefasciatus) and Anopheles gambiae sensu lato (*An. gambiae* s.l.) across various habitats and collection methods. In terms of mosquito abundance by habitat, Cx. quinquefasciatus overwhelmingly dominated, comprising 98.5% of the total catch, with *An. gambiae* s.l. representing only 1.5%. This finding is consistent with previous studies that have shown that Cx. quinquefasciatus, a primarily urban species, is more likely to be found in human-associated habitats like houses (Muturi et al., 2010). The house habitat (C) contributed 94.8% of the mosquito catches, emphasizing the preference of this species for indoor environments where water containers and other anthropogenic breeding sites are abundant. In contrast, the deep shade (A) and grass scrub (B) habitats yielded far fewer mosquitoes, confirming that Cx. quinquefasciatus is not typically associated with environments lacking stagnant water (Sinka et al., 2010). The collection methods revealed that hand catching was significantly more effective than box shelters, capturing 62.6% of mosquitoes versus 37.4% for the box shelter method ($\chi^2 = 14.928$, $p = 0.0257$).

This difference is in line with findings from other studies suggesting that hand catching, due to its direct nature, provides higher mosquito catch rates (Omondi et al., 2016). The flexibility of hand catching also allows for the collection of mosquitoes from a wider variety of habitats, both indoor and outdoor, making it an ideal tool for routine mosquito surveillance. While monthly variations showed that rainfall correlated with peaks in Cx. quinquefasciatus populations, especially in May, which aligns with findings from other studies that note the reliance of this species on water accumulation following rainfall (Muturi et al., 2010). This seasonal peak in abundance highlights the importance of accounting for seasonal environmental conditions, as periods of heavy rainfall create ideal breeding conditions for Cx. quinquefasciatus.

However, the presence of *An. gambiae* s.l. in the house habitat was most pronounced in August, suggesting that environmental factors like increased indoor activity during rainy months, combined with elevated humidity, can drive the abundance of *An. gambiae* s.l. (Sinka et al., 2010). Variation across sites along the urban to rural transect revealed no significant differences in mosquito numbers, confirming that the primary factor influencing mosquito abundance was habitat type, not geographic location. This finding supports the idea that habitat-specific interventions (such as targeting indoor breeding sites) are likely to be more effective than site-based control strategies (Sinka et al., 2010). Similarly, abundance by collection method across sites revealed that hand catching generally yielded more mosquitoes from house habitats, further reinforcing that house habitats consistently supported higher mosquito populations. Meteorological conditions, particularly rainfall and relative humidity, were shown to correlate strongly with mosquito abundance, particularly for Cx. quinquefasciatus, which thrived during the peak rainfall months.

This supports other research that indicates Cx. quinquefasciatus populations are highly responsive to changes in weather patterns, particularly rainfall, which creates favorable breeding sites (Omondi et al., 2016). The findings on monthly rainfall, temperature, and humidity (Table 8) underscore the importance of considering meteorological factors when planning vector control programs, particularly in areas with marked seasonal changes. Peak rainfall, combined with humidity, likely facilitated mosquito breeding and contributed to the increased numbers observed in the study. Taken together, these results underline the importance of focusing on indoor habitats, especially during rainy seasons, and adopting flexible surveillance methods like hand catching, which offer a more comprehensive view of mosquito populations. The role of meteorological conditions should also be factored into vector control strategies to ensure timely interventions, particularly during peak breeding seasons. These findings contribute to the growing understanding of mosquito ecology, with significant implications for designing more targeted, seasonally adaptive vector control interventions.

CONCLUSION

This study provides valuable insights into the distribution, abundance, and influencing factors of Culex quinquefasciatus and Anopheles gambiae sensu lato across different habitats and collection methods, highlighting several key implications for vector control strategies. The overwhelming dominance of Culex quinquefasciatus in house habitats underscores the importance of focusing mosquito control efforts on indoor environments, particularly in urban settings where human-associated breeding sites are abundant. The study also demonstrates that the handcatch method is significantly more effective than box shelters for routine mosquito surveillance, given its higher catch rates and ability to capture mosquitoes across a broader range of environments. Seasonal variations in mosquito abundance, particularly in response to rainfall,

 DOI: 10. 39401/jpbm.003.01.0023

further emphasize the importance of considering meteorological factors when planning vector control interventions. The findings also show that Anopheles gambiae is more likely to be found in house habitats during specific months, such as August, highlighting the need for tailored interventions that take into account seasonal and environmental changes. The lack of significant variations between different sites along the urban to rural transect reinforces the idea that habitat type, rather than specific geographic location, is the primary factor influencing mosquito populations. These results point to the need for targeted vector control programs that focus on high-risk habitats, particularly indoor spaces, and adjust for seasonal and meteorological influences on mosquito populations. Overall, the study contributes to our understanding of mosquito ecology and offers practical guidance for improving vector surveillance and control strategies, especially in urban areas.

Author Contributions

Fawad Khan conceptualized the study. Data collection was carried out by Fawad Khan, Farman Ali, and Abid Iqbal. Najiya al-Arifa and Saffora Riaz conducted specimen identification and lab analysis. Statistical analysis was performed by Rehman Mehmood Khattak and Khayyam Khayyam. Irum Alam Sthanadar and Saira Saira assisted with data interpretation. Aftab Alam Sthanadar and Muhammad Younus contributed to manuscript drafting and critical revision. All authors reviewed and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgment

The authors are grateful to the Health Department, Khyber Pakhtunkhwa, and Abdul Wali Khan University Mardan for logistic and technical support. Special thanks to local community health workers and volunteers in Lower Dir for assisting in field data collection.

Significance of the Study

This study highlights the resting behavior and ecology of *Culex quinquefasciatus* and *Anopheles gambiae* in a relatively understudied district of Pakistan. The findings are relevant for designing targeted vector control strategies in rural and semi-urban environments.

Future Research Gap

Further research should focus on seasonal variation in breeding habitats, insecticide resistance patterns, and molecular identification of species complexes. Long-term surveillance studies are needed to assess the impact of ecological shifts and climate on mosquito populations in Dir Lower.

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