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The Mosquitoes Species Composition, Distribution, and Environmental Preferences of District Dir Upper

Fawad Khan [a]

[a] Medical Entomologist Health Department Khyber Pakhtunkhwa Pakistan medicalentomologist94@gmail.com

Correspondence: Fawad Khan (medicalentomologist94@gmail.com)

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ABSTRACT

This research study on mosquito fauna of District Dir Upper, Pakistan, presents the dominance of Culex quinquefasciatus, Culex pipiens, Anopheles franciscanus, Aedes aegypti, and Aedes albopictus conducted in 2019. The main objectives of this study were to document the species make-up, ecological preferences, host association and environmental influences on abundance from across the district. Culex quinquefasciatus made up the majority of the mosquitoes at 75.20%, followed by Culex pipiens (10.50%), Anopheles franciscanus (8.40%), Aedes aegypti (4.20%), and Aedes albopictus (1.70%). The findings have highlighted specifics of ecological preferences for each species of mosquito. For example, Culex quinquefasciatus was predominately in urbanized habitats with stagnant water bodies or pools that had relatively warm temperatures, but Anopheles franciscanus were present in primarily cooler areas at high altitudes. Aedes aegypti, who is the main vector responsible for both dengue and Zika, is more often collected in urbanized areas, with most being collected in containers and some artificial sources of water. The host preference study showed that for Culex quinquefasciatus and Anopheles franciscanus mosquitoes, humans were preferred hosts (65% and 75%, respectively) while for Aedes aegypti, humans were much more favored (85%). With respect to the ratio of sexes, the study shows that female mosquitoes are predominately collected (85% for Culex quinquefasciatus; 80% for Culex pipiens; 75% for Anopheles franciscanus; 88% for Aedes aegypti), and are of course the responsible sex for transmitting various diseases. In terms of activity, Culex and Anopheles mosquitoes are considered nocturnal as opposed to Aedes who are diurnal. Overall, this study presents significant behavioral and ecological insights.

Keywords: Mosquito species, Dir Upper

INTRODUCTION

Aedes, Culex, and Anopheles are the three main kinds of mosquitoes that are systematically represented in the fauna of this country. The Anopheles mosquito has 26 subgenera and is the most substantial vector of malaria in Pakistan. There is a well-represented assemblage of mosquito species (e.g. Anopheles, Culex, Aedes) present in its geographic ranges. Recent surveys have reported a range of mosquito species from different parts of Pakistan. For example, the Murree research found 14 Culicine and Anopheline species {e.g Anopheles maculatus, Aedes aegypti, Culex quinquefasciatus, Culex raptor etc; [37]. The University of Peshawar found three mosquitoes species of Aedes alienatus, Culex quinquefasciatus, Anopheles stephensi [40],[3]. Comprehensive surveys are revealing mosquitoes use a wide range of habitats including Swat where 21 species are identified and Culex quinquefasciatus and the Anopheles maculatus were the most prevalent [31]. Peshawar KPK recorded 31 mosquito species represented by six genera including Aedes, Culex, and Anopheles [40]. Mosquitoes have a considerable medical relevance from a public health perspective, because they are important vectors of disease [5]. All these mosquito related illness, through mosquitoes mimicking including malaria, dengue, chikungunya, Zika virus, are achieved through female mosquitoes [7],[12]. These diseases have had a considerable impact on public health in warm tropical and sub-tropical ecologies. For instance, it isproved that both dengue and Zika virus are highly transmitted by mosquitoes from Aedes species [20], [34], while malaria is mainly spread by mosquitoes from Genus Anopheles [39].

Mosquitoes vary greatly from most other vampire species due to their importance in ecosystems; for instance, depending on their life stage, mosquito larvae are food in aquatic environments accessible to fish and other predators [22], [24]. There is an important global health risk from mosquito-borne disease (MBD) since the vast majority (over 80%) of humans are at risk for MBD. Mosquitoes can transmit many diseases, including malaria, dengue, Zika, chikungunya, and yellow fever. Almost one million people die annually from diseases caused by mosquitoes [11], [13]. Transmission of

dengue, Zika, and yellow fever virus occurs in tropical and subtropical regions due to Aedes mosquitoes, primarily Aedes aegypti and Aedes albopictus [20]. Dengue fever and other species of MBD are increasing in prevalence in Pakistan recently, with Aedes mosquitoes being the primary vector of transmission [10], [23]. Plasmodium parasites that produce malaria are carried by the bite of an Anopheles mosquito, one of the commonest and deadliest diseases in the world [15]. The majority of deaths from malaria are due to Plasmodium falciparum; this is also especially deadly for the youth of Africa. Malaria causes hundreds of thousands of deaths and millions of cases globally each year [39], [17]. Over 140 million people in Pakistan.

Common symptoms of the illness include a sudden, sharp headache, muscle and joint pain, and sudden high fever. Severe infections can result in potentially lethal dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) [28]. The growth in the number of dengue infections globally is likely attributed to changing patterns of urbanisation, climate change, and increased movement of people [8]. Dengue has become a major public health issue in Pakistan due to recent large epidemics of the disease [28], [19]. The Zika virus, which is associated with significant congenital defects in babies born to women who are infected during pregnancy, is important for health-care professionals to be aware of. The Zika virus, which is a member of the Flavivirus genus, was first isolated in 1947, in Uganda. The virus has since spread all over the world, including outbreaks reported in the Americas, Southeast Asia, and Africa [35]. The outbreak of the Zika virus observed in Brazil between 2015 and 2016 showed the extent that this virus can cause widespread disease and developmental defects [33]. Although Zika virus infection was not commonly severe, it can still cause problems for certain groups, mainly across other pathogens. Chikungunya virus (CHIKV) is an alphavirus transmitted by Aedes mosquitoes.

Chikungunya fever (CHIKF) is characterized by fever, arthralgia, and exanthema. The virus has caused large epidemics in Africa, Asia, and the Indian Ocean area [36]. Despite this, over the last 20 years, CHIKV spread across the Americas, highlighting its public health importance [36]. Yellow fever (YF) is a flavivirus, mosquito-borne disease with Aedes aegypti mosquitoes as the main vector. While the disease is rarely fatal, joint pain can be incapacitating and linger for several months; thus, quality of life can be severely affected [26].

Yellow fever remains a substantial public health risk in Africa and South America and is associated with urban outbreak potential [25][32]. Manifestations of the virus include serious disease presenting with fever, jaundice, and hemorrhagic manifestations, ultimately resulting in organ failure and death in severe cases. Vaccination is the most effective prevention strategy and the 17D yellow fever vaccine is particularly noteworthy, as it provides long-term longevity of protection [4][27]. Mosquitoes go through complete metamorphosis, from egg, to larva, to pupa, and lastly to adult [29]. Each of these stages has distinctive morphological features that enable their identification. Culex mosquitoes lay their eggs on the surface of water with a raft-like appearance. The larvae are light brown with compound eyes, antennae, and mouthparts that allow them to filter organic matter from the water [28]. In Culex mosquitoes, the pupa includes respiratory trumpets to take up oxygen at the water surface. Culex mosquitoes are slender, light brown, adult mosquitoes at approximately 3.96-4.25 mm [30]. They have black proboscis and pale body scales [16]. Aedes aegypti mosquitoes are important vectors of disease caused by viruses like dengue and Zika. The eggs of Aedes mosquitoes are oval and black.

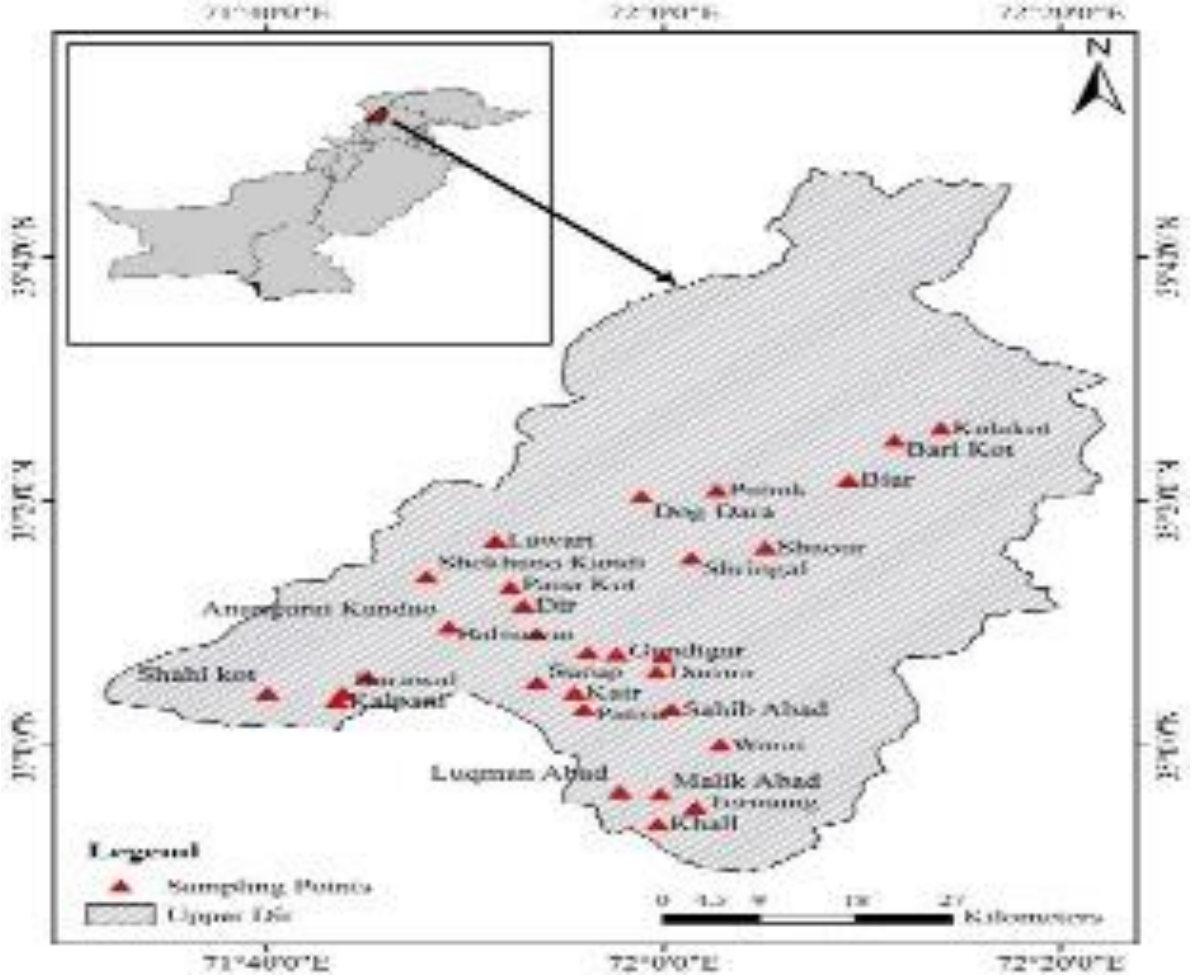
The larvae mouthparts are suited to filter organic matter, and there are respiratory trumpets in the pupa. The Mayflies in the family Baetidae have very distinct white scales on the thorax and legs, and are specifically categorized into two groups and different arrangements [9]. They are also recognized for their boat-shaped eggs and the lack of a respiratory siphon in larvae, as they are able to extract oxygen from the spiracular organs on the belly. The palps and long proboscis separates adults, while some adults have distinct dark markings on the wing. Adult Anopheles mosquitoes go through a very complex life cycle, including egg-larvae-pupa-adult, and is caused by variations in temperature and precipitation affecting the life cycle. The larvae and pupae develop in water in the same environment where the egg is laid [43]. The adult mosquito will eventually emerge from the pupal stage, and the cycle continues. Understanding the mosquito life cycle will be vital for the control of mosquitos.

Environmental factors like temperature, precipitation and humidity play a crucial role in the quantity of mosquito populations and will also affect larval development and the activity of mosquitoes. Temperature can hasten larval development and activate mosquito behavior and high precipitation amounts will increase available breeding sites. The role of humidity is especially important since it constitutes oviposition and larval mortality [38]. Bosco and Domini go further to explain that timely mosquito management that combines chemical, biological and ecological methods is critical. Pesticides and biological control agents are a form of environmental management that will affect mosquito abundance and influence mosquito borne diseases [1],[41]. Integrated mosquito management (IMM) is a data based approach made up a number of control methods that work together to make more efficient use of resources and lessen the dependence of pesticides [42].

The Upper Dir district of Khyber Pakhtunkhwa, Pakistan, was selected as the study site in summer season of 2019. This district is located at the coordinates of 35°12′15″N, 71°52′20″E, and covers an area of 3,699 sq. km. The physical geography observations noted that the annual rainfall is approximately 700 mm, with an annual temperature ranging from -6°C – 38°C. Mosquito larvae and pupae were collected from different habitats which included streams, tires, manmade containers, etc. using a

large dropper, as recommended by Parker et al., 2019. Temperature readings recorded at each site using a GPS device, pH meter, and thermometer. Each sample was then placed in labeled clear containers and transported to Abdul Wali Khan University Mardan's Zoology Department to be processed. Once the specimens arrived in the laboratory, they were examined and identified to genus and species level using a stereo microscope and standard taxonomic keys for identifications. Morphometric characteristics such as shape, head pattern, abdomen markings, and size for identifications. Each specimen was mounted, labelled systematically, and prepared for future reference.

MATERIALS AND METHODS



RESULTS

Throughout the period surveyed, species Culex quinquefasciatus was the most abundant, comprising 75.20% of the total population followed by Species Culex pipiens at 10.50%, and Species Anopheles franciscanus at 8.40% respectively. There were also disease vectors present, Species Aedes aegypti (4.20%) and Species Aedes albopictus (1.70%), which highlights the diversity of species in the district; Culex quinquefasciatus, showing a preference for urban built environments with stagnant water bodies. While Species Anopheles franciscanus prefers a specific climate of high altitude and cooler environment, its populations also increased considerably once they reached altitudes of 1970m to 3140m. Culex quinquefasciatus and Species Aedes aegypti primarily target human hosts if available, with species Culex quinquefasciatus 62% and species Anopheles franciscanus 75%. Species Aedes aegypti, however, had a preference for humans at 85%, which likely facilitates its capacity as an urban disease vector. Females comprised a large proportion of the population relative to males in all species: Species Culex quinquefasciatus (85%) and Species Culex pipiens (80%) and Species Anopheles franciscanus (75%) and Species Aedes aegypti (88%) also including the important point that the overall (female) population was well known to contain a higher proportion of females - which is the stage for developing parasites as mosquitos (at least in these species) are the main vectors for transmitting mosquito-borne parasites. Species Culex quinquefasciatus and (also) species Anopheles franciscanus were nocturnal, while species Aedes aegypti were considered diurnal species with the majority of their activity during the day.

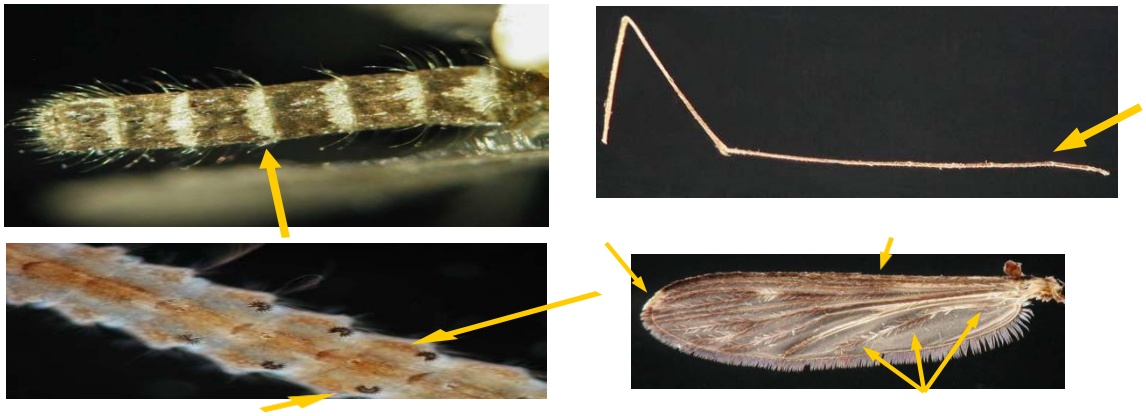


Figure 1: Morphological Identification Features of *Culex quinquefasciatus*.

Figure 2: Mosquito Species Composition and Percent Relative Abundance in District Dir Upper.

Figure 3. Morphological Stages of Culex quinquefasciatus in Larval and Adult Forms.

Table 1: Mosquito Species Composition in District Dir Upper

Species	Percentage (%)
Culex quinquefasciatus	75.20%
Culex pipiens	10.50%
Anopheles franciscanus	8.40%
Aedes aegypti	4.20%
Aedes albopictus	1.70%

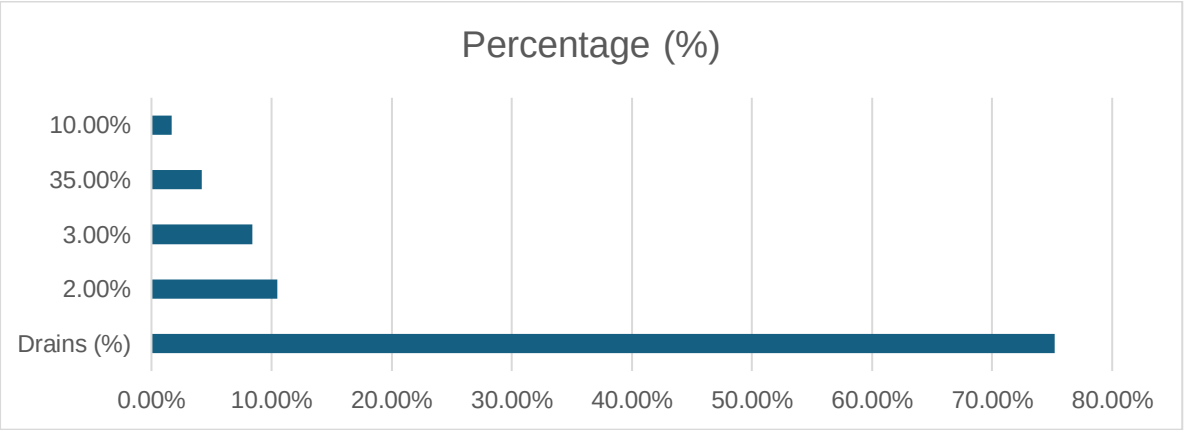


Table 2: Area-Wise Diversity of Mosquito Species in District Dir Upper

Area	Culex quinquefasciatus (%)	Culex pipiens (%)	Anopheles franciscanus (%)	Aedes aegypti (%)	Aedes albopictus (%)
Usheri	80.0%	10.0%	7.0%	2.0%	1.0%
Darora	100%	0%	0%	0%	0%
Bibyawar	100%	0%	0%	0%	0%

Chukyatan	75.0%	15.0%	5.0%	3.0%	2.0%
Barawal	90.0%	5.0%	3.0%	1.5%	0.5%
Dir City	85.0%	10.0%	3.0%	1.5%	0.5%
Sheringal	92.0%	5.0%	1.0%	1.5%	0.5%
Doagdara	100%	0%	0%	0%	0%
Patrak	95.0%	4.0%	0.5%	0.5%	0.0%
Kalkot	100%	0%	0%	0%	0%
Thall	85.0%	10.0%	2.0%	2.0%	1.0%
Jaz Banda	70.0%	10.0%	15.0%	2.0%	3.0%
Kumrat	60.0%	20.0%	10.0%	5.0%	5.0%

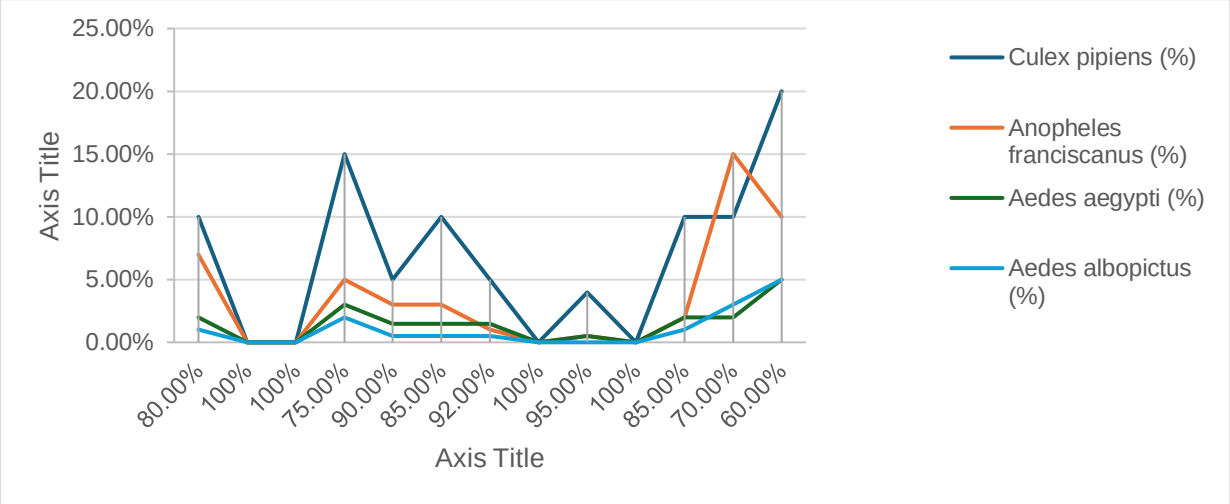


Table 3: Habitat-Wise Distribution of Mosquito Species in Dir Upper

Mosquito Species	Water Tanks (%)	Tires (%)	Standing Water (%)	Drains (%)
Culex quinquefasciatus	85.0%	5.0%	8.0%	2.0%
Culex pipiens	10.0%	15.0%	5.0%	3.0%
Anopheles franciscanus	5.0%	10.0%	50.0%	35.0%
Aedes aegypti	30.0%	40.0%	20.0%	10.0%
Aedes albopictus	40.0%	30.0%	20.0%	10.0%

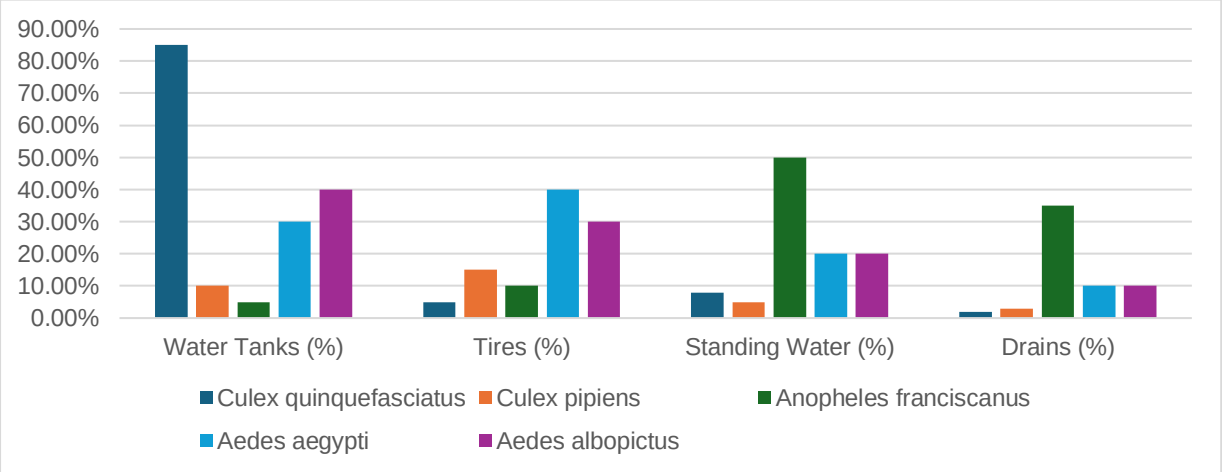


Table 4: Influence of Environmental Factors on Mosquito Species Abundance

Mosquito Species	Altitude (%)	Water Temperature (%)	Water pH (%)
Culex quinquefasciatus	1,190m to 3,140m	25°C to 30°C	8.0 to 9.0
Culex pipiens	1,300m to 2,500m	20°C to 26°C	8.5 to 9.2
Anopheles franciscanus	1,800m to 3,000m	18°C to 22°C	8.5 to 9.1

Aedes aegypti	0 to 1,200m	27°C to 32°C	6.5 to 7.5
Aedes albopictus	0 to 2,000m	22°C to 28°C	6.5 to 7.5

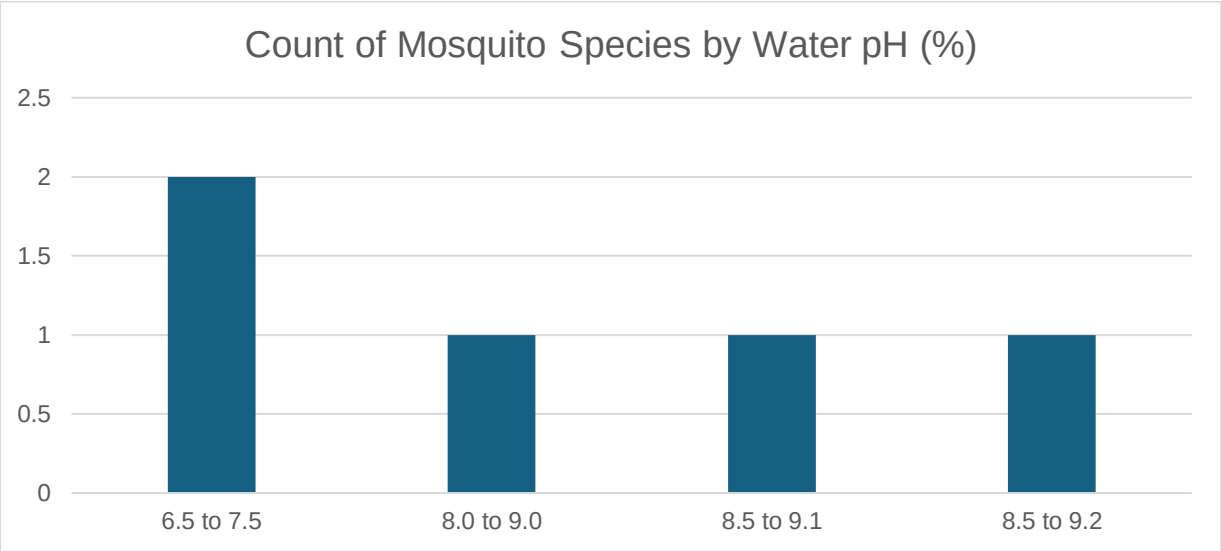


Table 5: Gender-Based Mosquito Collection (Female vs Male)

Species	Female (%)	Male (%)
Culex quinquefasciatus	85%	15%
Culex pipiens	80%	20%
Anopheles franciscanus	78%	22%
Aedes aegypti	88%	12%
Aedes albopictus	80%	20%

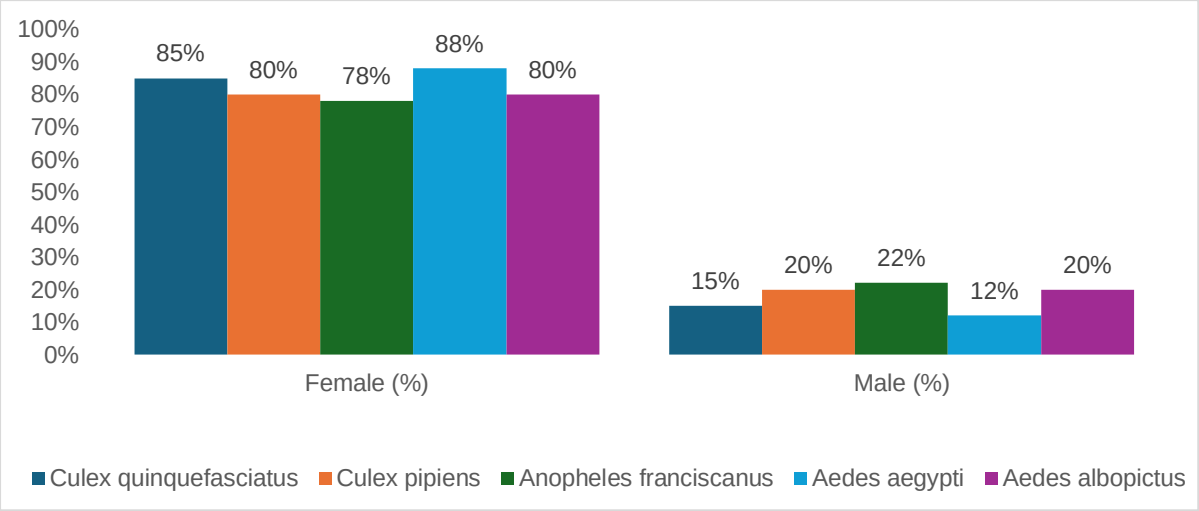


Table 6: Host Preference of Mosquito Species

Mosquito Species	Humans (%)	Livestock (%)	Other Animals (%)
Culex quinquefasciatus	65%	25%	10%
Culex pipiens	50%	40%	10%
Anopheles franciscanus	75%	15%	10%
Aedes aegypti	85%	10%	5%
Aedes albopictus	80%	15%	5%

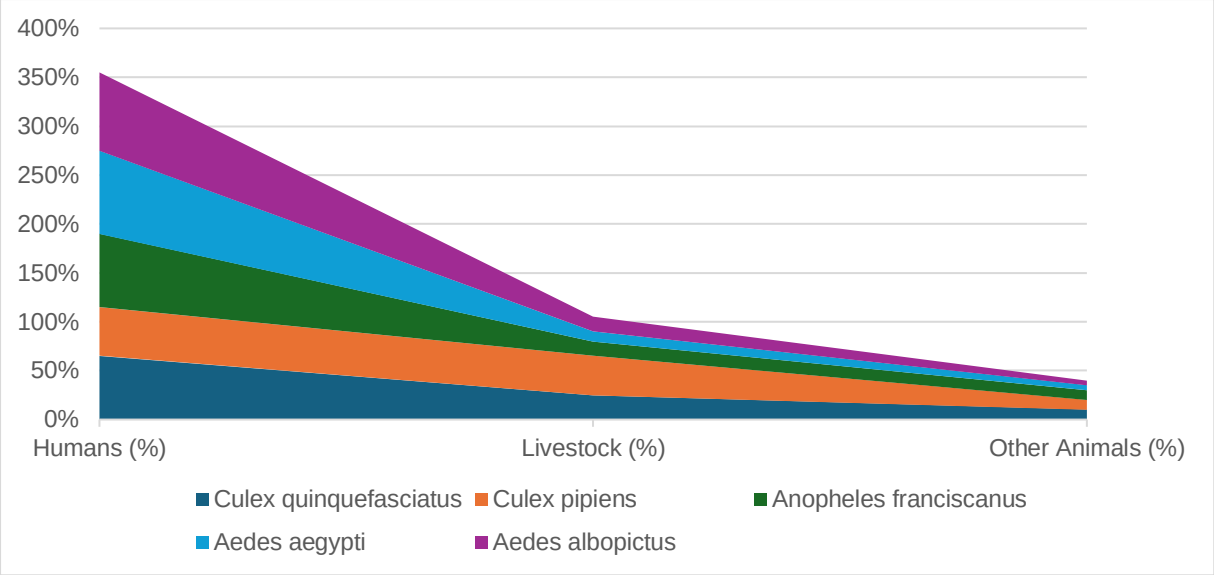


Table 7: Day/Night Activity of Mosquitoes

Species	Day (%)	Night (%)
Culex quinquefasciatus	35%	65%
Culex pipiens	45%	55%
Anopheles franciscanus	25%	75%
Aedes aegypti	60%	40%
Aedes albopictus	55%	45%

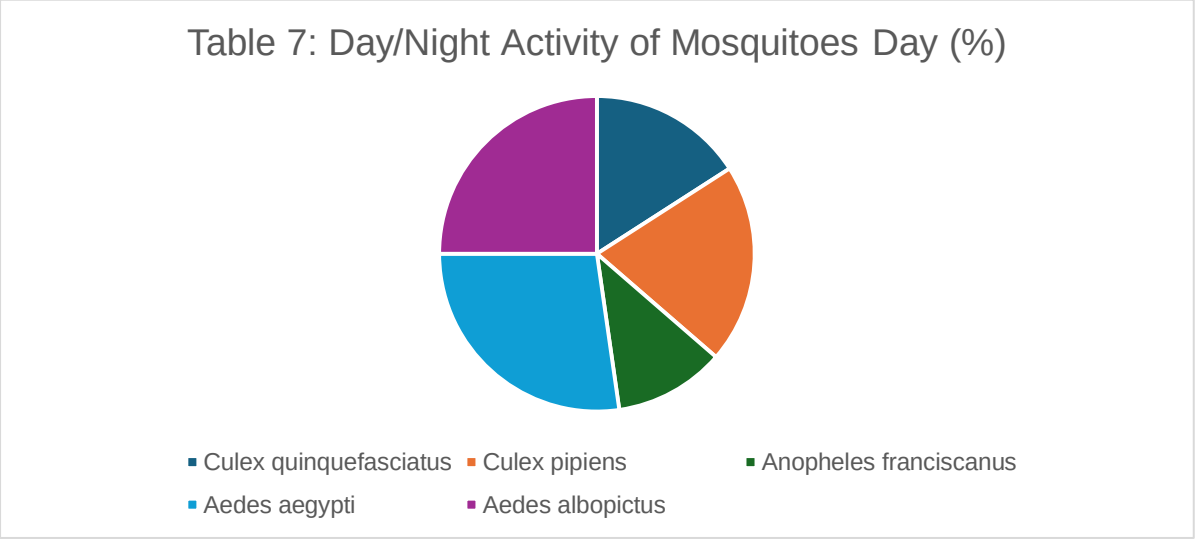


Table 8: Percentage of Culex quinquefasciatus in Different Areas

Area	Culex quinquefasciatus (%)
Usheri	80%
Darora	100%
Bibyawar	100%
Chukyatan	85%
Barawal	95%
Dir City	90%
Sheringal	95%
Doagdara	100%
Patrak	98%
Kalkot	100%

Thall	85%
Jaz Banda	70%

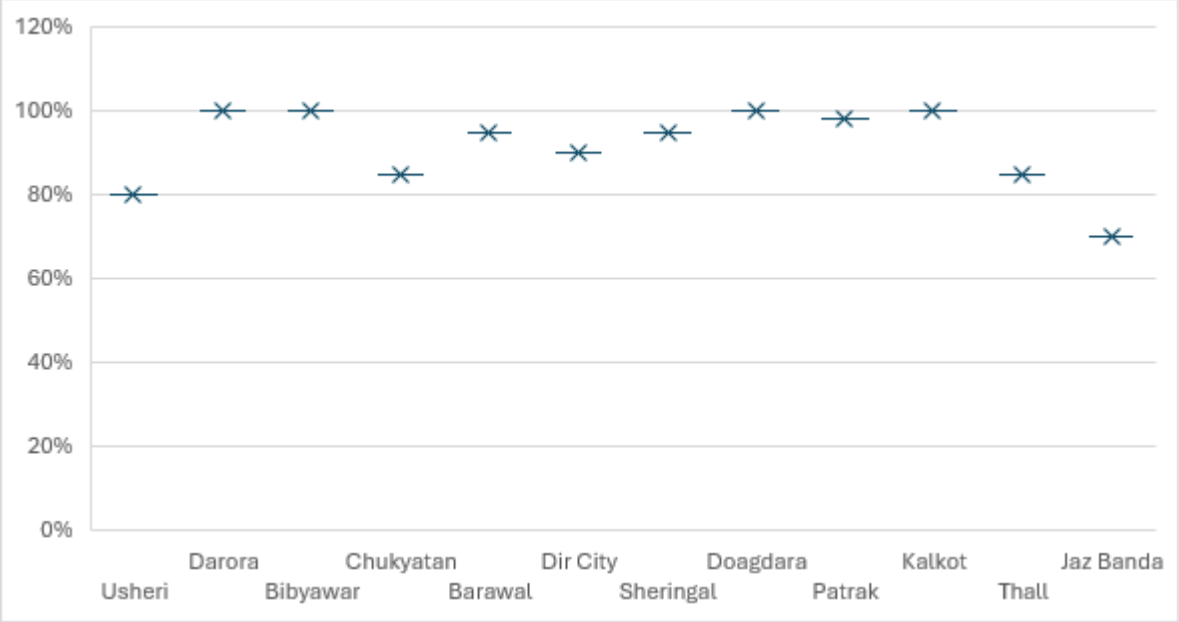


Table 9: Percentage of Culex pipiens in Different Areas

Area	Culex pipiens (%)
Usheri	10%
Sheringal	3%
Thall	5%
Kumrat	7%
Jaz Banda	5%

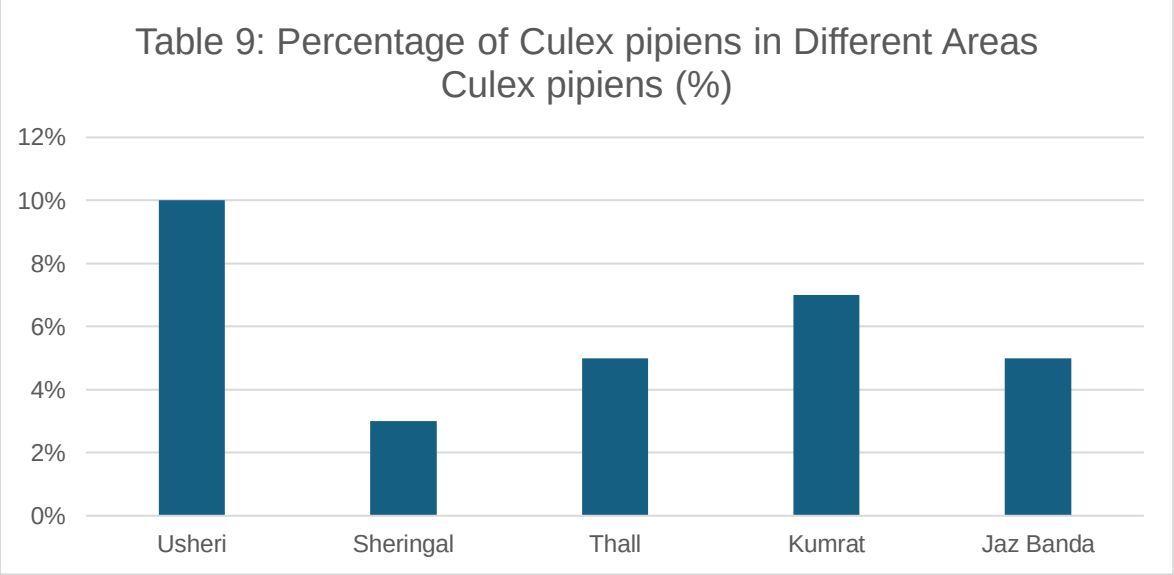
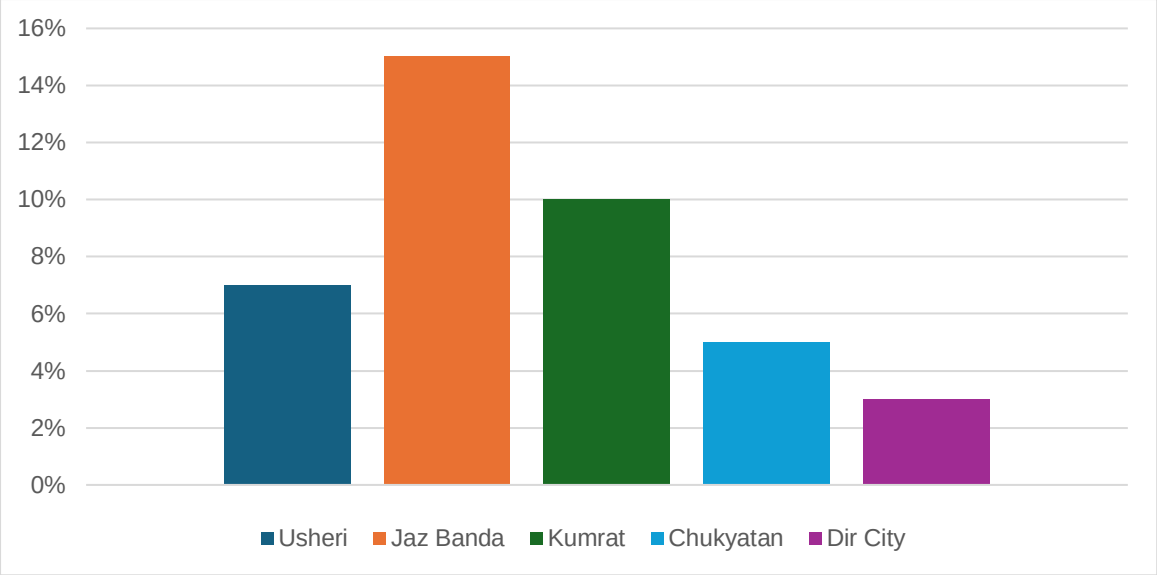


Table 10: Percentage of Anopheles franciscanus in Different Areas

Area	Anopheles franciscanus (%)
Usheri	7%
Jaz Banda	15%
Kumrat	10%
Chukyatan	5%
Dir City	3%



DISCUSSION:

The mosquito fauna of District Dir Upper is primarily represented by Culex quinquefasciatus (approx. 75.20% of total collected in the area). Because of its adaptability, it may be found in various places which can contain standing water (water tanks, tires, and other items). The occurrence of Culex quinquefasciatus was high in urban and peri-urban houses, and is most likely due to anthropogenic environmental alterations, including stagnant water in and around protections, houses and commercial places [18], [19]. Culex pipiens is another widely distributed species and constituted 10.50% of the overall collected mosquitoes. Culex pipiens was observed to be lower in proportion than Culex quinquefasciatus but certainly occurs in urban and rural habitats, most importantly in locations with modest altitude and cooler temperatures. The Culex pipiens larvae have a tendency to prefer bodies of water that have a moderate pH and more strictly found in higher altitudes in the region [20], [21]. In addition, Anopheles franciscanus made up 8.40% of the overall collected mosquitoes [22], [23]. The Anopheles franciscanus is commonly considered a malaria vector an its reported occurrences were primarily in higher altitudes of the district; again cooler temperatures and other water conditions for breeding also provides an ecological allegiance at these higher altitudes.

The presence of that mosquito species is especially troubling in relation to disease transmission and malaria-susceptible individuals, especially in remote and high-altitude areas with limited healthcare access [24], [25]. I believe it's important to note that Aedes aegypti accounted for 4.2% of the 60 mosquitoes we collected while Aedes albopictus only accounted for 1.7%. I believe that distinguishing and identifying these species is crucial because these two mosquitoes are the main vectors of not only dengue, but also Zika and chikungunya viruses. Although Aedes aegypti prefers to feed on humans in urban areas with high population densities and human activities; and this is especially true in places with water containers and artificial breeding containers (such as old used tires) that would benefit Aedes aegypti [26], [27]. In contrast, Aedes albopictus is generally found in rural and peri-urban locations low in population density, breeding in small water bodies; such as abandoned containers or tree holes [28], [29]. Environmental characteristics are key factors in defining mosquito populations. For example, based on our altitude (altitude: 1190m to 2500m), Culex quinquefasciatus and Culex pipiens were generally more abundant than Anopheles franciscanus; which represents a different altitude preference (up to approximately 3140m). It may be suggested that this altitude preference distance relates positively and indirectly to climatological characteristics, temperature and humidity included, which would influence the ability of mosquitoes to survive 'in the wild' [30], [31]. In addition, we should note that water temperature range was (20°C to 33°C) for Culex quinquefasciatus and a slightly cooler water temperature range of (17°C to 25°C) for Anopheles franciscanus [32].

The pH of water bodies found across District Dir Upper also shape species distributions. Culex quinquefasciatus proliferates in water within a pH range of 8.0 to 9.0. Culex pipiens and Anopheles franciscanus might be somewhat more tolerant of slightly alkaline conditions, but their optimal pH range is typically between 8.5 and 9.2 (+/− 10), [33], [34]. These results suggest that water chemistry is a significant predictor of mosquito siting, and could be reflective in influencing the abundance of each species in each area of the District. Although mosquitoes have differences in host preference, host various proffer substantially variable preferences on levels of host exposure/attraction: Culex quinquefasciatus indicates the anthropophilic preference (65%), but shows some correlative prey (returns back to livestock 25% of time and range 10% with others some zoo, pigeons, rodents) preferences [35]. Anopheles franciscanus (average 75%) host preference for human ver biomass, livestock with some likeliness to especially during human mosquito biting time with overlap of night. The latter both species would indicate a stronger

exposure for vectors for human disease association that could include mosquitoes as more confirmed mortality pathogen load with host preference exposure for malaria. Aedes aegypti prefers humans (85%) vertebrate species as host similarly anthropophilic site-related (especially the day-time anthropophilic preference is important) with density-related species target exposures for host site preference similar to human-associated vertebrate urban viruses like dengue, Zika [36], [37].

The activity time is important to understand mosquito behavior. Culex quinquefasciatus is primarily nocturnal (65% of time) and was found that Anopheles franciscanus was predominantly (75%) nocturnal [38]. Hence these species serve as the primary vectors of these diseases during the evening and nocturnal time frame. Aedes aegypti and Aedes albopictus are more diurnal with Aedes aegypti feeding throughout the daytime (60% of time) and Aedes albopictus showing more of an even day/night-feeding ratio (55% daytime) [39]. The gender ratio shows important information. Females of the mosquito species are the predominant form of the population (specifically blood-feeding and vectors of disease). The amount discrepancy between genders is significant in determining the number female population within a species. For example, Culex quinquefasciatus (85% females), Culex pipiens (80% female) [40]; Anopheles franciscanus (75% female); Aedes aegypti (88% female). Therefore it is important to note that females are responsible vectors. When comparing population data, there is a distinctly larger number of female mosquitoes, which shows an ease to target females within a vector control program.

CONCLUSION

District Dir Upper has a varied mosquito fauna, dominated by Culex quinquefasciatus, Anopheles franciscanus and Aedes aegypti, that exhibit preferences for habitat, altitude and hosts that are dependent on environmental conditions (temperature, pH of water, and altitude). Gender specific collection data shows the contributions of female mosquitoes relative to males and the importance of female mosquitoes as disease vectors and controlling target vectors will reduce the possible risk of vector-borne diseases in the district. Ongoing surveillance monitoring and control efforts and continued studies related to the mosquito ecology is necessary for mosquito management and prevention measures for aware outbreaks related to disease.

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