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Effect of Different Breeding Habitats on Abundance and Distribution of *Culex* Larvae in the Urban Areas of Bhaktapur Municipality, Nepal

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Abstract

This study investigated the distribution and abundance of *Culex* larvae in various breeding habitats in Bhaktapur, Nepal, and assessed the effects of different water conditions on larval mortality. A cross-sectional study was conducted across 35 breeding habitat, where larval surveys were carried out using the standard dipping method. The breeding habitats found in the study sites were categorized into five types, and environmental factors such as the state of water, light conditions, vegetation, and turbidity were recorded. Sewage canals had the highest larval abundance (47.44%). The presence of *Culex* larvae was significantly associated with breeding habitats and state of water ($p < 0.05$). A mortality experiment was conducted by introducing five *Culex* larvae into different water types (oil-water, detergent water, vinegar water, muddy water, clean water, and salt water), with survival monitored over two days. *Culex* larvae were detected in 10 (28.57%) of the breeding habitats, with the highest abundance in sewage canals (47.44%), followed by animal shelter puddles (28.85%) and rice fields (23.72%). Stagnant water had significantly higher larval presence (96.15%, $p < 0.05$) than flowing water. The overall Larval Productivity Index (LPI) was 64.1%, while the Pupal Productivity Index (PPI) was 79%, indicating higher survival in the pupal stage Mortality experiments revealed 100% larval mortality in oil-water, detergent water, and vinegar water within two days, and 80% mortality in salt water. Clean and muddy water resulted in lower mortality rates (40% and 20%, respectively). *Culex* larvae predominantly breed in stagnant, organically rich water bodies, particularly sewage canals and animal-related puddles. Household substances like detergents, oils, and vinegar show high larvicidal potential, offering eco-friendly control options. These findings support targeted larval source management and integrated vector control strategies to reduce *Culex*-borne disease risks.

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Keywords: *Culex* larvae, breeding habitats, larval productivity, pupal productivity, mortality rate, vector control.

Background

Vector-borne diseases remain a significant public health concern, particularly in tropical and subtropical regions (Rocklöv & Dubrow, 2020). Despite efforts to mitigate their spread, diseases such as lymphatic filariasis, Chagas disease, malaria, and dengue continue to rise. Mosquitoes of the family Culicidae, comprising approximately 3,727 species (Harbach, 2025), serve as key vectors of these diseases (Wilkerson et al., 2021).

Among them, *Culex* mosquitoes are capable of transmitting a wide range of medically significant arboviruses, including Japanese encephalitis virus (JEV) (Wispelaere et al., 2017), West Nile virus (WNV) (Kilpatrick et al., 2008) in turn, can be influenced by temperature and viral genetics. West Nile virus (WNV, and Western and Eastern equine encephalitis viruses (WEEV/EEEV) (Madhav et al., 2024). In addition to arboviruses, *Culex* mosquitoes also serve as vectors for parasitic diseases, such as lymphatic filariasis caused by nematodes and avian malaria (Ferraguti et al., 2021; Nchoutpouen et al., 2019) especially those transmitted by mosquitoes, have severe impacts on public health and economy. West Nile virus (WNV). *Culex pipiens* and *Culex quinquefasciatus* are among the most important species, as they are capable of transmitting multiple viruses and exhibit opportunistic feeding behavior, targeting both humans and animals as hosts (Bhattacharya & Basu, 2016; Farajollahi et al., 2011).

Culex species are widely distributed in Nepal, ranging from the lowland Terai to the mid-hill regions, and are particularly abundant in urban and peri-urban areas with poor waste management and stagnant water (Dhimal et al., 2014). Entomological surveys have confirmed the dominance of *Culex quinquefasciatus* in the Kathmandu Valley, Bharatpur, and the southern plains of the Terai, where it commonly breeds in polluted drains, septic tanks, and rice fields (Dhimal et al., 2014). The high density of *Culex* mosquitoes in these areas poses a persistent threat for the transmission of Japanese encephalitis virus (JEV) and lymphatic filariasis, especially in communities lacking effective mosquito control measures.

Recent entomological surveillance conducted across elevations ranging from 62 to 3,840 meters above sea level further confirmed the presence of *Culex* mosquitoes from the lowland Terai to high hills and even high mountain regions. *Culex pipiens* was found to be the most abundant species, while *Culex sasai*, newly recorded for Nepal, was primarily detected at high altitudes up to 3,840 m asl (Sukupayo et al., 2025). This wide ecological distribution highlights the adaptability of *Culex* mosquitoes and their potential to sustain disease transmission across diverse environmental conditions.

The female *Culex* mosquito lay eggs at night on the surface of stagnant water, forming rafts that contain 100-300 eggs. The larvae, commonly referred to as “wrigglers,” are highly active and visible in aquatic habitats. They feed on various organic materials present in the water and undergo multiple moults before pupation. During rest, the larvae position themselves at an angle relative to the water surface, and when swimming, they move upward at an inclined angle (CDC, 2024).

Factors such as inadequate waste disposal, poor sanitation, inefficient drainage systems, uncontrolled urban expansion, and limited resources have contributed to the creation of numerous ideal breeding grounds for mosquitoes (Wilke et al., 2021). These conditions, along with various climatic factors, have contributed to a high mosquito population density, increasing the risk of vector-borne disease transmission (Yan et al., 2024).

Culex mosquitoes are widespread in urban and suburban areas of tropical and sub-tropical areas (Krambrich et al., 2024). They typically develop in polluted stagnant water bodies such as ponds, puddles, rice fields, sewage canal, and swamps (Djoufounna et al., 2022), although some studies suggest that *Culex* larvae can also develop in clean stagnant water (Goselle et al., 2018).

Understanding larval habitats is essential for designing effective vector management strategies (Wilson et al., 2020). However, limited research has been conducted on *Culex* species and their larval habitats in Bhaktapur. Given the city's high population density and abundance of potential breeding habitats, targeted mosquito control strategies are essential. This study aims to assess the impact of breeding habitats on the abundance and distribution of *Culex* larvae in urban Bhaktapur. It also seeks to evaluate larval survival in various water types, determine the effects of household substances on larval mortality, and identify effective control measures. Furthermore, the study will calculate the Larval Productivity Index (LPI) and Pupal Productivity Index (PPI) for different sites.

METHODS

Study area

The study was conducted in the urban area of Bhaktapur Municipality, Nepal, focusing on 35 breeding habitat within a one kilometer radius of Bhaktapur Durbar Square (Figure 1). They included different breeding habitats like marshy land, sewage ditches, drainage systems, irrigation canals, river banks, pools, ponds, and other stagnant water sources (Figure 2), where the possibility of breeding of *Culex* mosquitoes is high.

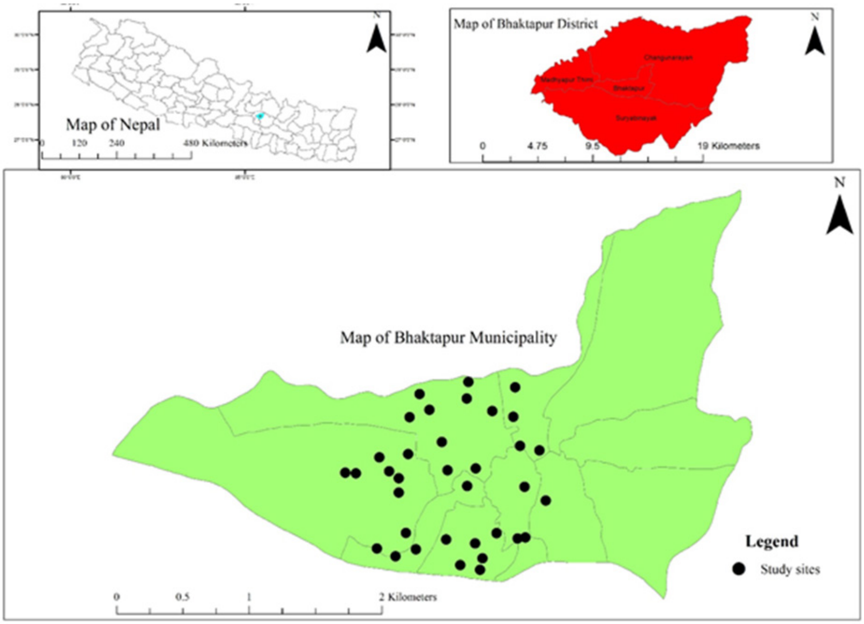


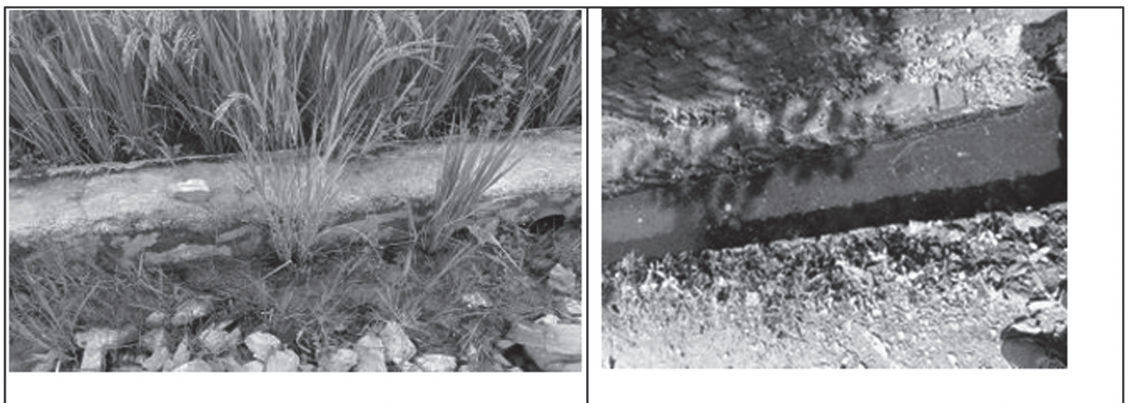
Figure 1. Map of study area with location of studied breeding habitats

Characterization of larval habitats

Mosquito study sites were assessed on six factors – 1. Breeding habitat type (sewage canals, rice field, puddles, and ponds), 2. Water turbidity (clear or turbid), 3. Vegetation (present or absent), 4. Sunlight exposure (shaded or exposed) and 5. State of water (stagnant of flowing).

Entomological surveys

This study conducted entomological surveys across 35 breeding habitats to assess the abundance and distribution of *Culex* mosquitoes. The survey was carried out during the post-monsoon season, from September to October 2022, focusing exclusively on outdoor environments.



A. Rice field | **B.** Sewage canal

C. Puddle in the residential area

D. Puddle in animal shelter

E. Pond

Figure 2. Breeding habitats of *Culex* spp.

Larvae collection and rearing

Larvae were collected using the standard dipping method (Amusan & Ogbogu, 2020), with three dips per breeding habitat. Third and fourth instar larvae, along with pupae, were collected from positive breeding habitats. These collected specimens were systematically recorded, and their development from larval to pupal and pupal to adult stages was monitored.

Species identification

Once the mosquitoes emerged as adults, species-level identification was carried out under laboratory conditions using standard morphological keys (Rueda, 2004). Identification at the adult stage allows for more accurate species confirmation compared to immature stages.

Evaluation of various water types on *Culex* larvae mortality

To determine the mortality rate of *Culex* larvae, an experiment was conducted using six different types of water: clean water, muddy water, detergent water, oil water, salt water, and vinegar water, with water collected from the larval habitat serving as the control. Clean water was obtained from tap water, while muddy water was prepared by adding soil to the tap water. Detergent water was prepared by mixing 10 grams of detergent powder into 100 ml of clean water, and oil water was created by adding 10 ml of vegetable oil to 100 ml of clean water. Salt water was prepared by dissolving 10 mg of salt in 100 ml of clean water, and vinegar water was made by mixing 10 ml of vinegar with 100 ml of clean water. Five *Culex* larvae were introduced into each container containing the respective water types and observed over a period of two days in the laboratory to assess their survival and mortality rates.

Data analysis

The distribution of *Culex* larvae across the study area was analyzed using distribution classes, as outlined in previous research (Bashar et al., 2016). The distribution class (C) was calculated using the formula:

$C = n/N \times 100\%$

Where, C = distribution, n = number of sites of the species, N = number of all sites.

The following distribution classes were adapted (Bashar et al., 2016):

The distribution classes were categorized as follows:

C1 – Sporadic (0–20%): Larvae found in fewer than 20% of sites.

C2 – Infrequent (20.1–40%): Larvae found in 20.1% to 40% of sites.

C3 – Moderate (40.1–60%): Larvae found in 40.1% to 60% of sites.

C4 – Frequent (60.1–80%): Larvae found in 60.1% to 80% of sites.

C5 – Constant (80.1–100%): Larvae found in 80.1% to 100% of sites.

Larval density was quantified by using the formula (Villarreal-Treviño et al., 2015):

$$D = \frac{the\ total\ number\ of\ larvae}{total\ number\ of\ dips}$$

Additionally, the Larval Productivity Index (LPI) and Pupal Productivity Index (PPI) were calculated to assess developmental success rates:

LPI = Number of pupae developed/Total number of larvae × 100

PPI = Number of adults developed/Total number of pupae ×100

Statistical analyses, including Fisher's Exact Test and the Chi-square test, were conducted to assess the association between the presence of larvae and their habitat, as well as larval mortality and water type, at a 5% significance level ($p < 0.05$). Data were analyzed using SPSS and Microsoft Excel 2007.

RESULTS

Distribution and abundance of *Culex* larvae

A total of 35 study breeding habitats were studied. Out of these, 10 breeding habitats tested positive for *Culex* larvae, with a distribution class (C) of 38.28%. Sewage canals had the highest larval abundance (47.44%), followed by puddles in animal shelters (28.85%) and rice fields (23.72%). No larvae were found in ponds or puddles in residential areas. The presence of *Culex* larvae was significantly associated with breeding habitats and state of water ($p < 0.05$), while no significant associations were observed with light condition, vegetation, or turbidity ($p > 0.05$) (Table 1).

Table 1. Characterization of breeding habitats with the presence of *Culex* larvae

Variable	Number of habitats		p-value	Number of larvae collected	Larvae (%)
	without larvae	with larvae			
Types of breeding habitats					
Sewage canal	2	5	0.015	74	47.44
Ponds	7	0		0	0
Rice fields	5	3		37	23.72
Puddles in animal shelter	4	2		45	28.85
Puddles in residential areas	7	0		0	0
Total	25	10		156	100
Sunlight exposure					
Sunny	12	5	0.725	59	37.82
Shady	11	7		97	62.18
Total	23	12		156	100
Vegetation					
Absence	12	8	0.489	92	58.97
Presence	11	4		64	41.03
Total	23	12		156	100
Water turbidity					
Clean water	9	3	0.467	5	3.21
Turbid Water	14	9		151	96.79
Total	23	12		156	100
State of water					
Current water	10	1	0.007	6	3.85
Stagnant water	14	11		150	96.15
Total	24	11		156	100

Larval Productivity Index (LPI) and Pupal Productivity Index (PPI)

Among the 10 breeding habitats positive for *Culex* larvae, the overall Larval Productivity Index (LPI) was 64.1%. The highest LPI was recorded at study site one with 88.88%, while the lowest LPI was observed at study site 19 with 45% (Figure 3). Similarly, the overall Pupal Productivity Index (PPI) was 79%, with the highest PPI observed at study site six at 94.12%, and the lowest PPI was found at both in study site one and study site 20, each with 50% (Figure 4).

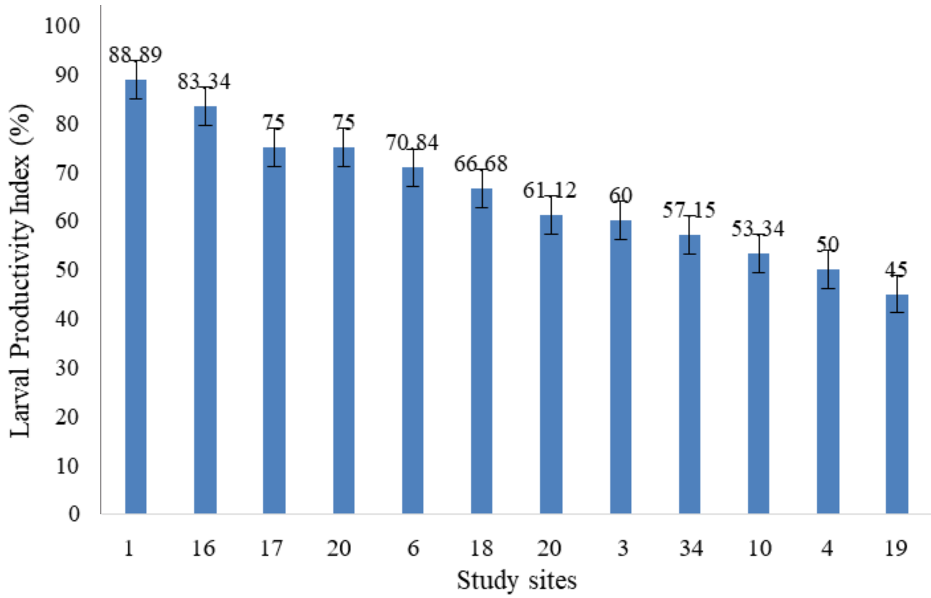


Figure 3. Larval Productivity Index (Number of pupae developed from larvae)

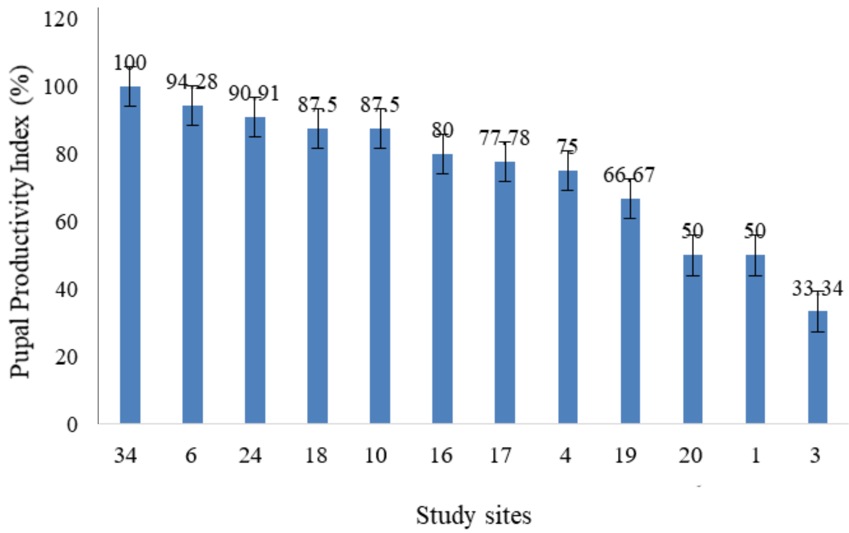


Figure 4. Pupal Productivity Index (Number of adults developed from pupae)

Mortality rate of *Culex* larvae on various types of water

Out of the five larvae introduced into each water type, 100% mortality of *Culex* larvae was observed within a few hours to two days in oil-water, detergent water, and vinegar water. In muddy water, a 20% mortality rate was recorded, while clean water resulted in a 40% mortality rate. Salt water caused an 80% mortality rate for *Culex* larvae. In contrast, no larval deaths were observed in the control group during the study period **(Figure 5)**.

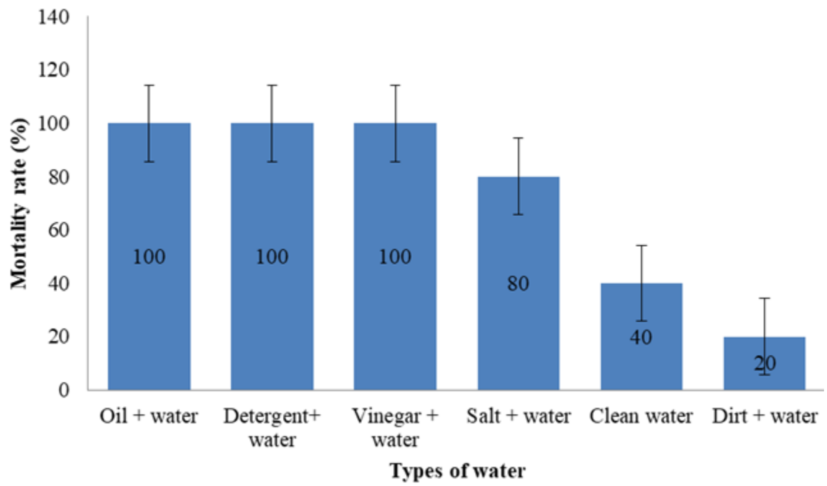


Figure 5. Mortality rates of *Culex* larvae in various types of water

DISCUSSION

The present study highlights the distribution, abundance, and survival of *Culex* larvae across various breeding habitats, as well as the effects of different water conditions on larval mortality. The findings reveal that *Culex* larvae were present in 10 out of 35 surveyed breeding habitats, with a distribution class (C) of 38.28%, indicating an infrequent presence. This aligns with studies conducted in other regions, where *Culex* larvae were found to be unevenly distributed across breeding habitats (Goselle et al., 2018). Sewage canals exhibited the highest larval abundance (47.44%), followed by puddles in animal shelters (28.85%) and rice fields (23.72%). These results are consistent with previous research identifying sewage canals and animal-related water bodies as primary breeding habitat for *Culex* mosquitoes due to the availability of organic matter and stagnant water (Wang et al., 2020). In contrast, no larvae were found in ponds or residential puddles, likely due to factors such as predation, water quality, or human intervention, as observed in similar studies (Bashar et al., 2016). This observation is also supported by other researches, which found that *Culex* mosquitoes prefer polluted, stagnant water over cleaner and more open water bodies (Djoufounna et al., 2022) where predation risk is higher (Liu et al., 2019).

The absence of a significant association between larval presence and factors such as sunlight exposure, vegetation, and water turbidity aligns with studies suggesting that *Culex* mosquitoes exhibit adaptability to diverse environmental conditions (Liu et al., 2019). However, stagnant water was significantly associated with larval presence ($p < 0.05$), reinforcing earlier findings that highlight its critical role in *Culex* breeding (Djoufounna et al., 2022). The low presence of larvae in flowing water (3.85%) further confirms the species' preference for stable aquatic environments conducive to larval development. While some studies have suggested that factors like sunlight and vegetation influence mosquito breeding (Mo'awia et al., 2020), the lack of such associations in this study may be attributed to regional environmental variations or species-specific behaviors.

The overall Larval Productivity Index (LPI) of 64.1% and Pupal Productivity Index (PPI) of 79% indicate that a higher proportion of pupae successfully developed into adults compared to larvae developing into pupae. This is consistent with findings from other studies, which suggest that the pupal stage is more resilient and has a higher survival rate than the larval stage (Goselle et al., 2018).

The mortality rate experiments demonstrated that oil-water, detergent water, and vinegar water caused 100% mortality of *Culex* larvae within a few hours to two days. These findings align with studies showing the lethal effects of oils, detergents, and acidic substances on mosquito larvae (Goselle et al., 2018; Madavi & Netam, 2020), demonstrating the ability of household detergents and oils to disrupt larval respiration and development. Similarly, the 80% mortality rate in salt water, likely due to osmotic stress, as reported by National Institute of Virology-Kerala Unit, India (Balasubramanian et al., 2019). In contrast, clean water and muddy water resulted in lower mortality rates (40% and 20%, respectively), indicating that these conditions provide a more favourable environment for larval survival (Goselle et al., 2018; Ukubuiwe et al., 2020). The absence of larval mortality in the control group further validates the experimental setup and highlights the effectiveness of the tested substances.

The study underscores the importance of targeting specific breeding habitats, such as sewage canals, puddles in animal shelters, and rice fields, for effective larval source management (LSM). The high mortality rates observed in oil-water, detergent water, and vinegar water suggest that these substances could be used as eco-friendly larvicides in integrated vector management (IVM) programs. Additionally, the strong association between stagnant water and larval presence emphasizes the need for proper water management, including the elimination of stagnant water bodies, to reduce breeding habitats of *Culex* mosquitoes

CONCLUSIONS

The study revealed that *Culex* larvae are unevenly distributed across different breeding habitats, with significant variation in abundance. Sewage canals, puddles in animal shelters, and rice fields were identified as the primary productive habitats, collectively accounting for the majority of larvae collected. Larval presence was significantly influenced by the type of breeding habitat and the state of water, with stagnant water favouring higher larval abundance. Conversely, light condition, vegetation, and water turbidity showed no significant association with larval presence. The mortality experiments demonstrated that certain water types such as oil-water, detergent, and vinegar water are highly lethal to *Culex* larvae, suggesting potential avenues for control. These findings contribute valuable insights into the ecology of *Culex* mosquitoes, highlighting critical habitats that should be targeted for vector control efforts to reduce mosquito populations and associated disease risks.

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