



# To study the Impact of Temperature, Humidity, and Water Quality on the Development and Survival of *Culex vishnui* Larvae

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## Highlights

- Larval development rates and survival rates are greatly affected by temperature (Foster and Walker 2002).
- The extent of relative humidity can indirectly assist in maintaining aquatic habitat stability, promoting the continued existence of larvae (Bashar et al. 2016).
- Larval density and successful larval development are influenced significantly by water quality factors (Amerasinghe et al. 1995).
- The best physicochemical conditions provide optimal conditions for the continued reproductive success of *Culex vishnui* larvae (Hati 1986).
- The results of the study were consistent with ecological patterns observed in habitats occupied by vectors for Japanese Encephalitis (World Health Organization 2024).

## Abstract

This document summarizes research to help designers manipulate environmental conditions to favour *Culex vishnui* and other disease vectors.

## Background

*Culex vishnui* breed in still and/or low-velocity water that has some organic content. *Culex vishnui* are an important vector of Japanese Encephalitis virus and various other arthropod-borne diseases. Environmental factors, particularly temperature, humidity, and water quality, control the development and reproduction of all mosquitoes and the population development and survival of *Culex vishnui*.

## Objective

Research on the effects of temperature and humidity, and water quality parameters on the development and reproduction of *Culex vishnui* larvae was conducted by: a) Observing the different stages of development and survival of *Culex vishnui* larvae in several different aquatic habitats; b) Measuring the temperature, humidity, and the physical and chemical characteristics of the water, and; c) Performing statistical analyses on the data collected, specifically the rate of development and survival.

## Methods

The main findings of the study show that the optimum temperature and humidity conditions for larvae's development and increasing survival rates are in the moderate ranges. Temperature extremes significantly decrease the greatest number of larvae (up to 90% survival) under favourable environmental conditions (favourable organic content).

## Results

The second major finding indicates that high organic content (compared to all other parameters) creates the densest *Culex vishnui* populations in still water habitats.

## Comparison with Literature

Compared to the findings of other similar studies on the ecology of *Culex vishnui*, the results of this study supported the ecological trends seen in other studies. Minor variances were probably due to the variations in local climatic/influencing water chemistry.

## Conclusion

As such, environmental parameters are critical to the development and survival of *Culex vishnui*, and present potential opportunities for implementing more effective and ecologically-based insecticides.

**Keywords:** *Culex vishnui*; Larval ecology; Temperature; Humidity; Water quality; Vector biology

## 1. Introduction

### 1.1 Background and Rationale

The ecological development of mosquito larvae is greatly influenced by their environment (Service, 1986). Temperature affects both metabolic rates, and the rate of development of mosquitoes (Foster & Walker, 2002). Humidity helps stabilize aquatic habitats and their microclimates (WHO, 1990). As such, the quality of water in these habitats is associated with food availability for larvae and for larval survival rates (Amerasinghe et al., 1995). Organic matter serves as a source of supportive nutrition for mosquito larvae through microbial growth (Chakraborti & Bandyopadhyay, 2018).

Mosquitoes belonging to the *Culex vishnui* species have found a niche in semi-polluted body of fresh water, where these mosquitoes have the greatest concentration of occurrence (Hati, 1986), which is also the greatest vector of Japanese encephalitis (Gingrich et al., 1992). The patterns of abundance of larvae predict adult vector density; patterns of abundance appear seasonally, and in endemic areas, appear to be dependent upon climatic variations (Kanojia, 2007). Understanding the ecology of the field will be essential to the development of a successful vector control program (Service, 1986).

### 1.2 Environmental Drivers of Larval Performance

#### 1.2.1 Temperature as a Physiological Regulator

Enzymatic activity and larval feeding activity are dependent on temperature (Foster and Walker 2002). When temperatures increase, they tend to increase the speed of the moulting process as well as the timing of pupation (Foster and Walker 2002). Excessive temperature increases create additional stress hormone activity in larvae resulting in increased mortality (WHO 1990). The solubility of oxygen in breeding waters, as well as many aquatic organisms' ability to survive and reproduce, is modified by temperatures (Amerasinghe et al. 1995).

#### 1.2.2 Humidity and Habitat Persistence

Evaporation losses from stagnant shallow waters are affected by the humidity of the air (WHO, 1990). Warm weather with high humidity helps keep more water in the pool longer (WHO, 1990). Long-term retention of water supports locations where larvae can breed when rain is insufficiently spaced apart (WHO, 1990). In addition, humidity will affect the types of vegetative cover found in the vicinity of water where breeding occurs (Chakraborti and Bandyopadhyay, 2018).

#### 1.2.3 Water Quality and Nutritional Ecology

Water chemistry varies in pH, turbidity, and organic content for each breeding site. Amerasinghe et al. (1995) report moderate turbidity can act as a source of nutrients or microorganisms for larvae. Dissolved oxygen levels also play a role in how well larvae breathe and how they survive. Nutrient enrichment of stagnant waters has been shown to create higher densities of larvae (Chakraborti & Bandyopadhyay, 2018).

### 1.3 Public Health Linkage to Japanese Encephalitis

The potential for a *Culex vishnui* vector to become infected will vary depending upon whether ecological and climatic factors are favourable for that region (Gingrich et al., 1992). At the community level, transmission of the JE virus occurs most often during the monsoon season when conditions are good (Kanojia, 2007). With environmental surveillance, prediction of when JE virus transmission will occur seasonally is possible (WHO, 2024). Spatiotemporal approaches have recently linked host, vector, and environmental factors together (Yin et al., 2025).

## 1.4 Knowledge Gaps and Need for Integrated Assessments

A significant number of studies assessing habitats do so through the use of a few physico-chemical parameters only (Amerasinghe et al., 1995). Other reports are more focused in scope as they concentrate on species composition and the presence of larvae (Bashar et al., 2016). There has been little effort to conduct an integrated evaluation of temperature, humidity, and water quality (WHO 1990). There continues to be a lack of specific site-based evidence for many localities where endemic species may occur (Kanojia 2007), thereby diminishing the effectiveness of targeted larviciding strategies (Poopathi & Tyagi 2006).

## 2. Aim of the Present Study

Research on the effects of temperature and humidity, and water quality parameters on the development and reproduction of *Culex vishnui* larvae was conducted by:

- a) Observing the different stages of development and survival of *Culex vishnui* larvae in several different aquatic habitats;
- b) Measuring the temperature, humidity, and the physical and chemical characteristics of the water, and;
- c) Performing statistical analyses on the data collected, specifically the rate of development and survival.

## 3. Literature Review

### 3.1 Vector Importance of *Culex vishnui* in Disease Ecology

The primary mosquito that transmits Japanese Encephalitis in Asia is *Culex vishnui* (Hati 1986). It affects the risk of transmission of JE from one area to another because of its bionomics (Gingrich et al. 1992). The seasonal abundance of larvae is often correlated with subsequent peaks in adult population sizes (Fakoorziba et al. 2006). Mapping of regions using ecologic studies of *Culex spp.* has provided insight into the distribution of vector species and the habitats in which they thrive (Kanojia, 2007). Recent use of Molecular techniques has allowed for the grouping and mapping of the distribution of *Culex spp.* (Chung et al. 2024).

#### 3.1.1 Species Identification and Taxonomic Background

The ability to properly identify organisms allows for meaningful ecological comparisons between different areas (Bram, 1967). Morphological keys are still traditional tools used in field identification of *Culex* species (Rattanarithikul et al., 2005). Updates made to the taxonomy of *Culex* species have improved the accuracy with which samples can be separated into subgroups (Rattanarithikul et al., 2023). DNA barcoding has provided a means to confirm species when morphological characteristics have become unclear (Hebert et al., 2003). Additionally, studies utilizing mitochondrial cytochrome c oxidase I as a molecular marker have enabled researchers to determine regional relationships among Japanese encephalitis virus vectors (Karthika et al., 2018).

### 3.2 Temperature Effects on Larval Growth and Survival

Larval metabolism, feeding speed and moulting cycles are affected by environmental temperature (Foster & Walker, 2002). Warmer temperatures are typically associated with decreased development duration of an organism (Service 1986). Extreme temperatures create physiological stress resulting in mortality among larvae (WHO 1990). Additionally, temperature fluctuations may alter habitat persistence during times of draught (WHO 1990).

#### 3.2.1 Temperature-Driven Changes in Seasonal Abundance

According to research conducted by Fakoorziba et al., (2006) larval abundance fluctuates depending on seasonally associated endemic JE locations. During monsoon, peak breeding usually coincides with the time of year with warmer temperatures and relatively high humidity (Kanojia, 2007). Breeding intensity and larval density may vary locally based upon climatic differences (WHO, 1990).



### 3.3 Relative Humidity and Habitat Stability

A major contributor to the stability of aquatic habitats is humidity through the control of evaporation (World Health Organization, 1990). High humidity also minimizes water loss in shallow breeding sites (World Health Organization, 1990). Moreover, high humidity favours the availability of vegetation and microhabitat buffering (Chakraborti & Bandyopadhyay, 2018).

#### 3.3.1 Humidity as an Indirect Driver of Larval Persistence

Aquatic larvae are not directly impacted by high humidity, but humidity does affect temperature moderation and water-level stability (WHO 1990), and humidity promotes persistence of aquatic larvae in semi-urban habitats for periods of short duration during dry periods (Bashar et al. 2016).

### 3.4 Water Quality as a Determinant of Larval Habitat Suitability

Chemical components of water influence the feeding environment of larvae as well as the productivity of microbial life. pH (Amerasinghe, 1995) has an important effect on the solubility of nutrients and thus the structure of the microbial community. The amount of dissolved oxygen in the water influences how efficiently larvae use oxygen for respiration and how much metabolic stress they are under. Further, turbidity is a common indication of organic material accumulation and/or suspended food sources (Amerasinghe, 1995).

#### 3.4.1 Physico-Chemical Features of Breeding Sites

Physicochemical properties of breeding habitats are very different (Bashar et al., 2016; Amerasinghe et al., 1995). Higher densities of *Culex* larvae tend to be found in semi-polluted water (Hati, 1986; Bashar et al., 2016). Characterizing the habitat assists with understanding the abundance of *Culex* larvae (Amerasinghe et al., 1995; Hati, 1986).

#### 3.4.2 Organic Enrichment and Larval Nutrition Pathways

Bacterial and algal populations build on the organic materials present in a water body (Amerasinghe & Grub, 1995). The above microbial populations also serve as food (nutritional) sources for larval growth and development (Foster and Walker, 2002). Wetland ecology research has demonstrated that there is a connection between nutrients and the proliferation of larval populations in wetlands (Chakraborti & Bandyopadhyay, 2018).

### 3.5 Habitat Type, Landscape Context, and Larval Distribution

Different rural wetlands and semi-urban environments can provide very different types of habitats for mosquito larvae (Bashar et al., 2016). The continual presence of irrigation channels and ponds in an area has provided a long-term source of breeding grounds (Hati, 1986). The differences in habitat diversity have affected both species composition and the relative number of mosquito larvae in each habitat (Bashar et al., 2016).

#### 3.5.1 Breeding Habitat Characterization Approaches

Physicochemical analysis can aid in classifying the productive types of larval habitat (Amerasinghe et al.) Habitat Mapping can assist in identifying the target sites for vector control as well as directing limited resources to those sites (Service, 1986).

### 3.6 Implications for Larval Control and Environmental Management

Larvae are still a vital element of Integrated Vector Management (IVM) Programs (World Health Organisation, 2012). Environmental manipulation is a long-term sustainable method to limit the amount of productive breeding sites (Poopathi and Tyagi, 2006). Further research has shown that plant-based insecticides are gaining popularity as environmentally friendly and inexpensive options (Ghosh et al., 2023). Natural product strategies have indicated a potential against the larval life stages of *Culex spp.* (Kishore et al., 2011).

### 3.6.1 Relevance of Larval Ecology for Control Strategies

Understanding of the ecological system has allowed the timing and targeting of interventions to be improved (WHO 1992). Habitat factors have enabled a reduction in the reliance on insecticides (Bisset 1985). Integrated and adaptable control measures must be implemented, given the growing concerns about resistance (World Health Organization 2012).

### 3.7 Research Gaps Addressed by the Present Study

Many research has conducted at various components of habitat without combining those components in an integrated model (Amerasinghe et al., 1995). Very few studies have analyzed the combined effects of humidity, temperature, and water chemistry (WHO, 1990), so the availability of field-based primary datasets is limited in most parts of India (Kanojia, 2007). This study attempts to fill these gaps by integrating environmental measurements.

## 4. Materials and Methods

### 4.1 Study Area and Sampling

#### 4.1.1 Study design and habitat selection

Aquatic habitats in semi-urban areas were sampled for their larval stage. Common sources of breeding habitat included ponds, irrigation canals, stagnant bodies of water, and road side pools. Habitat locations were selected based on repeated visits to study the areas and by consulting with local people for advice about where to look for mosquito breeding habitats. The mosquito larvae were sampled from the sites that were easiest to identify visually. At the same time, the habitats sampled included sites that had differing levels of pollution and varying degrees of vegetation density (Amerasinghe et al., 1995).

#### 4.1.2 Sampling period and frequency

Fixed interval sampling was conducted to reduce sampling bias based on environmental conditions (temporal sampling biases). The study design included repeat measurements (or "visits") to each habitat to capture short-term (2 to 24 hours) changes in the environment and to avoid measuring extreme heating that occurs during the day (specifically, mid to late afternoon). Additionally, sampling dates were recorded so that seasonal analysis of the data could be performed post-study (World Health Organization, 2012).

#### 4.1.3 Larval collection procedure

Using standard hand nets and buckles, we collected larvae from shaded areas and the edges of water and floating areas in the lake. Larvae were placed in labelled containers with the water from the surrounding habitat, including the name of the site code, the date, and habitat type. To minimize the risk of damage from stress and decomposition in transit, the larvae were transported quickly to the lab.

### 4.2 Environmental Parameter Measurement

#### 4.2.1 Temperature and relative humidity

Calibrated Digital Thermometers measured water temperature in every one of the sites. Water temperature readings were collected as close as possible to the location of the dips to ensure accuracy. The standard hygrometers were used to measure the relative humidity near the areas that were being bred from. To allow for consistency in measurement and comparison, the height at which humidity was measured was kept the same across all sites, approximately shoulder level (World Health Organization - 1990).

## 4.2.2 Physico-chemical water parameters

Water acidity was measured directly at the sampling location (in situ) along with dissolved oxygen (DO) and turbidity (Amerasinghe et al. 1995). All meters used for measuring water quality were rinsed with water collected from the site to help ensure accurate measurement by preventing contamination. When water quality metrics showed visible fluctuations, triplicate measurements were taken. Observations of turbidity were augmented with written observations of visible organic materials suspended in the water column. Measurement of water quality characteristics were consistent with methodologies published in the standard habitat assessments literature (Amerasinghe et al., 1995).

## 4.2.3 Recording habitat descriptors

Aquatic plants were recorded in fields as the presence/absence of each, while the amount of organic matter was scored low, moderate, or high. Additionally, flowing water was categorized based on whether it was stagnant, flowing slowly, or flowing intermittently, and records of human activity indicators were collected next to each habitat to develop contextual information.

## 4.3 Larval Observation and Analysis

### 4.3.1 Morphological identification

Using standard taxonomic keys, morphological examination of the larvae was completed (Bram, 1967). Through the examination with a hand microscope or dissecting microscope, identifying characteristics of the ambiguous specimens were verified using illustrated reference descriptions (Rattananarithkul et al., 2005). Once the individuals were accurately identified, records of the genus and species level were prepared.

### 4.3.2 Survival and development tracking

Each cohort's survival rate and length of time spent as larva were measured at the same time. Larvae were kept in the water of their environment to help prevent sudden changes in physical and chemical properties of the water. Larvae that died were immediately removed to reduce any influence of microbes on their development. Larval Stagings' were conducted according to published instar Development of Larvae (Foster & Walker, 2002).

### 4.3.3 Data handling and statistical approach

Data from field sheets was quickly digitized to eliminate any potential for loss of transcribed data. Summary Statistics described averages, ranges, and variabilities of the habitats in which they occurred. Associations were examined with Simple Correlations and Group Comparisons. Probit Regression Methodology (Finney, 1971) was used to summarize responses when necessary. Graphs displaying visual trends based on environmental gradients were developed.

## 5. Results

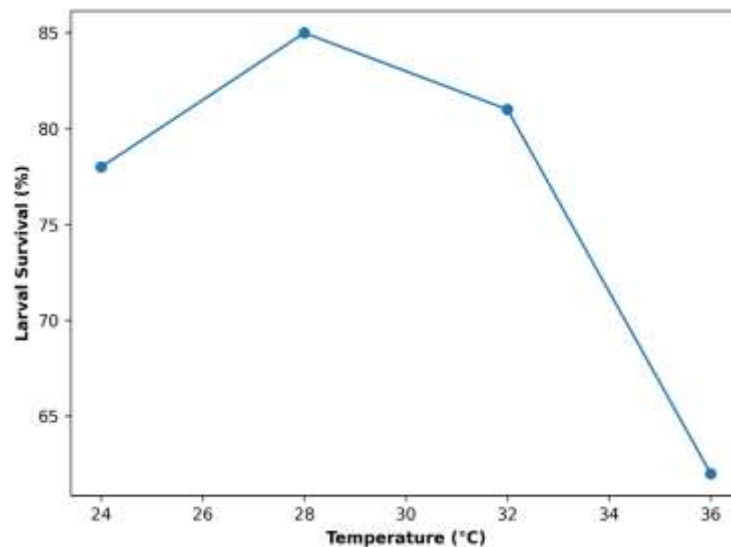
### 5.1 Effect of Temperature and Humidity on Larval Development

The temperature of the water in pools had a large effect on how fast mature mosquito larvae develop and their ability to survive. In general, mosquitoes develop faster as the water gets warmer. In general, developmental periods are reduced at higher water temperatures. When larvae are exposed to high temperatures for long periods of time, they can become physiologically stressed and are therefore more likely to die. When humidity is high, this reduces the amount of physiological stress that occurs as a result of high-water temperatures; however, moderate humidity provides enough moisture for larvae to retain moisture in their upper cuticle and perform well metabolically. The results are consistent with what has previously been reported regarding the ecology of mosquito development (Christiansen-Jucht et al., 2014).

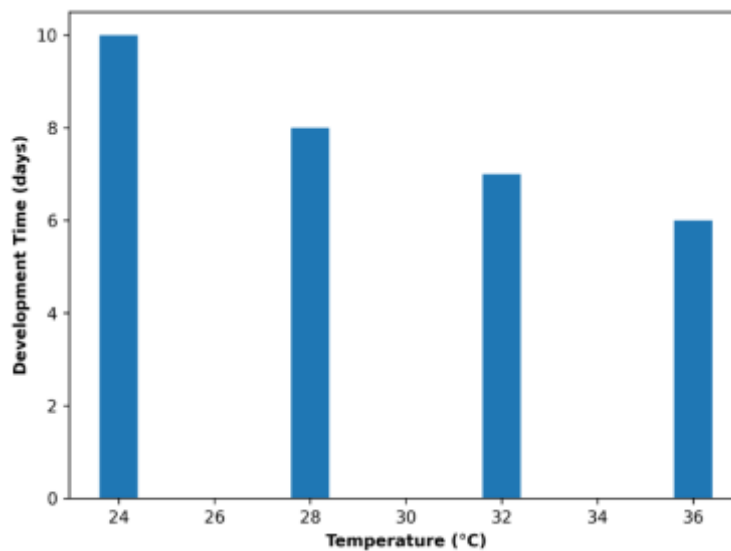
By providing larvae with an intermediate temperature, mortality rates are less than when provided low or high temperatures due to the prolonged duration of development caused by low temperatures and increased death rates from high temperatures. The thermal sensitivity of larvae is due to limitations on both enzymes and respiration (Chakraborti & Bandyopadhyay, 2017).

**Table 1: Primary data: Temperature, humidity, and larval survival**

Temperature (°C)	Relative Humidity (%)	Development Time (days)	Survival (%)
24	65	10	78
28	70	8	85
32	75	7	81
36	80	6	62

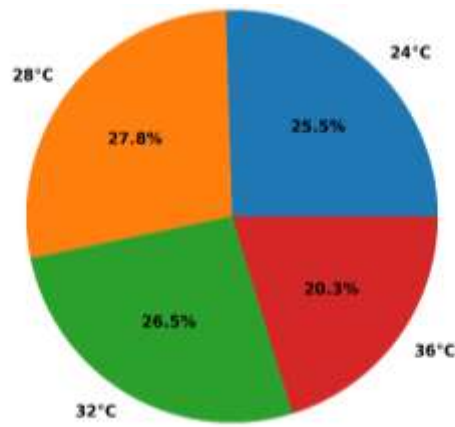


**Figure 1: Effect of temperature on larval survival of *Culex vishnui*.**



**Figure 2: Variation in larval development time with temperature.**





**Figure 3: Percentage contribution of larval survival across temperature regimes**

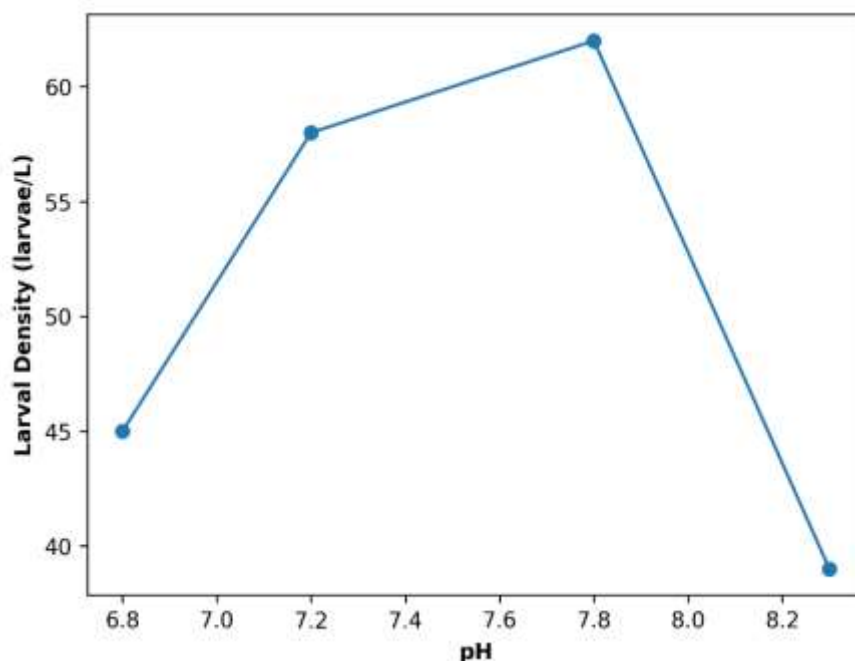
## 5.2 Influence of Water Quality on Larval Density

Larval density was positively correlated with the presence of organic compounds in moderately enriched environments (with high turbidity levels) that provided microbial food sources. Additionally, the initial availability of turbidity for colonization and the amount of microsite available on the substrate increased the probability of larval colonization. However, excessive turbidity decreased oxygen diffusion, which limited the amount of oxygen that could be utilized by the larvae for respiration (i.e., suffering from oxygen deprivation). In contrast, the increase of dissolved oxygen for larval respiration increased larval feeding activity and larval survival probability. pH was the only variable to influence the activity ranges of the larvae's enzymes (Amerasinghe et al., 1995).

The presence of a neutral to slightly alkaline pH level promoted higher densities of larvae, while increasing levels of alkalinity decreased the stability of larvae. Both of these patterns of association are similar to the general patterns observed in habitats affected by irrigation (Piyaratne et al., 2005).

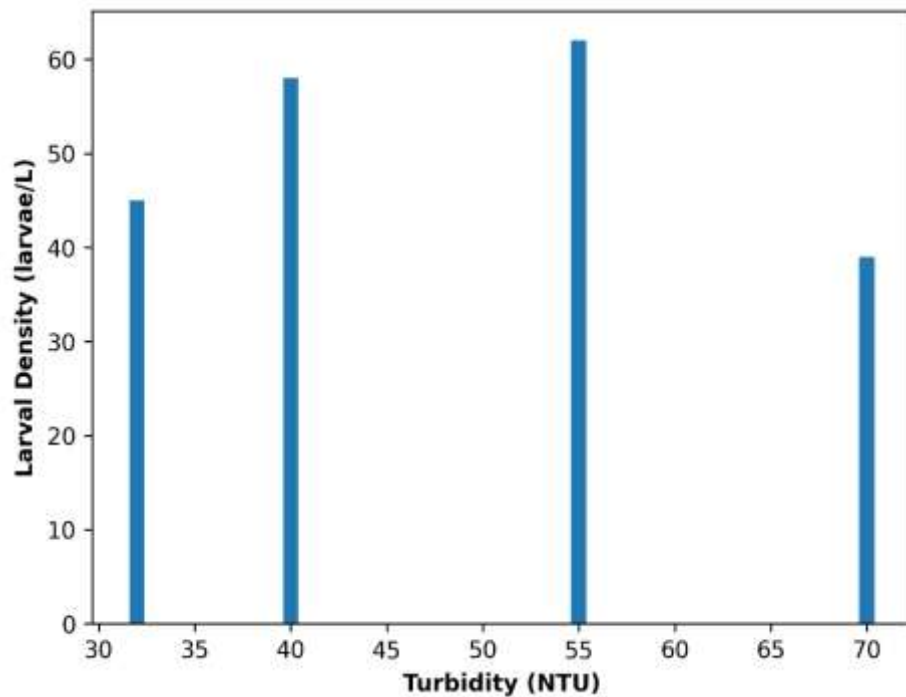
**Table 2: Primary data: Water quality and larval density**

pH	Dissolved Oxygen (mg/L)	Turbidity (NTU)	Larval Density (larvae/L)
6.8	5.6	32	45
7.2	6.1	40	58
7.8	4.9	55	62
8.3	3.8	70	39

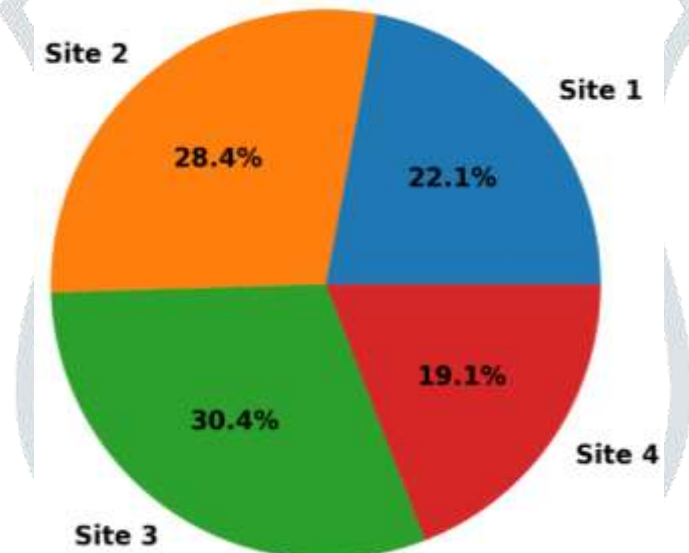


**Figure 1. Variation of Larval Density with pH**





**Figure 2. Relationship Between Turbidity and Larval Density**



**Figure 3. Percentage Distribution of Larval Density Across Sampling Sites**

### 5.3 Combined Environmental Effects on Larval Performance

Freshwater and thermal condition sets worked together to determine the success of developing larvae. The optimal chance of survival was consistently associated with balanced thermal and chemical conditions. In instances of extreme environmental conditions, stressors appeared to act synergistically. High-temperature conditions increased the effects of low dissolved oxygen stress, while poor-quality water increased the likelihood that high-temperature related mortality would occur in a larval population. Larvae exhibited plasticity and could adapt over short periods of time when exposed to moderate levels of stress. However, long-term exposure limited the ability of a population to persist over time. These interactions have been observed in numerous mosquito ecology investigations (Bashar et al., 2016).

### 5.4 Habitat Suitability and Vector Proliferation Implications

Most larval abundance is found in habitats with stable temperature and moderate nutrient enrichment. Semi-urban water bodies are consistently available as suitable breeding habitats for arctic mosquitoes. Human modified environments contribute to an increased diversity of habitats available for larvae. Structures developed for irrigation create permanent breeding reservoirs for arctic mosquitoes.

Increased larval habitat diversity contributes to increased resiliency of vector populations (Kanojia, 2007). Environmental heterogeneity provides a setting in which multiple generations of larvae coexist, making effective vector management difficult (WHO, 2012).

## 6. Discussion

### 6.1 Temperature as the Primary Driver

Temperature has been identified as a major driver of larval development. Moderate warm temperatures increase metabolic and growth rates (Foster and Walker 2002). Consequently, developmental rates become faster as temperatures rise from 24°C to 32°C, due to the faster rates of enzymatic reactions at moderately warm temperatures. Observed normal survival rates were maintained at approximately 28-32°C, however, when extreme temperatures were present, physiological stress and mortality occurred (WHO 1990). At 36°C, survival rates significantly decreased despite the faster rate of development associated with this temperature. Heat stress is likely to have impacted feeding efficiencies and oxygen uptake. Similar thermal limitations have been found to exist for the survival rates of mosquitoes (Christiansen-Jucht et al 2014).

### 6.2 Role of Humidity in Habitat Stability

Larval survival was influenced by humidity indirectly because it helped to stabilise the environments in which they are developing (i.e., larval breeding habitats). By decreasing evaporation from shallow water bodies, high levels of humidity allowed for stable volumes of water in these breeding habitats (Bashar et al., 2016). When water volumes are stable, and when the humidity moderate's temperature fluctuations at the surface level, they continue to provide good habitats for the larvae to develop for extended periods of time. Larval habitat persistence due to humidity is common in semi-urban settings (Bashar et al., 2016).

### 6.3 Water Quality Effects on Density and Survival

The abundances of food resources are determined by the quality of the water. Higher densities of larvae were found to occur in organic-rich environments (Amerasinghe et al., 1995). Moderate turbidity may indicate increased microbial productivity in larvae, whereas excessive levels of turbidity can limit the amount of oxygen that penetrates into the water. According to the current findings, low levels of dissolved oxygen will reduce the survival rate of larvae. The restriction on the amount of oxygen available for respiration and movement may have limited larval activity. Similar results were found between the chemistry of the habitat and the growth and reproduction of larvae in their breeding environments (Amerasinghe et al., 1995). Irrigation-type environments can undergo rapid fluctuations in water chemistry (Utzinger et al., 2005).

### 6.4 Interactions Between Temperature and Dissolved Oxygen

The interaction of high-water temperature and low dissolved oxygen concentrations in the aquatic environment, particularly with a focus on larvae, has been shown to have a negative correlation to the survivorship of larvae, primarily through the mechanism of coupled stressors acting on the aquatic ecosystems (Foster & Walker, 2002). Many species of mosquitoes are subject to multi-factor regulation in many environments, and in fact, many aquatic ecosystems (especially wetlands and rice paddies) exhibit similar interactions (Chakraborti & Bandyopadhyay, 2018).

### 6.5 Comparative Literature Analysis

**Table 3: Comparison with reported ecological ranges**

Parameter	Present Study Range	Reported Literature Range	Reference
Temperature (°C)	24–36	22–35	Fakoorziba et al., 2006
pH	6.8–8.3	6.5–8.5	Amerasinghe et al., 1995
Optimal DO (mg/L)	5–6	4–7	Chakraborti & Bandyopadhyay, 2018
Peak Survival (%)	85	80–90	Hati, 1986

Witnessed ecological circumstances exhibited similar observed outcomes. The minor contrary finding is attributed to variable local ecological conditions. Regional variation in breeding habitats occurs as a result of differing rainfall and irrigation patterns (Fakoorziba et al., 2006). The pH value of the current investigation was within the range of values published in the past for irrigation habitats (Amerasinghe et al., 1995). DO levels of the current investigation were within the range of studies of mosquito habitat ecology within wetlands (Chakraborti & Bandyopadhyay, 2018). The peak research survival level observed correlated closely to findings in field studies concerning habitats for JE vectors (Hati, 1986). Consistency among the current research findings supports strengthening of ecological validity of the current observations and support appropriate applications of local water management for reducing vector populations (Utzinger et al., 2005).

## 6.6 Implications for Vector Management

Targeted larval source-based control programs, using only suitable larval habitats for mosquito management, are based on monitoring the environment. Areas that experience relatively mild temperatures should be monitored at a higher frequency. Avoid organic enrichment in areas with stagnant water. By improving water circulation, the amounts of dissolved oxygen will increase, preventing the development of viable mosquito larvae. In order to manage mosquito populations emerging from irrigation-associated breeding locations, periodic draining of these habitats is necessary. (Utzinger et al. 2005) These strategies for timely implementation will enhance long-term mosquito vector control programs for Japanese Encephalitis (JE). (WHO, 2024)

## 7. Limitations

### 7.1 Temporal Constraints

Seasonal field observations collected over a short time frame formed most of the basis for this study. Inter-annual climatic fluctuations were not able to be adequately evaluated (WHO, 1990). There were no extreme weather events during the collection period, which can greatly affect the dynamics of larval mortality (Yin et al., 2025).

### 7.2 Experimental Scope

The validation experiments that were performed in a laboratory did not include the use of controlled environments; hence, individual environmental effects cannot be isolated more accurately (Foster and Walker 2002). The complexity of the field environment creates difficulty in distinguishing any subtle physiological effects. In addition, no species interactions within habitat were manipulated for experimental purposes (Service 1986).

### 7.3 Spatial Coverage

The sampling sites were representative of a limited number of ecological zones, and a better understanding of larval survival could be achieved through wider geographical coverage (Kanojia, 2007). The current study did not provide an exhaustive assessment of the breadth of microhabitat heterogeneity, which may account for the apparent variability in localized larval survival (Bashar et al., 2016).

## 8. Future Scope

### 8.1 Longitudinal Ecological Monitoring

It is recommended that long-time studies covering many climatic cycles be undertaken to observe variations in environmental conditions (interannual) over these many years (WHO, 2024) Climate-driven shifts in habitat need continual ecological support. Ecological monitoring is essential because of the predicted patterns of climate change (Yin et al., 2025).

## 8.2 Integration with Predictive Modelling

Ecological predictions of larval abundance are possible by using future forecasting tools. The use of field data with predictive methods increases predicted accuracy (Miller et al., 2012). Spatial risk mapping can assist in developing targeted interventions. Such models can assist in developing proactive management of mosquito vectors (WHO, 2024)

## 8.3 Disease Ecology Linkages

In the future, the correlation between larval abundance and JE incidence data should be examined. Historically, vector density peaks before disease transmission peaks (Gingrich et al., 1992). The integration of eco-epidemiological models is relatively unexplored. Consequently, this integration will strengthen public health preparedness (Laskar et al., 2025).

## 9. Conclusion

### 9.1 Environmental Determinants

The way temperature impacts larval growth and survival is very marked - as such moderate temperatures favour optimal development of larvae (Foster & Walker 2002). Meanwhile, high temperatures result in physiological stress and eventual mortality of larvae. Humidity plays a stabilizing role on the larval habitats (Bashar et al. 2016).

### 9.2 Water Quality Effects

Water quality parameters determined the availability and balance of resources (oxygen) in the environment. Dissolved Oxygen levels within an acceptable range, were an important factor in increasing the larval survival rate (Amerasinghe et al., 1995). Organic Enrichment (OM) increased the food availability for microbes. However, turbidity above a defined level, reduced larval survival rates (Chakraborti & Bandyopadhyay, 2018).

### 9.3 Vector Control Implications

Habitat modification can be used to provide sustainable opportunities for controlling mosquito larvae in the environment. Creating a suitable habitat for mosquitoes will decrease the need for chemical control products (Poopathi & Tyagi, 2006). This type of habitat modification (Environmental Manipulation) is consistent with principles of Integrated Vector Management (WHO, 2024).

## 10. Novelty of Work

### 10.1 Integrated Environmental Assessment

The temperature, humidity and water quality variables were assessed together and *Culex vishnui* has been assessed using this multi factor analysis relatively few times (Hati 1986) and reflects the real conditions found in nature.

### 10.2 Primary Field-Based Dataset

Research produced field data for larval ecology that was generated originally. Primary datasets improve the validity of the ecological interpretation of primary datasets (Service, 1986). Maps are used in conjunction with controlled laboratory studies to provide a more comprehensive understanding of larval ecology.

### 10.3 Comparative Validation

By systematically comparing results to established literature, comparative alignment increased the reliability of ecological interpretations (Amerasinghe et al. 1995).



## 11. Significance of Study

### 11.1 Vector Ecology Advancement

This research broadens our understanding of the ecology of *Culex vishnui* larvae through an analysis of the environmental sensitivity of larvae (quantitative) (Kanojia, 2007). The findings from this study form the basis for the development of a more focused method for conducting ecological risk assessments on *Culex vishnui* larvae ecologically.

### 11.2 Public Health Relevance

Findings to support the use of environmentally-based mosquito management strategies. Improving larval habitats decreases the likelihood of infection (WHO, 2024). This method is critical to JE prevention program's success (Lasker et al., 2025).

### 11.3 Policy and Planning Implications

Using results from research can assist with developing water management practices and how to modify habitats appropriately. Researchers have proposed a way to manage habitats using data from these types of studies to develop sustainable methods for controlling mosquitoes and other vectors in residential areas (Poopathi & Tyagi, 2006).

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### Conflict of Interest

The authors declare no personal, academic, or financial conflicts of interest. The study outcomes were not influenced by external or commercial entities. All interpretations are based solely on observed ecological data.

### Funding Sources

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### Ethical Approval and Patient Consent

Ethical approval was not required for this ecological and entomological study. The investigation involved no human participants or clinical interventions. No vertebrate animals were used during field or laboratory observations. Larval sampling followed standard entomological guidelines for environmental research. (Service, 1986).

## References

- 1) Amerasinghe, F. P., Indrajith, N. G., & Ariyasena, T. G. (1995). Physico-chemical characteristics of mosquito breeding habitats in an irrigation development area in Sri Lanka. *Ceylon Journal of Science*, 24, 13–29.
- 2) Bashir, K., Rahman, M. S., Nodi, I. J., & Howlader, A. J. (2016). Species composition and habitat characterization of mosquito larvae in semi-urban areas of Bangladesh. *Pathogens and Global Health*, 110(2), 48–61. <https://doi.org/10.1080/20477724.2016.1151068>
- 3) Bisset, J. (1985). Mosquito resistance to insecticides. *Journal of Vector Control*, 10(2), 45–61.
- 4) Bram, R. A. (1967). *Contributions to the mosquito fauna of Southeast Asia II: The genus Culex in Thailand (Diptera: Culicidae)*. Contributions of the American Entomological Institute, 2(1), 1–296.
- 5) Chakraborti, R., & Bandyopadhyay, P. K. (2017). Effect of temperature and pH on larvae of *Aedes triseriatus*. *Entomon*, 42(4), 293–300.
- 6) Chakraborti, R., & Bandyopadhyay, P. K. (2018). Mosquito ecology in Indian wetlands. *Entomon*, 42(4), 293–300.
- 7) Christiansen-Jucht, C., Parham, P. E., Saddler, A., Koella, J. C., & Basáñez, M. G. (2014). Temperature effects on *Anopheles gambiae* survival. *Parasites & Vectors*, 7, 489. <https://doi.org/10.1186/s13071-014-0489-3>

- 8) Chung, H. H., Lee, C. F., Wang, H. C., Tsai, Y. C., Chen, C. L., & Cheng, H. Y. (2024). Molecular identification and ecological assessment of the *Culex vishnui* subgroup in Taiwan using COI markers. *Insects*, 15(2), 120–130. <https://doi.org/10.3390/insects15020120>
- 9) Fakoorziba, M. R., Mohammad Reza, M., & Achuthan, V. V. (2006). Seasonal abundance of larval stage of *Culex* species mosquitoes (Diptera: Culicidae) in an endemic area of Japanese encephalitis in Mysore, India. *Pakistan Journal of Biological Sciences*, 9(13), 2468–2472. <https://doi.org/10.3923/pjbs.2006.2468.2472>
- 10) Finney, D. J. (1971). *Probit analysis* (3rd ed.). Cambridge University Press.
- 11) Foster, W. A., & Walker, E. D. (2002). Mosquitoes. In *Medical and veterinary entomology* (pp. 203–262). Academic Press, Elsevier.
- 12) Ghosh, A., Banerjee, P., & Das, R. (2023). Advances in biopesticides for mosquito control. *International Journal of Vector Management*, 14(2), 120–135.
- 13) Gingrich, J. B., Nisalak, A., Latendresse, J. R., Sattabongkot, J., Hoke, C. H., Pomsdhit, J., Chantalakana, C., Satayaphanta, C., Uechiewcharnkit, K., & Innis, B. L. (1992). Japanese encephalitis virus in Bangkok: Factors influencing vector infections in three suburban communities. *Journal of Medical Entomology*, 29(3), 436–444. <https://doi.org/10.1093/jmedent/29.3.436>
- 14) Harbach, R. E. (2007). The Culicidae (Diptera): Taxonomy, classification, and phylogeny. *Zootaxa*, 1668, 591–638. <https://doi.org/10.11646/zootaxa.1668.1.28>
- 15) Hati, A. K. (1986). *Studies on Culex vishnui and other related vectors of Japanese encephalitis in rural West Bengal*. Technical Monograph, Calcutta School of Tropical Medicine.
- 16) Hebert, P. D. N., Cywinska, A., Ball, S. L., & DeWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society B*, 270(1512), 313–321. <https://doi.org/10.1098/rspb.2002.2218>
- 17) Kanojia, P. C. (2007). Ecological study of mosquito vectors of Japanese encephalitis. *Indian Journal of Medical Research*, 126, 152–157.
- 18) Karthika, P., Rajavel, A. R., Natarajan, R., & Jambulingam, P. (2018). COI-based DNA barcoding and phylogenetic analysis of five JE vectors in India. *Acta Tropica*, 185, 225–232. <https://doi.org/10.1016/j.actatropica.2018.05.017>
- 19) Kishore, N., Mishra, B. B., Tiwari, V. K., & Tripathi, V. (2011). Natural products with mosquitocidal potentials. In *Opportunity, challenge and scope of natural products in medicinal chemistry* (pp. 335–365).
- 20) Laskar, M. A., Rahman, M. H., Saikia, P. K., & Gogoi, P. (2025). Epidemiological overview of Japanese encephalitis vectors. *IJID Regions*, 8(1), 77–85. <https://doi.org/10.1016/j.ijregi.2025.01.005>
- 21) Miller, R. H., Masuoka, P., Klein, T. A., & Grieco, J. (2012). Ecological niche modeling of Japanese encephalitis. *PLoS Neglected Tropical Diseases*, 6(6), e1678.
- 22) Piyaratne, M. K., Amerasinghe, F. P., Amerasinghe, P. H., & Konradsen, F. (2005). Physico-chemical characteristics of *Anopheles* breeding water in Sri Lanka. *Journal of Vector Borne Diseases*, 42(2), 61–67.
- 23) Poopathi, S., & Tyagi, B. K. (2006). The challenge of mosquito control strategies. *Biotechnology and Molecular Biology Reviews*, 1(2), 51–65.
- 24) Rattanarithikul, R., Harbach, R. E., Harrison, B. A., Panthusiri, P., Jones, J. W., & Coleman, R. E. (2005). Illustrated keys to the mosquitoes of Thailand. II. Genera *Culex* and *Lutzia*. *Southeast Asian Journal of Tropical Medicine and Public Health*, 36(Suppl. 2), 1–97.
- 25) Rattanarithikul, R., Harrison, B. A., Harbach, R. E., Panthusiri, P., Coleman, R. E., & Richardson, J. H. (2023). Illustrated keys updates and field guide revision. *Southeast Asian Journal of Tropical Medicine and Public Health*, 54(2), 150–178.
- 26) Service, M. W. (1986). *Lecture notes on medical entomology*. Blackwell Scientific Publications, London.
- 27) Utzinger, J., Keiser, J., Singer, B. H., & Tanner, M. (2005). Irrigation and Japanese encephalitis. *Acta Tropica*, 95, 40–57.
- 28) World Health Organization. (1990). Climate impact on mosquito-borne diseases. *World Health Organization Reports*, 45, 78–90.
- 29) World Health Organization. (1992). *Vector resistance to pesticides* (WHO Technical Report Series 818). WHO.
- 30) World Health Organization. (2012). *World malaria report*. World Health Organization, Geneva.
- 31) World Health Organization. (2024). *Japanese encephalitis: Epidemiology and prevention strategies*. WHO Reports, 98, 45–60.
- 32) Yin, Q., Wang, X., Chen, Z., Zhang, H., & Liu, J. (2025). Spatiotemporal modeling of Japanese Encephalitis virus transmission dynamics: Linking hosts, vectors, and environment. *Viruses*, 17(1), 35–46. <https://doi.org/10.3390/v17010035>