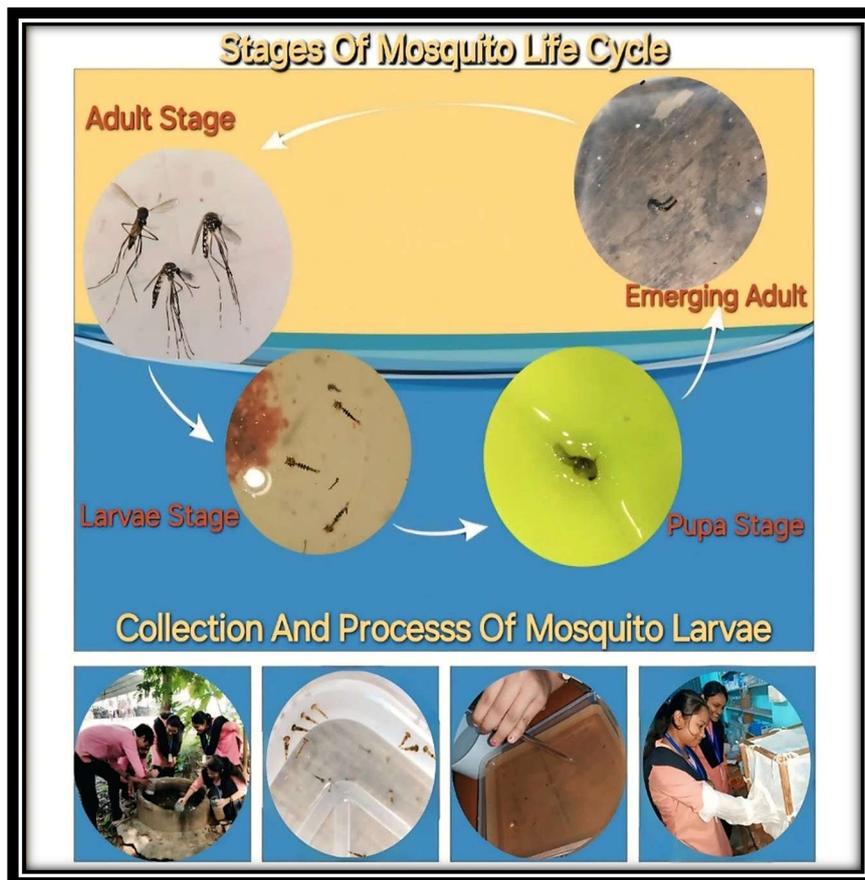


**A STUDY ON SURVIVAL RATES OF VARIOUS LIFE STAGES
OF MOSQUITO, *Culex Quinquefasciatus* COLLECTED
FROM CAMPUS OF NAYAGARH AUTONOMOUS COLLEGE,
NAYAGARH**



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A Project Report On

“A STUDY ON SURVIVAL RATES OF VARIOUS LIFE STAGES OF MOSQUITO, *Culex Quinquefasciatus* COLLECTED FROM CAMPUS OF NAYAGARH AUTONOMOUS COLLEGE, NAYAGARH”

This project report submitted to

DEPARTMENT OF ZOOLOGY

Nayagarh Autonomous College, Nayagarh for the partial fulfilment of the B.Sc. course

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2025

CERTIFICATE

This is to certify that the project entitled “A STUDY ON SURVIVAL RATES OF VARIOUS LIFE STAGES OF MOSQUITO, *Culex Quinquefasciatus* COLLECTED FROM CAMPUS FROM CAMPUS OF NAYAGARH AUTONOMOUS COLLEGE, NAYAGARH” submitted to the Nayagarh Autonomous College in partial fulfilment of the degree of B.Sc. in Zoology is a record of project work carried out by MR. SHIBASIS RATH bearing Exam Roll No. 5901NA22037 and Regd. No. 2202010870010255. He is a bona fide student of this department and the project work has been carried out under my supervision and guidance.

No part of the work has been submitted for any other degree.

Head of the Department

Signature of the Supervisor

DECLARATION



I hereby declare that the report titled “A STUDY ON SURVIVAL RATES OF VARIOUS LIFE STAGES OF MOSQUITO, *Culex Quinquefasciatus* COLLECTED FROM CAMPUS OF NAYAGARH AUTONOMOUS COLLEGE, NAYAGARH” is the result of my independent research, conducted at the Department of Zoology, Nayagarh Autonomous College, Nayagarh. This report is a summary of my findings and knowledge acquired through relevant literature and references from various sources. It is submitted in partial fulfillment of the requirements for Semester VI, as per the CBCS syllabus recommended by the UGC, Government of India.

I further declare that no part of this project has been published or submitted for any degree or certification at any university, college, or institution. I take full responsibility for any errors or omissions in the text and sincerely welcome valuable suggestions for improvement in my future research endeavors.

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ABSTRACT:-

This paper explores the dangers of mosquitoes, particularly *Culex quinquefasciatus*, which are the main carriers of various diseases and thus represent a major challenge of public health. The Research in this area is paramount for developing control strategies that are efficient. This study investigates the survivability of *Culex quinquefasciatus* across its life cycle—egg, larva, pupa, and adult—by analyzing samples taken from the campus of Nayagarh Autonomous College, Nayagarh. At the college, both the productivity and mortality rates that are caused by the bio-control agents or predatory predators are investigated. Here, the research will involve field sampling and controlled laboratory observations in order to assess mortality rates, environmental influences, and biological factors affecting life stage transitions. The main factors like temperature, humidity, water quality, predation, and density-dependent effects were examined in order to determine their time of the breath of the mosquito. The purpose of the study is to provide a complex description of the species of this animal and its adaptation patterns to its ecological surroundings. The findings provide useful insights into mosquito population dynamics by facilitating the design of aimed-based vector management strategies to lessen the spreading of mosquito-transmitted diseases.

CHAPTER-1

INTRODUCTION

INTRODUCTION

Mosquitoes are not just the pests; they are the main agents for the transmission of several deadly diseases that have a great impact on the public health of the whole world. The link of their actions and the spreading of the diseases such as malaria, dengue fever, yellow fever, and various forms of encephalitis are the one importance of putting in place the effective or efficient mosquito control measures.

Culex quinquefasciatus, which is also known as the southern house mosquito, is an intermediate-sized brown mosquito whose habitat is mostly in tropical and subtropical regions. The species' place of origin is uncertain. It may have been native to the lowlands of West Africa, or to Southeast Asia. *Culex quinquefasciatus* is now found throughout subtropical and tropical areas worldwide, including the America, Australia and New Zealand, except for exceedingly dry or cold regions.

This species is the primary transmitter of a great many pathogens that make humans sick with the diseases like St. Louis encephalitis, West Nile virus, and lymphatic filariasis.

The taxonomic status of the southern house mosquito, *Culex quinquefasciatus* (Say, 1823) is as follows:

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Order: Diptera

Family: Culicidae

Genus: *Culex*

Species: *quinquefasciatus*

Scientific name: *Culex quinquefasciatus* (Say, 1823)

In the US Southern States, it is the leading vector of the St. Louis encephalitis virus, and in India and Southeast Asia, it is the primary vector of *Wuchereria bancrofti*, a nematode causing lymphatic filariasis.

Culex quinquefasciatus are commonly associated with both domestic and peridomestic habitats. Typical habitats are organically rich water including sewers, groundpools, ditches, agriculture seepage pits, and sewage treatment plant holding tanks.

Adults: The adult *C. quinquefasciatus* is a medium-sized mosquito and is brown in colour. The body is about 3.96 to 4.25 mm long. While the main body is brown, the proboscis, thorax, wings, and tarsi are darker than the rest of the body. The head is light brown, with the lightest portion in the center. The antennae and the proboscis are about the same length, but in some cases, the antennae are slightly shorter than the proboscis. The abdomen has pale, narrow, rounded bands on the basal side of each tergite. Males can be differentiated from females in having large palps and feathery antennae. Male have no proboscis while female have needle like proboscis for biting. male *Culex* mosquitoes feed mostly on plant nectar and sugar sources, while females need a blood meal for egg production. The pupa emerges into adults within 1-2 days. The adult rest on the surface of the water till their body dry and hardens.



Plate 1. Adult stage of *Culex quinquefasciatus*

Egg: The egg stage of *Culex* mosquitoes is a pivotal life cycle that features specific oviposition methods and impressive resistance to environmental changes. Female *Culex* mosquitoes display very specific oviposition behaviours, which involve laying eggs in characteristic floating rafts at the surface of standing water. These egg rafts usually comprise 100 to 300 eggs, although this number may differ with species and environmental conditions (Crans & McNelly, 1997).

It is an engineering wonder in nature the way *Culex* eggs are aligned vertically within rafts that float because of surface tension held together by a gelatinous substance. There are several adaptive reasons for forming such rafts: protection from desiccation of individual eggs,

enhancement of survival chances as eggs are held together, and oxygen exchange to be efficiently distributed for developing embryos.

Culex mosquitoes are very particular about their choice of oviposition sites. This greatly affects the survival rate of eggs and larvae. Several species of Culex have developed a liking for certain kinds of aquatic environments. For example, *Culex quinquefasciatus* is known to have a preference for anaerobic water habitats, often choosing polluted water bodies rich in organic content. This preference is thought to be an adaptive strategy for reducing predation risks because most aquatic predators shun such habitats (Suman *et al.*, 2011).



Plate 2. Egg stage of *Culex quinquefasciatus*

Larva: Emerging from the egg rafts following hatching, Culex larvae, called "wigglers," promptly start their life in water. This stage may last from 5 to 23.5 days, with a wide variability depending on the environmental conditions of temperature and resources.

These aquatic larvae are well adapted to their environment. They have structures that are specialized for feeding and respiration. They filter-feed on microorganisms, algae, and organic detritus suspended in the water column using brush-like mouthparts. For respiration, Culex larvae use a special adaptation in the form of a siphon located at the posterior end of their body. With this siphon, they can breathe atmospheric oxygen while submerged, a feature of behavior commonly observed when larvae hang upside down from the water surface (Clements, 1992).

Temperature fluctuations also affect larval development. Diel temperature ranges significantly enhance larval growth compared to constant temperatures. In the light of this, environmental factors like temperature and habitat quality showed a strong sensitivity during larval

development, which would mean a possible impact of climate change and urbanization on the future population dynamics of *Culex*.



Plate 3. Larvae stage of *Culex quinquefasciatus*

Pupa: The pupal stage is considered a crucial transitional phase of the *Culex* mosquito's development, representing a period from an aquatic larval phase to the terrestrial adult phase. In the pupal stage, such extensive morphological and physiological transformations are seen during mosquito metamorphosis. These larvae are generally referred to as "tumblers" since their movement is characteristically within the water body as they develop under various types of aquatic settings while preparing to emerge as adults.

Culex pupae are non-feeding and entirely depend on their energy reserves for the transformation, which they acquired during the larval stage. Despite being non-feeding, pupae are very mobile and quite sensitive to environmental stimuli. Their comma bodies are streamlined for quick motion through water; they can dive or tumble out of the way of a threat. This tumbling is very significant for avoiding predators, and this aspect is considered as an evolutionary adaptation that has resulted in improved survival rates in deeper water bodies (Clements, 2000).

Specialized structures, known as respiratory trumpets, facilitate respiration in the *Culex* pupae. The trumpet-shaped appendages of the cephalothorax enable the pupa to acquire atmospheric oxygen even though it is submerged. Pupae rise to the surface periodically and break the surface tension using their trumpets to refill their air supply. This respiration mechanism makes *Culex*

pupae highly adaptable to any kind of aquatic environment, which varies in its level of dissolved oxygen.

Environmental factors, especially temperature, are closely associated with pupal development and survival. Percentage of successful pupation greatly differs with varying temperature regimes. Temperature variability is crucial for the regulation of *Culex* population dynamics and imply that climate change would significantly affect the pattern of development of mosquitoes (Guidzavaï *et al.*, 2021).

Pupae of *Culex* are well adapted to polluted water environments. Such a tolerance for bad water quality has allowed colonizers like *Culex* mosquitoes to gain a foothold in a wide variety of habitats, including areas around urban and industrial development where water pollution is ubiquitous. Such an adaptation makes it difficult for vector control measures to succeed against *Culex* mosquitoes in human-altered environments (Crans and McNelly 1997). The pupal stage of *Culex* mosquitoes is essential for the development of effective vector control strategies.



Plate 4. Pupa stage of *Culex quinquefasciatus*

These complete four-stage development (egg, larva, pupa, adult) influenced by various degree of environmental elements and species-dependent adaptation.

If areas which are prone to *Culex quinquefasciatus* breeding are to be controlled, it is important to fully understand the life cycle of *Culex quinquefasciatus*. The cycle of life of this species consists of four developmental phases: the egg, the larva, the pupa, and the adult. The females lay the eggs at the water surface, either fresh or stagnant water, every raft bears from 100 to 300 eggs. The "wigglers" which refer to the larvae, are actually the ones that get hatched from the eggs and spend their days in the water, feeding on organic material. These instars four of larval stage are within five to eight days, as time goes by the environmental conditions dictate.

After the larval period, pupae were developed, from where adult mosquitoes emerged after about 36 hours at 27°C. The entire life cycle is often completed in just seven days when the temperature and humidity are the most ideal.

It can be seen that the rates of thriving of *Culex quinquefasciatus* at different life stages are different, and it depends on environmental factors and geographic location. Environmental conditions have associated with significant variations in both adult and immature stages characteristics of insect, including larval growth rate, developmental times, body size, fecundity, and longevity (Shelton 1973, Loetti et al., 2011). Temperature is a particularly important abiotic factor for mosquito and other arthropods, as it directly affects mortality, lifespan, and development rates that can cause changes in morphology (Su and Mulla 2001, Debat et al. 2003, Gunay et al. 2011). The majority of insect function optimally within a narrow range of temperatures, and deviations from this range can cause stress during development, which may result in developmental inconsistencies (Mpho et al. 2002). In addition, there are often trade-offs between developmental time and both adult size and fitness, which have significant downstream effects on survival, feeding behaviour, and fecundity (Shelton 1973, Mohammed and Chadee 2011). Alterations to these life history traits can lead to substantial variations in vectorial capacity of mosquito that harbour and transmit pathogens (Dye 1992, Delatte et al. 2009)

Climate change and environmental changes have profound effects on mosquito population dynamics and the transmission of vector-borne diseases. Changes in temperature may speed up mosquito development but may also increase mortality at extreme levels.

CHAPTER-2

OBJECTIVE

OBJECTIVE

This project aimed to evaluate the survival rates of different life stages of Culex species of larvae, pupae, and adults by analysing their response to ambient temperature variations. Besides this mosquito sex ratio (male : female) and their mean development time was also evaluated which provides a insights into their growth dynamics.

CHAPTER-3
REVIEW OF LITERATURE

REVIEW OF LITERATURE

Rueda et al., (1990) studied how six constant temperatures—15°C, 20°C, 25°C, 27°C, 30°C, and 34°C—influence the growth, development, and survival of *Culex quinquefasciatus* and *Aedes aegypti*. The researchers applied the Sharpe & DeMichele four-parameter model based on high-temperature inhibition to depict temperature-dependent median developmental rates in both mosquito species. Their findings indicated that an increase in temperature resulted in a general decrease in body size for both species. Head capsule widths at all instars were significantly wider at 15°C compared to higher temperatures (30–34°C), and larval body lengths were also significantly longer at 15°C than at 34°C. Furthermore, all instars, pupae, and adult *Culex quinquefasciatus* were significantly heavier at 15°C compared to those at 27–34°C. Temperature played a crucial role in survival rates. For *Culex quinquefasciatus*, survival to adulthood was highest (85–90%) between 20°C and 30°C but dropped drastically to 38% at 15°C and 42% at 34°C. Similarly, *Aedes aegypti* exhibited the highest survival at 20°C (92%) and 27°C (90%), but survival at 15°C was drastically low, reaching just 3%. These results highlight the complex relationship between temperature and mosquito life history traits. Although lower temperatures (15°C) support larger body size and mass, they significantly reduce survival, particularly for *Aedes aegypti*. This temperature-dependent shift in development and survival is crucial for understanding the ecological dynamics of these species and has significant implications for vector control strategies.

Tahara et al., (2002) conducted a study comparing the egg hatchability, insemination rates, and longevity of Japanese *Culex pipiens pallens* Coquillett and Japanese *Culex quinquefasciatus* Say at 25°C and 30°C. Their findings revealed that *Cx. p. pallens* exhibited high egg hatchability at 25°C, but this dropped significantly at 30°C due to the near absence of inseminated females. In contrast, *Culex quinquefasciatus* maintained high egg hatchability and insemination rates even at 30°C. Furthermore, the longevity of adult females and males was generally shorter in *Cx. p. pallens* compared to *Culex quinquefasciatus* at both temperatures. These results suggest that high temperatures may limit the distribution of *Cx. p. pallens*, making its establishment in Okinawa highly unlikely even if it were to spread to the region.

Krishnamoorthy et al., (2004) studied *Culex quinquefasciatus* survival and *Wuchereria bancrofti* infection in 2,187 mosquitoes fed on 69 human volunteers (59 infected, 10 uninfected). Mortality was higher in mosquitoes feeding on microfilaraemic individuals (50%) than amicrofilaraemics (29%). Mosquitoes dying before 13 days post-feeding had higher

parasite loads, with 45% carrying 10 larvae, while survivors had fewer (2.2 larvae on average). Infection rates varied with human microfilarial density, reaching 90% in high-density cases. Mosquitoes feeding on infected hosts had 11–15 times higher mortality risk, with death odds doubling for every 60–70 parasites. These findings highlight parasite-induced mortality, reducing transmission, and inform lymphatic filariasis control strategies.

Liu et al., (2005) stated that mosquito-borne infectious diseases are a significant global health concern, accounting for millions of cases annually. Among various mosquito species, *Culex quinquefasciatus*, commonly known as the southern house mosquito, is a primary vector of several debilitating diseases. Notably, Krishnamoorthy et al. (2004) identified it as a major transmitter of lymphatic filariasis, a parasitic infection that leads to irreversible and permanent disability. Additionally, Swain et al. (2008) implicated *Culex quinquefasciatus* in the spread of the West Nile virus, which can cause severe neurological diseases in humans. Given the adaptability and widespread distribution of this mosquito species, Okuda (2007) emphasized the importance of understanding its biology and ecology to develop effective and sustainable vector control strategies.

Liu et al., (2005) stated that this study aims to assess the survival rates of *Culex quinquefasciatus* across different life stages within a university campus environment. By conducting a comprehensive analysis from egg to adult, this research seeks to identify key factors influencing mosquito survivability in this specific setting. The findings will contribute to a deeper understanding of local mosquito ecology and support the development of more effective and context-specific vector control strategies. Ultimately, this study aspires to reduce the risk of mosquito-borne diseases for students, residents, and surrounding urban communities.

Liu et al., (2005) examined permethrin resistance in *Culex quinquefasciatus* from Mobile (MAMCqG0) and Huntsville (HAMCqG0), Alabama. Resistance increased significantly after one generation in Mobile (MAMCqG1) and three generations in Huntsville (HAMCqG3). As a major vector of lymphatic filariasis and West Nile virus, *C. quinquefasciatus* thrives in diverse environments, yet its ecology on university campuses remains understudied. Local climate, human activity, and landforms influence mosquito survival, requiring context-specific vector control. This study investigates *C. quinquefasciatus* survival across life stages in a university setting to identify key ecological factors. Findings will enhance targeted control strategies, reducing disease risks for students and urban communities.

Okuda (2007) investigated haematophagy in insects like mosquitoes, bedbugs, and fleas, highlighting adaptations for blood detection, ingestion, and digestion. In *Culex quinquefasciatus*, blood meals trigger midgut epithelial apoptosis, with regenerative cells replacing lost digestive cells. However, after multiple feedings, regeneration declines, reducing longevity, reproduction, and vectorial capacity. To prevent overdistension, haematophagous insects use abdominal stretch receptors and initiate rapid diuresis, expelling up to 40% of the meal. Blood digestion releases cytotoxic haem, managed through species-specific mechanisms: *Rhodnius prolixus* crystallizes haemozoin, *Anopheles darlingi* forms haem aggregates, and *Aedes aegypti* utilizes ferritin. *Culex quinquefasciatus* primarily relies on apoptosis, but limited regeneration after repeated feedings may hinder survival and disease transmission, highlighting physiological constraints in vector ecology.

Swain et al., (2008) studied temperature effects on *Culex quinquefasciatus* strains with and without malathion resistance under laboratory conditions. Fourth-stage larvae were exposed to heat stress (37°C–41°C), revealing that resistant larvae had higher heat tolerance, with LT50 values confirming greater survival. Thermal stress significantly affected mortality rates, pupation, and adult emergence. Heat-exposed larvae showed increased pupation rates but delayed metamorphosis, and adult emergence was lower. Malathion-resistant females exhibited longer lifespans post-heat exposure, while susceptible females had reduced longevity, suggesting a thermal advantage for resistance. These findings imply that climate change may favor resistant mosquito populations, complicating vector control. The study underscores the need for adaptive strategies in Integrated Vector Management (IVM) that consider both insecticide resistance and environmental stressors to develop sustainable mosquito control programs.

Atkinson et al., (2010) conducted a genomic analysis of *Culex quinquefasciatus*, a key vector of West Nile virus and lymphatic filariasis, revealing extensive genetic adaptations. The mosquito's genome, with 18,883 protein-coding genes, is significantly larger than those of *Aedes aegypti* and *Anopheles gambiae*, primarily due to expansions in sensory and detoxification-related genes. It has the largest olfactory receptor repertoire among dipterans (180 genes) and an expanded gustatory receptor family, enhancing host-seeking and oviposition. The study also identified a substantial expansion of cytochrome P450s and glutathione transferases, linked to insecticide resistance and adaptation to polluted habitats. These findings highlight *C. quinquefasciatus*'s resilience and the genetic mechanisms driving its widespread distribution, emphasizing the challenges in vector control.

Gokhale (2013) studied *Culex quinquefasciatus* mosquitoes from Gorakhpur (endemic for lymphatic filariasis) and Pune (nonendemic) to assess environmental influences on life history traits and insecticide susceptibility. Life table analysis revealed significant differences in development time, survival rates, and fecundity, indicating local adaptations. Morphological distinctions, such as siphon structure, saddle, anal gills, and pecten teeth count, further differentiated the populations. Principal component analysis confirmed these variations. Pesticide sensitivity tests showed larvae from both locations were most susceptible to deltamethrin, suggesting a potential target for vector control. The study highlights the role of ecological factors in mosquito adaptation, morphology, and insecticide response, providing crucial insights for disease control strategies. These findings emphasize the need for location-specific interventions to combat vector-borne diseases like lymphatic filariasis more effectively.

Chandel et al., (2013) investigated the midgut microbial communities of *Culex quinquefasciatus* mosquitoes collected from various regions across India. Utilizing 16S ribosomal DNA amplicon sequencing, the researchers identified 83 bacterial species spanning 31 genera, all belonging to the phyla Proteobacteria, Firmicutes, and Actinobacteria. Notably, Proteobacteria was the most dominant phylum, with the genus *Enterobacter* being the most prevalent, detected in nearly all sampling locations except Leh. This study underscores the significant diversity in gut bacteria among individual mosquitoes, suggesting that such variation may influence differences in disease transmission rates or vector competence within mosquito populations. These findings highlight the importance of further research into the role of midgut microbiota in the transmission dynamics of pathogens like West Nile virus and lymphatic filariasis.

Chandel et al., (2013) analyzed the midgut microbiota of wild *Culex quinquefasciatus* mosquitoes across India using 16S rDNA sequencing. They identified 83 bacterial species from 31 genera within Proteobacteria, Firmicutes, and Actinobacteria. Proteobacteria, especially γ -proteobacteria, dominated, with *Staphylococcus* being the most diverse (11 species) and *Enterobacter* the most widespread, absent only in Leh. Bhuj had the highest bacterial diversity (22 species), while Leh had the lowest (8 species). Minimal bacterial overlap among mosquitoes suggested a highly diverse microbiota, potentially affecting vector competence and disease transmission. This was the first large-scale study highlighting the need for further research on microbiota roles in mosquito physiology. These findings provide insights into *Culex quinquefasciatus* adaptation and resistance, emphasizing the need for integrated vector

control. Future research should explore environmental interactions, genetic resistance, and microbiota influences to develop sustainable strategies for reducing disease transmission.

Noori. N, et al., (2015) Carried out an experiment to describe the isolated effects of each nutrient (PO₄, NO₃, NH₄) on pre-adult development. This results showed that breeding sites with higher PO₄ or NO₃ concentrations had higher larval survival rates. High NO₃ concentrations favor the development of male mosquitoes and suppress the development of female mosquitoes, but those adult females that do emerge develop faster in containers with high NO₃ levels compared to the reference group. The addition of PO₄ in the absence of nitrogen sources to the larval habitat slowed larval development, however, it took fewer days for larvae to reach the pupal stage in containers with combinations of NO₃ and PO₄ or NH₄ and PO₄ nutrients.

Samy, A.M. et al., (2016) examined the potential global distribution of *Culex quiquefasciatus* under current and future climate conditions. Researchers found that the species currently thrives in low-latitude regions, with suitable habitats in parts of North Africa and Western Europe. Future projections suggest a similar distribution but with increased suitability in southern Australia. The species' stability was highest between 30°S and 30°N, though model predictions varied for North and Central Africa, southern Asia, central USA, and southeastern Europe. Disparities in predictions were most notable in Saudi Arabia and Europe. These findings help anticipate future shifts in mosquito distribution due to climate change.

Yousafzai et al., (2020) investigated the effects of these heavy metals (Cu,Pb,Cd)on the development of *Cx. quinquefasciatus* at concentrations set by Pakistan Environmental Protection Agency (Pak-EPA) The 2nd instar larvae of *Cx. quinquefasciatus* were exposed to different concentrations of Pb, Cd and Cu and their effects on oviposition preference, egg hatching rate and larval development were studied. The LC₅₀ values of Pb, Cd and Cu were 12.6, 6.3 and 2.6 ppm, respectively. Gravid female mosquito adults deposited a significantly lower number of egg rafts in containers containing 0.50 ppm Pb or 1.0 ppm Cu in water. Each of the heavy metals in water resulted in significantly ($p < 0.05$) lower egg hatching rate, prolonged time to pupation, lower pupation rate, prolonged time to adult emergence, lower adult emergence rate and higher female to male ratio. It is concluded that the 2nd instar larvae of *Cx. quinquefasciatus* are susceptible to Pak-EPA permissible levels of Pb, Cd and Cu in municipal and liquid industrial effluents.

Bell, M. J. (2020), examined that the impact of latrine construction on synanthropic fly populations, bacterial transmission, and *Culex quinquefasciatus* densities in relation to diarrhoeal disease and lymphatic filariasis (LF). Despite assumptions that improved sanitation reduces disease vectors, results showed no significant reduction in fly or mosquito densities between intervention and control groups. However, during the monsoon season, 40% fewer flies were caught in intervention areas. Most flies belonged to the Muscidae family, with 60.3% carrying pathogens like *E. coli*, *V. cholerae*, *Salmonella*, or *Shigella*. *C. quinquefasciatus* was rarely infected with *Wuchereria bancrofti* (LF prevalence: 0.0034%). While latrines alone did not significantly impact vector populations or pathogen transmission, reducing open defecation remains crucial. Effective public health improvement requires both latrine coverage and behavior change to limit bacterial spread.

Villena, O.C. et al., (2024) examined the environmental and geographical factors influencing the occurrence and abundance of *Culex quinquefasciatus* in Hawai'i, a key vector of avian malaria threatening native Hawaiian honeycreepers. Researchers analyzed factors such as temperature, precipitation, elevation, site location, and trap type using statistical models. The results showed that mosquito occurrence was highest at mid-elevations, peaking between July and November, and increased with temperature and precipitation up to 580 mm. Mosquito abundance also peaked at mid-elevations, with the highest numbers recorded between August and October. Understanding these factors is crucial for mosquito control efforts to reduce the risk of avian malaria and protect endangered bird species.

Krishnakanth et al., (2024) examined how factors like NaCl concentration, ovitrap color, water source, and ovitrap size influenced egg-laying preferences. Results showed that egg-laying decreased with higher NaCl concentrations, and black ovitraps were the most preferred. Among different water sources, water from paddy fields was the most favored for oviposition.

Modak et al., (2024) found that *Culex quinquefasciatus* populations in the sub-Himalayan region of West Bengal are highly resistant to synthetic pyrethroids. This resistance is primarily due to the presence of the L1014F *kdr* mutation in the *vgsc* gene. The findings highlight the importance of understanding insecticide resistance mechanisms to improve vector control strategies and minimize errors in implementation.

Nayak et al., (2018) investigates mosquito species, particularly *Culex quinquefasciatus*, in Ganjam district, Odisha, focusing on their oviposition preferences. The research showed that *Culex quinquefasciatus* was abundant in the area. Laboratory tests revealed that gravid female

mosquitoes preferred ovipositing in black ovitraps, and their egg-laying decreased as the concentration of NaCl increased. Water from paddy fields was the most preferred among various water sources, and mosquitoes laid more eggs in larva-holding water than in distilled water. Additionally, larger ovitraps saw more egg rafts deposited. These findings help understand mosquito behavior, which could inform vector control strategies.

Acharya et al., (2018) evaluated the impact of thermal stress on the survival and development of cross-tolerance to *Bacillus thuringiensis israelensis* (Bti) toxins in *Culex quinquefasciatus* larvae. Larvae were exposed to various temperatures (39°C to 45°C) and survival times were recorded. All larvae survived 60 minutes at 39°C, while higher temperatures led to progressively shorter survival times, with complete mortality observed at 45°C after 10 minutes. The larvae were then treated with Bti at different concentrations, showing increased mortality with higher Bti concentrations, from 5% mortality at 0.5 ppm to 100% at 3.0 ppm. To assess cross-tolerance, larvae pre-adapted to 39°C for 60, 90, and 120 minutes were exposed to Bti solutions (1.0 ppm and 1.5 ppm). The pre-adapted larvae showed significantly lower mortality compared to control larvae, indicating that thermal stress contributes to the development of cross-tolerance to Bti toxins in wild *Culex quinquefasciatus*.

CHAPTER-4
MATERIALS AND METHODOLOGY

MATERIALS AND METHODOLOGY

4.1. Site of study



Fig 1. Nayagarh Autonomous College, Nayagarh, Odisha, India 752069

Latitude - 20.114637° Longitude -85.100507°

Materials

4.2. The following materials were used in the study on the survivability rates of various life stages of *Culex quinquefasciatus* mosquitoes collected from the campus:

- Mosquito larvae
- Standard dipper (350ml)
- Dropper
- Plastic containers
- Rearing trays
- Mosquito Cage (made up of wood & mesh)
- Feeding material: Fish food, 10% sucrose solution
- Cotton
- Data recording: Note book, Data Sheet
- Petri dish

Methodology

4.3.1 Collection of Mosquito Larvae

The *Culex quinquefasciatus* mosquitoes used in this experiment were initially collected as larvae from an abandoned concrete tank, a potential breeding sites within the Nayagarh Autonomous College campus. The tank contains stagnant water that facilitated mosquito breeding. Larvae were collected using a standard 350 ml dipper and carefully transferred to plastic containers. They were then transported to the Zoology Department's research laboratory at Nayagarh Autonomous College for rearing.



Plate 5. Standard mosquito Dipper

4.3.2 Rearing of Mosquitoes in Laboratory Conditions

In the laboratory, the larvae were transferred into rectangular plastic containers. Each developmental stage (*i.e.*, L1-L2, L3-L4) and pupae were identified based on size and morphological characteristics and carefully transferred using larval dropper into separated designated containers. During transfer their numbers are accurately counted and noted. To ensure proper nutrition and optimal growth, the larvae were fed fish food. Daily observations were conducted to monitor developmental progress and record mortality rates systematically.



Plate 6. Larval Dropper



Plate 7. Fish food applied to larva

4.3.3 Pupal Stage Observation

Once the larvae reached the pupal stage, they were carefully transferred to a mosquito cage made of fine mesh and wood to prevent the emerged adults from escaping. The number of pupae was recorded daily to monitor the larval-to-pupal conversion rate. To ensure successful adult emergence, the pupae were maintained under controlled conditions.



Plate 8. Mosquito Cage



Plate 9. Pupal transfer into the cage

4.3.4 Adult Emergence and Gender Identification

After pupal transformation, the number of successfully emerged adult mosquitoes was recorded. To ensure proper nutrition, they were provided with a 10% sucrose solution absorbed in a cotton swab and placed in a petri dish inside the mosquito cage. Mortality during

emergence was documented to evaluate survival rates. The adult mosquitoes were maintained under controlled conditions, and their activity and feeding behaviour were monitored regularly.



Plate 10. Cotton swab soaked with sucrose solution

4.3.5 Daily Observations and Data Collection

The survivability of mosquitoes was evaluated by monitoring the developmental progression from larvae to pupae and subsequently to adults. The number of larvae that failed to reach the pupal or adult stage was documented to determine mortality rates at each stage. The proportion of male and female mosquitoes was calculated to assess population dynamics, providing insights into sex-based survival differences and potential reproductive trends.

CHAPTER-5

RESULT AND DISCUSSION

RESULT AND DISCUSSION

Developmental progression and survival analysis of mosquito larvae

Significant differences in survival and developmental time were observed across different life stages of *Culex quinquefasciatus*. The survival rate varied between larval instars, with the highest mortality recorded in the early stages (L1-L2), while pupal and adult emergence rates remained relatively high. The transition from larvae to pupae showed a notable decline in numbers, with only a fraction of individuals successfully reaching the adult stage. Environmental conditions, competition for resources, and rearing practices played a crucial role in influencing survival percentages.

Table 1 presents the daily record of larvae, pupae, and adult numbers over the observation period. The initial larval population (L1-L2) started at 96 individuals on day 1, but a sharp decline was observed in the following days. By day 4, the L1-L2 stage had reduced to only 5 individuals, with a corresponding increase in the L3-L4 population. The survival rate of early instars was considerably lower compared to the later stages, suggesting that the highest mortality occurred during the transition from L1-L2 to L3-L4.

By day 5, the number of L1-L2 larvae had completely diminished, with only four individuals remaining in the L3-L4 stage. The pupal population increased slightly, indicating that a small proportion of larvae successfully reached the pupal stage. However, only a limited number of pupae transitioned into adults. The emergence of adults began on day 2, with a single mosquito reaching adulthood. The highest adult emergence was recorded on day 7, where five individuals successfully completed metamorphosis.

A steady decline in population was evident throughout the observation period, as shown in Table 1. The **cumulative survival rate** from larvae to adulthood remained low, with only a small fraction of the original larval population reaching maturity. The **bottleneck effect** was particularly evident in the early larval stages, suggesting that external factors such as nutrition availability, temperature, and larval density may have influenced survival outcomes.

The mortality pattern observed indicates that the early larval stages are highly susceptible to environmental stressors, while later developmental stages, particularly pupae, exhibited better survival. This aligns with previous findings on *Culex quinquefasciatus*, where similar trends in survival and mortality have been reported. Understanding these variations in developmental

success is crucial for vector control strategies, as interventions targeting early-stage larvae could significantly impact overall mosquito populations.

The Result of the experiment are tabulated below.

Table: 1(Summery of all the larva, pupa and adult data recorded)

Day	Time	L1-L2 Stage	L3-L4 Stage	Pupa	Adult
Day 1	10:00 am	96	38	3	0
Day 2	10:00 am	79	37	1	1
Day 3	10:00 am	25	24	4	2
Day 4	10:00 am	5	19	8	3
Day 5	10:00 am	0	4	4	5
Day 6	10:00 am	0	3	0	4
Day 7	10:00 am	0	1	2	5
Day 8	10:00 am	0	0	0	1
Day 9	10:00 am	0	0	0	1

The data illustrate a significant decline in early instar survival, with a progressive increase in pupal and adult emergence. The highest mortality was observed in the L1-L2 stage, while the survival rate stabilized in later stages. These trends indicate that environmental and rearing conditions played a crucial role in larval development and overall survival. The total number of successfully emerged adults recorded was 22, highlighting the overall survival percentage relative to the initial larval count.

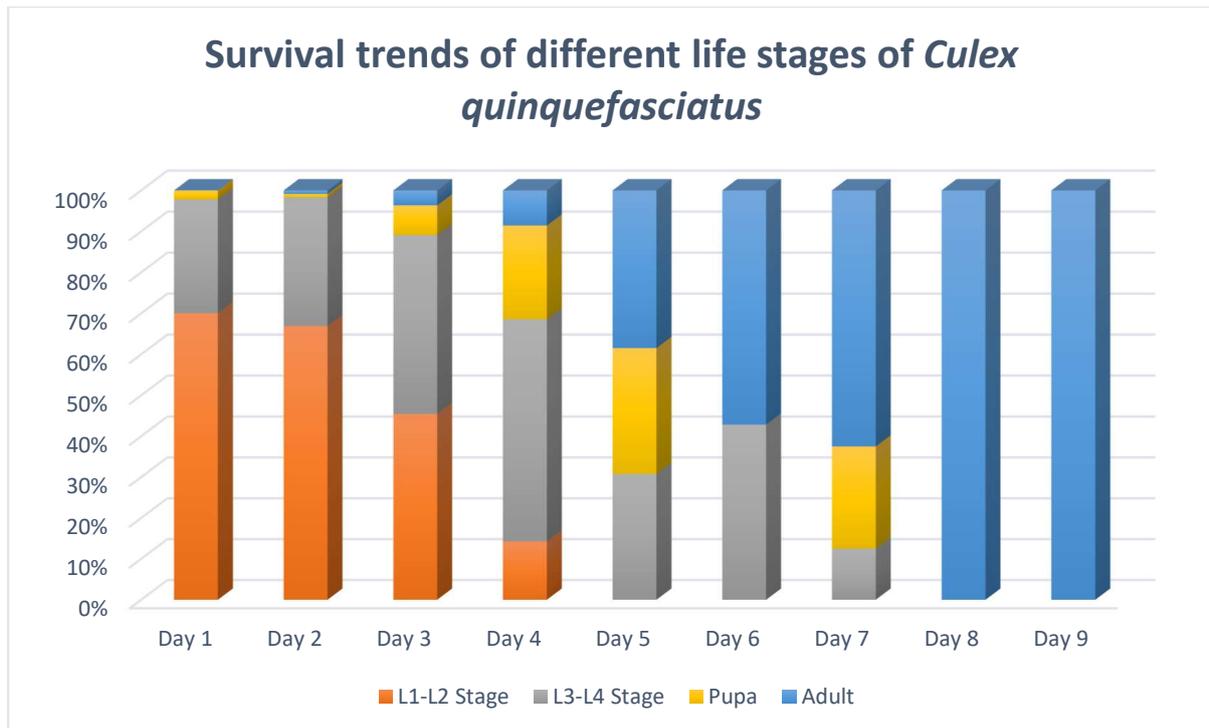


Fig 2. Survival trends of *Culex quinquefasciatus* from the larval (L1-L2, L3-L4) to the adult stage over time.

Mean Developmental Duration and Sex Ratio Analysis

The mean developmental time from the first larval instar (L1) to adult emergence exhibited variability across different life stages (Table 2). The early instars (L1-L2) requiring an average of 96 hours (4days × 24hours), late instars (L3-L4) taking 168 hours (7days × 24hours), and the pupal stage lasting 144 hours (6days × 24hours). The total duration for complete development was recorded as 9 days is 216 hours (9days × 24hours), the actual time usually takes to develop into an adult. The pupal stage was significantly shorter compared to the larval instars, allowing for a more rapid transition to adulthood.

Table 2: Mean developmental time of different life stages of *Culex quinquefasciatus*.

The complete development from the L1 stage to adult emergence required the following time durations.

Stage	Time (Hours)
L1-L2 Stage	96 hours
L3-L4 Stage	168 hours
Pupa	144 hours
Total Development Time	9 days (216 hours)

Sex ratio analysis of the emerged adults revealed a noticeable difference in male and female proportions (Table 3). Out of 22 emerged adults, 9 were males and 13 were females, resulting in a male-to-female ratio of 0.40:0.59. Sex differentiation was based on morphological characteristics, primarily antennal structure and body size. Statistical analysis showed a significant variation in sex distribution, with females comprising a larger proportion of the adult population.

Table 3: Ratio of total number of emerged adult (male and female) mosquitoes,

Category	Count	Ratio
Male	9	0.40 (40%)
Female	13	0.59 (59%)
Total	22	100%

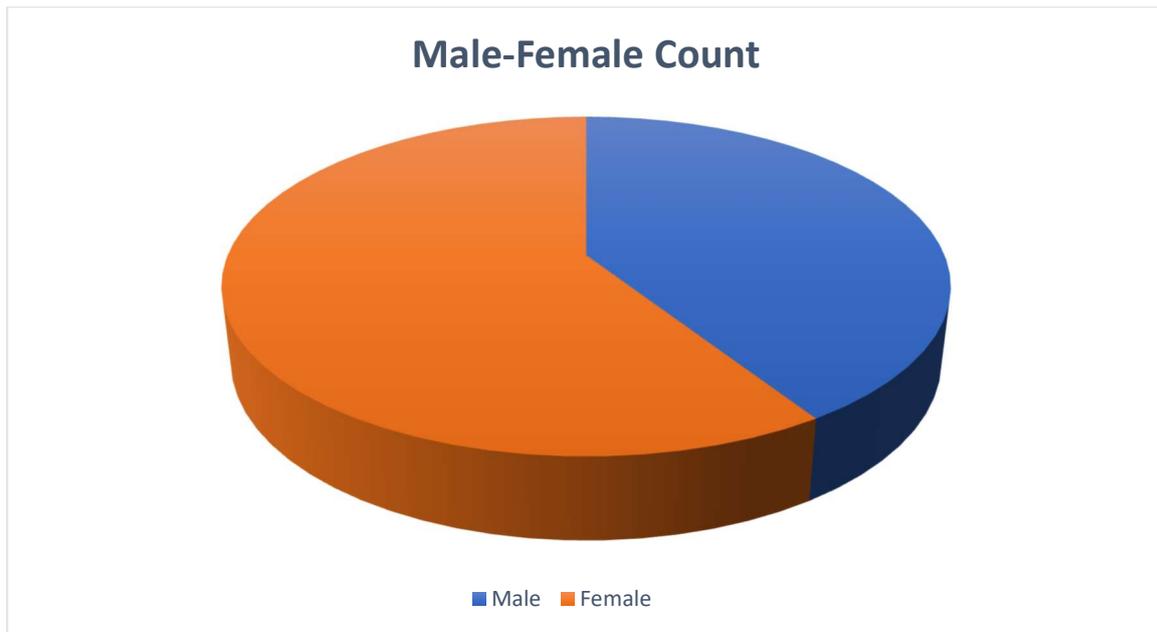


Fig 3. Percentage of emerged adult male and female.

Survival Rates and Developmental Trends

The survival rates at different life stages are presented in tabulation 4, illustrating a progressive decline in population across developmental stages. The highest mortality was observed during the L1-L2 stage, while pupal survival remained at 100%, indicating a significant drop in survivability during the early larval phases. Several external factors, including nutrition, larval density, and environmental conditions, contributed to these variations.

The mean survival percentage across different life stages showed a gradual decline, with an overall transformation rate of 16.05% from larvae to adulthood (Table 4). The survivability rate from early instars (L1-L2) to late instars (L3-L4) was calculated as $(38/96) \times 100 = 39.58\%$, whereas the transition from late instars (L3-L4) to pupa resulted in a survival rate of $(22/38) \times 100 = 57.89\%$. Notably, the pupal stage exhibited a 100% survival rate ($22/22 \times 100$). These findings are consistent with previous studies on *Culex quinquefasciatus*, reinforcing the critical role of environmental conditions in determining survivability rate.

Statistical analysis revealed significant differences in survival rates and developmental times across different life stages, indicating a strong correlation between growth progression and environmental stability. The observed developmental trends suggest that factors such as larval density, feeding frequency, and temperature regulation play a crucial role in shaping the life cycle dynamics of *Culex quinquefasciatus*.

The low overall survivability rate of 16.05% ($22/137 \times 100$) is primarily attributed to low temperature conditions during the larval stages, which negatively impacted early-stage survival.

The survival rates across different life stages, showing a decline from L1 to adulthood. The highest mortality occurred in early instars, while pupal survival reached 100%.

Table 4: Survival rates of different life stages of *Culex quinquefasciatus*

Life Stage	Initial Count	Survived Count	Survival Rate (%)
L1-L2	96	38	39.58
L2-L3	38	22	57.89
Pupa	22	22	100.00
Total	137	22	16.05

The Total survival rate was 16.05%, influenced by environmental factors.

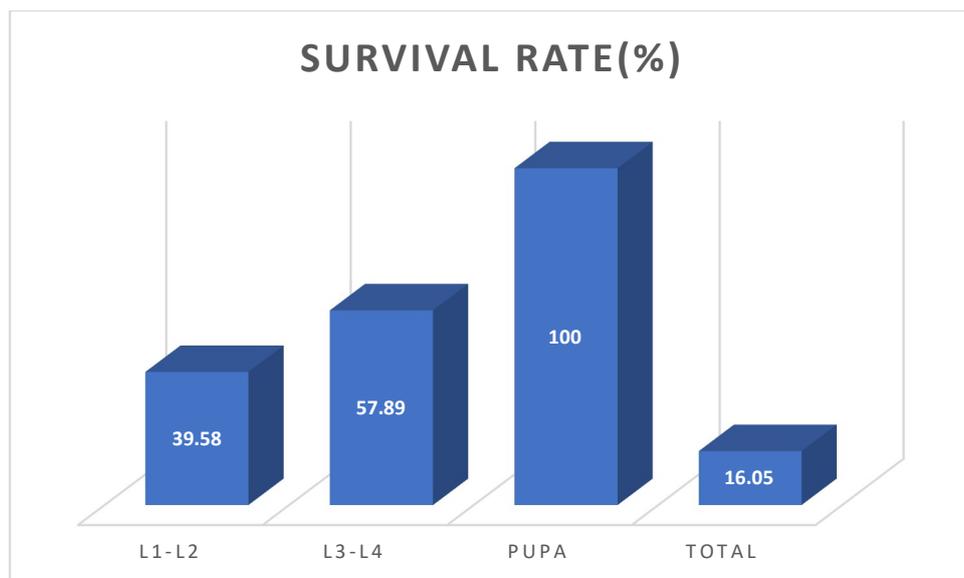


Fig 4: Survival rate distribution across different life stages of *Culex quinquefasciatus*.

These results highlight the critical role of environmental factors in shaping mosquito life cycle dynamics, emphasizing the need to account for seasonal variations when developing vector control strategies.

CHAPTER-6

CONCLUSION

CONCLUSION

The findings of this study demonstrate that the survival patterns and developmental variations observed across different life stages of *Culex quinquefasciatus* were significantly influenced by the prevailing cold climatic conditions during the experimental period. Mosquito development requires an optimal temperature range of approximately 25-30°C for efficient growth and survival. However, during the study, the temperature was lower, coupled with reduced humidity, as commonly observed in winter seasons. These suboptimal conditions led to higher mortality in early larval instars, prolonged developmental duration, and a reduced overall transformation rate from larvae to adults. Additionally, the absence of a temperature-regulated chamber further exacerbated these effects, as fluctuating environmental factors directly impacted larval survivability and developmental progression. These results highlight the critical role of temperature and humidity in shaping mosquito life cycle dynamics, emphasizing the need to account for seasonal variations when developing vector control strategies.

CHAPTER-7

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