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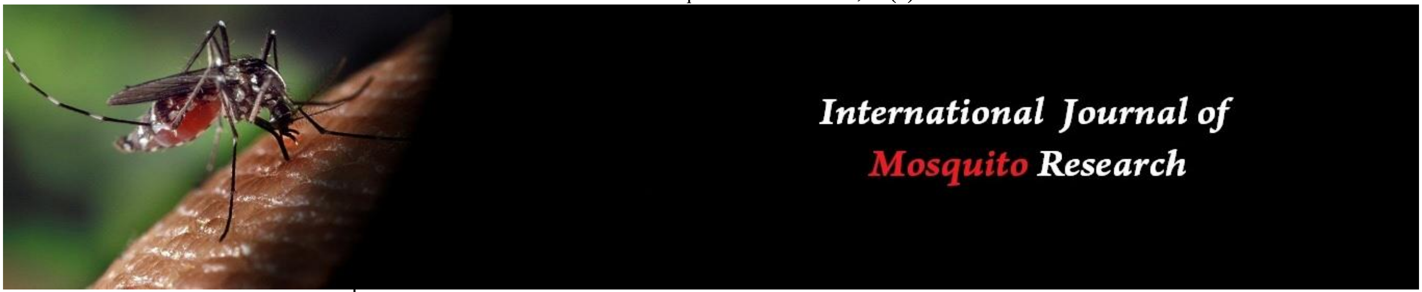

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Unveiling the underwater story: The secret lives of *Aedes* mosquito larvae

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Abstract
Mosquitoes (Diptera: Culicidae) are prominent vectors for the transmission of various life-threatening diseases, including dengue fever, chikungunya, Zika virus, malaria, filariasis, and yellow fever. *Aedes* mosquito-borne diseases (ABDs), such as Zika, dengue, chikungunya, and yellow fever, have become significant global public health issues in recent years. Annually, mosquito-borne diseases impact around 700 million individuals and result in over one million fatalities globally. Despite extensive research on the behaviour and vectorial capacity of adult *Aedes* mosquitoes, a significant information silence persists about their larval stages. This review seeks to deliver a thorough examination of *Aedes* larval life, analysing their developmental stages, the environmental and biological elements affecting their growth, and the consequences of larval biology for adult mosquito characteristics and disease transmission potential. Although larvae play a crucial role in the mosquito life cycle, few research has thoroughly examined these initial stages, and there is currently no comprehensive review on *Aedes* larval behaviour and development. This research aims to address that deficiency and facilitate the formulation of targeted mosquito control tactics by pharmacological or genetic interventions designed to impair larval development, hence diminishing the incidence of *Aedes* borne infections.

Keywords: *Aedes*, larval life, mosquito

Introduction
One of the most important vectors for spreading illnesses including dengue fever, chikungunya, zika virus, malaria, filariasis, and yellow fever to vertebrates is the mosquito (Diptera: Culicidae) [1]. Mosquitoes have a detrimental effect on both the surrounding environment and human well-being [2]. *Aedes* mosquito-borne diseases (ABDs), including Zika, dengue, chikungunya, and yellow fever, have grown in importance as a worldwide health concern in recent years [3-5]. Almost 700 million people are afflicted by mosquito-borne illnesses each year, which result in over a million fatalities globally [6]. The most common viral disease spread by *Aedes* mosquitoes among them is dengue [7-9]. Dengue is spread by several species of female *Aedes* mosquitoes. *Ae. albopictus* (Skuse, 1894) often known as the Asian Tiger mosquito, alone has been confirmed to be the prime vector in some of the recorded dengue outbreaks [10]. Additionally, this species has contributed significantly to the global spread of chikungunya [11], and may be connected to the transmission of the Zika virus [12]; it is among the mosquito species that spreads very fast around the world [13]. *Ae. albopictus* is quite anthropophilic, which means it bites people a lot, and it can adapt well to a variety of situations [14, 15]. This species of mosquito may reproduce in a variety of environments, including both man-made containers like abandoned tires and coconut shells and natural places like tree holes and leaf axils. On the other hand, *Aedes aegypti*, sometimes referred to as the forest day-biting mosquito, is a major carrier of urban yellow fever and the primary vector of dengue fever [16, 17]. This species is widely distributed around the world and does well in urban settings.
The dispersion and establishment of *Aedes* mosquito populations depend critically on the selection of suitable breeding sites for egg laying. Key behaviours like as reproduction, aggregation, and offspring production are regulated by chemical signals.

Mosquitoes are attracted to particular smells according on the kind of stimuli they come across. The ability to smell is a crucial sense for female mosquitoes to locate areas that supply the nutrients needed for larval development [18]. Mosquitoes have a detrimental effect on both the surrounding environment and human well-being [2]. Mosquitoes in urban environments frequently develop their immature stages in containers filled with water. Numerous mosquito species choose shaded locations for egg laying [19]. *Aedes aegypti* breeding success is largely dependent on the kind of containers used, the physicochemical characteristics of the water, and the state of these containers. Mosquito survival rates are significantly influenced by the chemical properties of aquatic environments [19]. A few examples of mosquito oviposition signals are water temperature, pH, and the presence of compounds such as phosphates, ammonia, nitrates, sulphates, and dissolved solids [20, 21]. Mosquito larvae consume debris and related bacteria to obtain nutrition as they go through four instar stages. The physicochemical qualities of the water, the availability of nutrients, and the environmental factors at breeding sites all have an impact on the larval development of mosquitoes, which in turn affects their health and vectorial capacity (VC). Because the fitness of adult mosquitoes depends on their larval stages, water pollution has a deleterious impact on the aquatic insect ecology, especially on insects that are important in medical point of view. The dynamics of mosquito-borne illness transmission are thus affected by aquatic environment [22-24]. *Aedes* embryos go through a latent phase after development, which allows them to survive for up to six months, depending on relative humidity [25-28]. The larvae break their hibernation and emerge when the environment is right for their development [25, 26, 28]. The regulation of *Aedes* mosquitoes in both their larval and adult stages is greatly influenced by several environmental conditions. These factors have a major effect on the developmental processes of *Aedes* species, according to numerous research. More precisely, warmer temperatures tend to hasten the *Aedes* larvae's growth and shorten their maturity period. The reason for this acceleration is that higher temperatures cause metabolic rates to rise, which promotes quicker growth and development [29-34]. Furthermore, it has been discovered that prolonged exposure to light speeds up the growth of mosquito larvae. The impact of light on physiological processes and behavioural patterns that affect growth rates is thought to be responsible for this effect [34, 35]. The availability of nutrients in aquatic environments is vital, as increased nutrient levels can decrease competition among larvae. This reduction in competition often results in faster growth and shorter development times since larvae experience less stress from limited resources [31, 34, 35]. The nutritional value of the larval diet of mosquitoes influences both their vectorial capacity (VC) and life history features [36]. Moreover, since these dynamics might affect adult body size, it is crucial to comprehend how larval populations are impacted by density dependency and competition. Consequently, this influences the traits of the adult mosquito's life cycle and its capacity to spread illness [37]. Based on empirical data, life history features of mosquito species are influenced by intraspecific and interspecific competition, especially when resources are few [38, 39]. The number and quality of resources in *Ae. albopictus* and *Ae. aegypti*'s environments determine how their larval rivalry plays out [40]. The fitness of adult mosquitoes can also be influenced by the relative density of developing larvae in addition to the quantity and quality of nutrients [41]. The pace of development and general fitness of individual mosquitoes are significantly influenced by the density of developing larvae in a particular location [42, 43]. The growth rate and biomass accumulation of individual mosquitoes can be affected by variations in the density of developing individuals within the same area and resource conditions, which can then result in variances in the per capita resource availability.

This review aims to examine the current understanding of *Aedes* larval life, focusing on the various larval stages, factors influencing their development, and how larval life impacts adult mosquitoes and their vectorial capacity. While considerable knowledge exists about the behavior of adult *Aedes*, there is comparatively limited information regarding their larvae, with only a few research papers addressing different aspects of *Aedes* larvae. During the literature review, it was found no comprehensive review paper specifically focused on *Aedes* larval life or behavior. Therefore, the authors have undertaken the initiative to draft the first review of its kind, detailing the life of *Aedes* larvae in relation to various influencing factors from egg to emergence and how survive against the odds to the best of our knowledge. This review aims to provide readers with an overview of *Aedes* larval secret life and assist in developing future strategies for controlling *Aedes* mosquitoes and *Aedes*-borne diseases (ABDs).

Material and methods: We conducted a comprehensive search for peer-reviewed studies examining the larval behaviors of *Aedes aegypti* and *Aedes albopictus*. The aim of this investigation was to gather extensive data on the characteristics of these two mosquito species. We utilized the terms *Aedes aegypti* and *Aedes albopictus*, along with keywords such as larval biology, bionomics, behavior, habitat, water quality, nutrition, development, factors influencing larval behavior, and larval competition. Our search included the Science directory, PubMed database, and Google. This extensive review aimed to uncover relevant information on various aspects of the biology and life cycles of these larval mosquito species. From the vast body of literature on these species, we chose papers, articles, and reviews written in English that provide thorough details on the behaviour and larval life of *Aedes aegypti* and *Aedes albopictus*. This method made guaranteed that important study results were thoroughly examined. Additionally, we used the references in these selected publications to track down and look for particular data sources, which helped us cross-check generally acknowledged truths that were devoid of supporting data. A systematic search turned up a total of 181 papers discussing the life, behaviour, and development of larvae as well as other aspects like climate, nutrition, habitat quality, competition between larvae, and the function of certain genes in *Aedes aegypti* and *Aedes albopictus*. These articles were part of the review process for further analysis. After being excluded based on (i) language criteria and (ii) title and abstract inspection, 150 articles were left for full-text assessment. After carefully comparing the full-text articles with the exclusion and inclusion criteria, 105 research publications met the inclusion criteria. Based on these 105 papers, the final evaluation assured a comprehensive analysis of pertinent research on the larval lives of *Aedes albopictus* and *Aedes aegypti*, with a focus on *Aedes aegypti*.

Life cycle of *Aedes* mosquito

When a mosquito gets close to water, it uses a variety of senses to determine if the conditions are right for egg laying. These elements include food availability, salinity, dangerous toxin concentrations, and chemical cues given out by other animals. These signals may be used to detect the presence of larvae, pupae, or possible predators nearby [44-49]. It is unclear what physiological mechanisms mosquitoes use to sense water and judge its suitability for raising their young. A mosquito "tastes" the water by dipping its legs and mouthparts into it; this causes sensory neurons to fire and send information to its brain, allowing it to determine which spot is ideal [50]. The precise mechanisms by which mosquitoes distinguish between fresh and saltwater, however, remain unknown. A gene known as ppk301, which is necessary for mosquitoes to successfully lay their eggs in the right kind of water, is one significant component that has been found. The PPK gene family, which is less well studied, is found in insects and encodes receptor proteins linked to chemosensory processes [50-55]. This receptor proteins usually have two transmembrane domains and an extracellular loop with a highly conserved cysteine-rich region [51, 56]. Certain neurons identified in mosquito legs and mouthparts were found to harbour the gene *ppk301*. But even in the absence of *ppk301*, these neurons responded to salt, indicating that other, unknown genes may also play a role in keeping mosquitoes from depositing their eggs in salty water [50].

Mosquitoes go through several stages of growth called holometabolous development, which includes the egg, larva, pupa, and adult stages. Individual eggs are placed on moist surfaces in *Aedes* species [57].

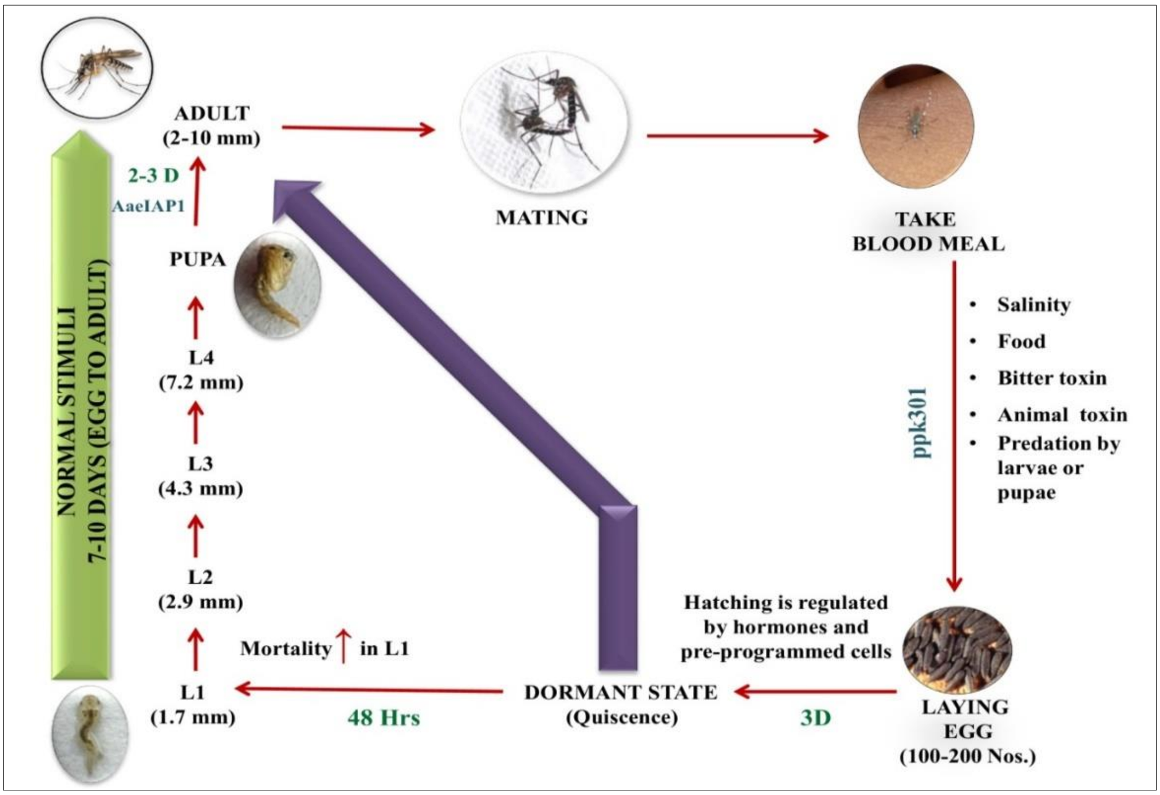

Fig 1: Life Cycle of *Aedes* mosquitoes.

When exposed to water, the majority of eggs hatch into larvae in 48 hours [58]. Notably, *Aedes albopictus* and *Aedes aegypti* eggs can withstand desiccation and endure for up to a year without water [59]. *Aedes* larvae may survive in even the smallest pools of water; they go through four instar stages before becoming pupae [60]. The lifecycle of a mosquito takes about seven to ten days, from egg to adult, with the normal adult emerging two to three days after pupation [61]. Adult female mosquitoes look for an appropriate blood meal after mating, while male mosquitoes do not consume blood. Although *Aedes albopictus* also feeds on humans, it is less selective in its selection compared to *Aedes aegypti*, which has a high preference for feeding on humans [58]. Females finish their life cycle (Figure 1) by producing eggs and depositing them on a damp surface two to three days after feeding [58]. Adult mosquitoes emerge after two to three days of the pupal stage.

Egg: A female mosquito can lay up from 100 to 200 eggs after feeding on blood [62, 63]. These eggs are laid on moist surfaces, frequently at different points in relation to the water [62, 63]. During oviposition, the eggs soon turn black after being initially white [64]. Every egg has a chitin-containing shell to protect it, and throughout development, a specific extracellular matrix called the serosal cuticle forms. The embryo is encased in this structure, which eventually develops into the eggshell [65]. Because of its protective cuticle, the egg is resistant to desiccation (ERD) and can survive for up to a year [66, 67]. Nevertheless, the length of time eggs can survive in dry environments varies depending on the species and the degree of ERD. For example, because of their high ERD, *Ae. aegypti* eggs may survive in arid conditions for several months [65]. The chorion, or outer shell, of the egg, is essential for defence, gas exchange, and reducing water loss [64]. The endochorion and exochorion are the two layers that make up the chorion [68]. The exochorion, which frequently exhibits distinctive ornamentation, is a helpful characteristic for species differentiation [69-71].

Larva: About three days after oviposition, embryonic development is finished, producing fully formed first instar larvae (L1) that are inactive inside the egg's chorion-a condition known as quiescence. Previously known as a pharate first instar diapause, this quiescent period is unique and does not directly correspond to any other phase in insects [72, 73]. In contrast to diapause, which is a hormonally controlled developmental pause lasting a certain amount of time, quiescent larvae hatch as soon as they encounter the right environmental stimulus [74]. Encased in an impermeable chorion, the fertilised mosquito egg is a closed system that depends on maternal lipid stores to finish embryonic development and maintain a quiescent metabolic state. Triglycerides make up more than 90% of the stored lipids in the egg and are probably essential for the synthesis of energy when first instars larvae remain quiescent [75, 76]. The period of quiescence and the physiological and nutritional state of newly hatched larvae seem to be directly correlated. After a protracted period of quiescence, first instars (L1) that hatch from eggs typically have fewer lipid reserves, which reduce their ability to withstand famine and delay their larval growth. Lipid oxidation to support fundamental metabolic processes is probably the cause of the drop in lipid reserves in larvae from older eggs [75]. The egg's energy stores are eventually depleted by prolonged quiescence in first instars larvae (L1), even though oxygen consumption and metabolism drop down after embryogenesis is complete [77].

Both maternally inherited reserves and nutrients that the larvae themselves collect are necessary for the growth and development of the larvae. According to the researchers, a first instar (L1) can survive long periods of quiescence, but doing so has drawbacks. The mosquito populations in the wild commonly live in conditions with suboptimal nutrition, the effect of first instar quiescence on larval development and starvation tolerance is crucial from both an ecological and epidemiological perspective. The fitness, ability to reproduce, and vector capacity of adult mosquitoes are all impacted by this variation [78-80]. The larvae's development depends on the maternal nutritional reserves, which also probably act as a buffer against unfavorable environmental conditions or variations.

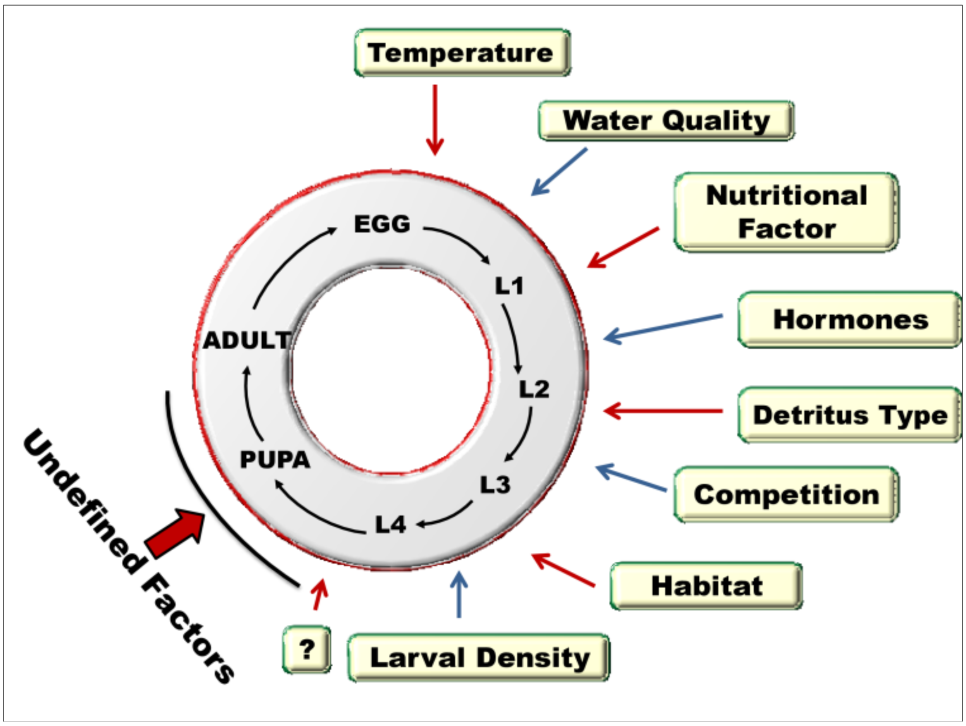

Fig 2: Several factors influencing the life of *Aedes* larvae.

An egg develops into an adult mosquito in seven to ten days under ideal circumstances. *Aedes* larvae can stay in this stage for a few days to several months, depending on their habitat. They feed on microorganisms that are present in the water. There is no moulting during the pupal or adult phases, however there are several moults throughout the larval stages. It simply takes three to five days to go from larva to pupa [81]. Only during the four instars of the larval stages—the first (L1), second (L2), third (L3), and fourth (L4)—does body size increase (Figure 2) [81]. The L1, L2, L3, and L4 larvae have average sizes of roughly 1.745 mm, 2.935 mm, 4.343 mm, and 7.202 mm, in that order [82]. The head, neck, thorax, abdomen, antennae, preclypeal spines, mentum, compound eyes, mouth brushes, median brushes (palatum), lateral brushes, comb spines, syphons, and anal papillae are among the physical features of L4 larvae that have been observed by researchers [82-86]. *Aedes* larvae are distinguished by the presence of a syphon and a single row of combs in the tail region [84]. First instars of Aedine mosquitoes that have just emerged and are formed from eggs that have been exposed to prolonged quiescence, such as those found during the dry season; on the other hand, offspring who do not experience extended periods of egg quiescence usually recover their ability to withstand metals throughout the rainy season [78]. Other stressors such as pollution, oxidative stress, pesticide metabolism, and competition between larvae can also cause the pattern of vulnerability [78, 87]. Consequently, prolonged quiescence could potentially improve the vector's ability to spread by raising the dengue virus load in adult females hatching from quiescent eggs [79], and it could also have an impact on vector control tactics. In particular, mosquito larvae that emerge during the changeover from the dry to the rainy season may have weakened physiology, rendering them more vulnerable to vector control strategies than larvae that hatch later in the wet season [74]. Therefore, for efficient vector and disease control, an understanding of the mosquito life cycle is essential. The amount of food consumed during the larval development phase is linked to the timing of pupation, adult emergence, and adult size [88, 89]. When food is abundant, the mosquito's development period from its first instar larval stage to adulthood is shortened [90]. Temperature and larvae density have an impact on growth and development as well [91]. Studies have indicated that *Aegypti* larvae grow faster when there are plenty of food sources available. It takes the same amount of time for an egg to mature into an adult under conditions of limitless food availability as it does for the larvae because larger food concentrations speed up larval growth but delay down the pupa's transition from the fourth instar (L4). When food supplies run out, the species can continue to reproduce by starting the pupation process, as long as the total amount of resources in the environment is still higher than a particular threshold. *Ae. aegypti* larvae most likely increase ecdysteroid levels in response to hunger, which influences the timing of pupation.

Pupa

For the 4th moulting stage, which indicates the transition from the larval to pupal stage, to occur successfully, there must be a notable drop in juvenile hormone (JH) levels and an increase in ecdysone [92, 93]. Pupae are relatively active throughout the pupal stage, responding to changes in light and using their tails to crawl or tumble into sheltered regions. However, this is a non-feeding, resting phase. Because the head, thorax, and their extended appendages are united into a structure called the cephalothorax, the pupae's adult shape is largely obscured. This arrangement, with the abdomen hanging down and the thorax in touch with the surface membrane, helps the pupa maintain buoyancy and enable it to float at the water's surface. It is positioned between the appendages. During the pupal stage, the terminal abdominal spiracles become useless for breathing due to the altered body form and posture. Rather, the mesothoracic spiracles—which open into enormous formations called "respiratory trumpets"—are responsible for breathing. The hydrophobic edges of these respiratory trumpets extend through the surface membrane when the pupa floats at the air-water contact. In this stage, adult organs form from undifferentiated embryonic cells, whereas other larval organs, such the alimentary canal, break down. The heart and fat body are examples of organs that continue throughout adulthood. When temperatures are high enough, metamorphosis can happen in as little as one or two days. When the adult is completely grown inside the pupal cuticle, it rests at the water's surface and inhales air to raise its internal pressure, which finally results in a break along the thoracic cuticle's midline. Restricting food during *Aedes aegypti* larvae's last instar stage can stop them from pupating [88], although if the larvae have stored enough food during previous stages, they usually pupate without food [41]. Therefore, when subjected to restricted but permissive feeding levels, *Aedes aegypti* larvae in the fourth instar stage may experience premature pupation, changing into pupae. When the pupa reaches adulthood, it emerges from the water, flies to regions where people are present, and starts to feed on them. The full metamorphosis from pupa to adult takes roughly two to three days [81].

Role of genes in larval development

In the growing *Ae. aegypti* pupal head, studies revealed sex-specific gene expression patterns. Doublesex was found to be a major regulator of sexually dimorphic gene expression during brain development. *Ae. aegypti* has been found to have both male and female splicing variants of the dsx gene [94].

The development of *Aedes* mosquitoes is influenced by several factors, including the AaeIAP1 gene and the expression of the sex-specific dsx gene. Throughout *Ae. aegypti*'s developmental stages, the expression of the AaeIAP1 transcript was tightly controlled; in comparison to the larval stage, levels were considerably greater in the pupal and adult stages. Additionally, AaeIAP1 expression considerably increased in response to several environmental stresses, including severe temperatures, UV light, and permethrin treatment. This shows that AaeIAP1 plays a vital role not only in the physiological development of *Ae. aegypti* but also in controlling stress-induced apoptosis [95]. Egg hatching is regulated by hormones and the pre-programmed state of cells, but AaeIAP1 plays a smaller role in this process. As a result, there is a higher mortality rate in L1 instars compared to L4 instars and the pupal stage [94]. It is still unclear how exactly *Aedes* larvae change from a dormant stage to pupae or adults. We don't yet know enough about the cues regulating this process to develop practical methods for controlling *Aedes* larvae or infections carried by mosquitoes. Because *Aedes* mosquitoes may find an endless number of places to spawn, controlling them could become a difficult issue if we ever figure out the complete process underlying this change. Ultimately, the emphasis switches to the dsx gene because, in contrast to AaeIAP1, it might provide more effective means of controlling *Aedes* in the future.

Adult

The head, thorax, and abdomen are the three separate components that make up the adult *Aedes* mosquito body. *Aedes* mosquitoes can vary in size overall, although their length usually ranges from 2 to 10 mm [96]. *Ae. aegypti* is distinguished by two narrow, white, horizontal lines that run along the middle of the thorax and are surrounded by a lute-like curve. On the other hand, *Ae. albopictus* has a single, thick, white line running down the centre of its thorax [63]. A unique mouthpart called the proboscis is designed for nectar feeding in males and blood feeding in females [97]. The remaining components of the mouth, which resemble stylets with needle-like structures, are protected by the labium, the outer sheath of the proboscis. The muscles at the base of the mandibles and maxillae in females can force them into tissue, flattening and toothing it and opening a channel for other stylets. These stylets have passages for drawing blood and delivering saliva. For energy, mosquitoes, both male and female, feed on sugars found in plant liquids, especially those from nectaries, though they can also eat honeydew and rotting fruit [58]. *Aedes* mosquitoes bite during the day and spend their nights in shadowy regions of homes and yards. They bite repeatedly and are capable of feeding on numerous persons during a single blood meal [98].

Environmental factors affecting larval development

Numerous biotic and abiotic environmental factors can have a substantial impact on the rate at which organisms develop and survive. Generally speaking, selection pressure for shorter development durations is higher than for other life history features [99]. Therefore, an essential component of comprehending how phenotypes adjust to various environmental circumstances is development time [31].

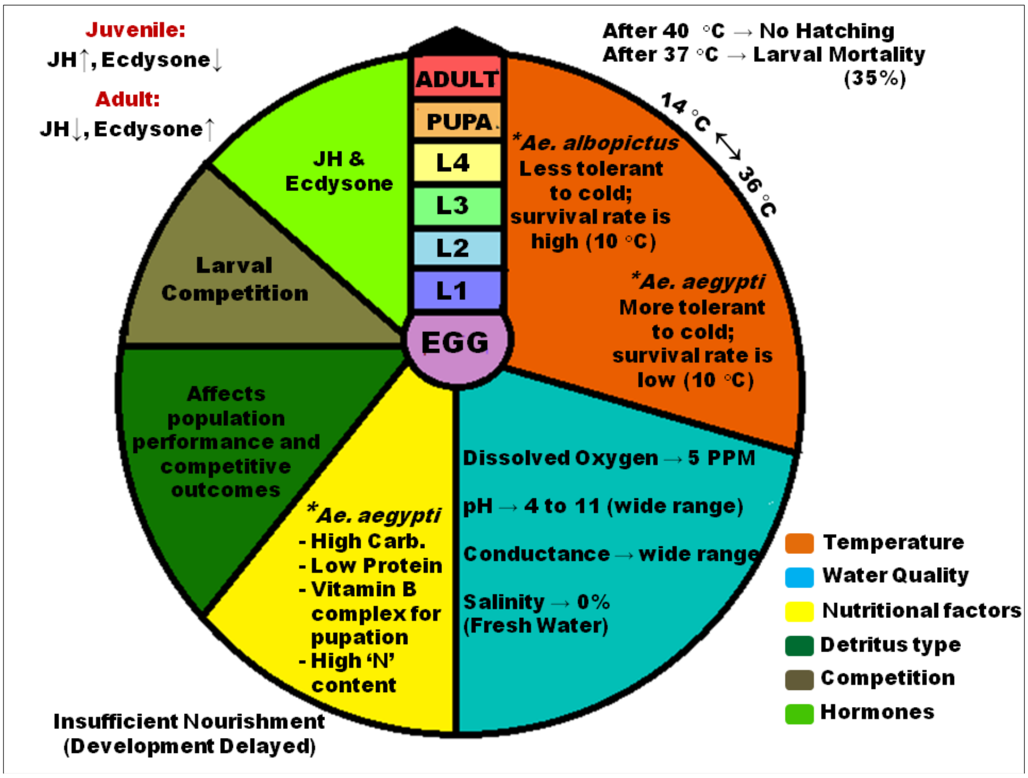

Fig 3: Different factors (Temperature, Water quality, Nutritional factors, Detritus type, Competition, and Hormones) have different impact on life of *Aedes* larvae.

Temperature

As poikilothermic animals, mosquitoes are directly impacted by ambient temperature (Ta) in terms of their general physiology, fitness, and seasonal activity [100]. The threat of disease outbreaks increases as a result of global climate change, which also increases the average Ta and encourages the geographic migration of important mosquito vectors [101-104]. The effects of low temperatures on immature stages of both *Ae. aegypti* and *Ae. albopictus* were explored in both laboratory and outdoor settings. The findings showed that *Ae. albopictus* was generally less tolerant of cold temperatures than *Ae. aegypti*. Specifically, *Ae. albopictus* first and fourth instar survival rates at 10°C were considerably greater than *Ae. aegypti* survival rates [105]. The acclimatisation of *Ae. aegypti* immatures was not significantly affected by brief exposure to low temperatures; on the other hand, continuous exposure improved their physiological adaption to the cold. *Ae. albopictus*, on the other hand, showed more acclimatisation after being exposed to low temperatures. Field trials conducted by Chang *et al.*, during the winter revealed that when exposed to cold fronts, *Ae. aegypti* larvae died at a considerably higher rate than *Ae. albopictus* larvae; these results imply that *Ae. aegypti* is negatively impacted by low temperatures in northern and central Taiwan, but this effect is not strong enough to stop the species from spreading throughout the area [105].

Though they can have varied impacts on the survival of immature stages, higher temperatures are generally associated with faster rates of development in insects [33, 106-108]. Studies show that an adult can develop from a newly hatched larva in a temperature range of approximately 14°C to 36°C (Figure 3) [109]. Larval development takes less time as temperatures rise, regardless of the conditions of food availability and population density [31]. Nonetheless, research indicates that no eggs hatch above 40°C and that hatching rates drastically decrease at 37°C (57% hatching rate) [110, 111]. Compared to groups at lower temperatures, there was a considerable increase in larval and pupal mortality at 37°C, reaching approximately 35%. Furthermore, the growth time from egg to adult emergence was dramatically decreased by higher temperatures and increased exposure to light [111]. *Ae. aegypti* (Rockefeller strain) eggs hatched at a high rate (>90%) at 22°C to 28°C, according to one study [27]. Nevertheless, *Ae. aegypti* eggs collected at 20°C from the La Reunion Islands in France had a lower hatching rate of about 67%, according to another study [112]. For *Ae. aegypti* eggs from Trinidad, Mohammed and Chadee [113] reported a 98% hatching rate at 24-25°C, whereas a recent study by Sukiato *et al.* [111], indicated that over 90% of eggs hatched at 31°C.

These differences in temperature responses across various *Ae. aegypti* strains may be explained by adaptations to their native settings. The timing of the developmental stages of *Ae. aegypti* has been found to have changed due to altering climatic trends, according to recent research [114]. It is commonly known that changes in the surrounding environment can have a substantial impact on the physiology of mosquitoes [115-117], which can therefore have an effect on the mosquitoes' ability to spread the dengue virus [94, 96]. Even current population dynamics models, however, frequently oversimplify the impact of environmental conditions, concentrating mostly on temperature in the case of *Ae. aegypti* and other mosquito vectors [117-119]. In comparison to the fourth larval instar (L4), which saw almost 75% of the growth, the first three larval instars (L1-L3) had lower growth rates and lower starvation resistance. The length of each instar was shortened by increasing temperatures, although starvation resistance showed a U-shaped response: it rose from L1 to L2 at 20°C and 30°C, stayed stable at 22°C and 28°C, and fell at 24°C and 26°C. Only at 26°C to 30°C did growth from L2 to L3 result in noticeably greater hunger resistance. L1 larvae consistently showed decreased starvation resistance at temperatures above 22°C, while L2-L4 larvae showed lower starvation resistance between 20°C and 24°C with increments of 2°C; however, the effects were less and unique to each instar above 24°C. Temperature may have an impact on how *Ae. aegypti* population density regulation is influenced by temperature since the anabolic and catabolic processes of larvae are temperature-dependent [120].

Despite the fact that temperature directly affects mosquito biology, little is known about the phenotypic and genetic aspects of thermal adaptation in disease-carrying mosquitoes. Determining the critical elements that contribute to the invasive success of *Aedes* species requires a fuller knowledge of the ways in which environmental temperature (Ta) influences the biology of these species and the potential heredity of temperature-related features. Additionally, novel population control techniques may be made possible by this information [121].

Water Quality

Few studies have examined the effects of environmental factors—specifically, the quality of the water at breeding sites—on mosquito distribution and dynamics to date, particularly in metropolitan areas [122]. Dissolved oxygen has been found to be a significant factor determining the quantity of *Aedes* larvae among a variety of water quality parameters. All breeding sites had dissolved oxygen concentrations above 5 parts per million, which is a sign of acceptable water quality. The *Aedes* larvae's varying abundance in different habitats implies that these mosquitoes might have particular oxygen needs for healthy growth. According to a study [123], different mosquito genera have different oxygen requirements, with *Aedes* mosquitoes being able to survive in environments with varying dissolved oxygen levels. In home sewage water with different pH levels, *Ae. aegypti* larvae can live and mature into mosquitoes [124, 125]. Increased total dissolved solids and high dissolved oxygen concentrations greatly increase the larval growth of both *Ae. aegypti* and *Ae. albopictus*. *Ae. albopictus* loves a pH range of 6.5 to 7.5, while *Ae. aegypti* prefers a pH range of 7.5 to 8.5 (Figure 3) [126]. The studies have revealed that *Aedes* larvae can withstand a broad pH range; pH levels in breeding habitats have been found to range from 4 to 9. But in a different study, found that mosquito larvae prefer breeding settings with pH ranges of 6.5 to 8 [127], whereas Clark *et al.*, claimed that mosquitoes prefer to breed in habitats with pH ranges of 4 to 11 [128]. The results of different study show that *Aedes* mosquitoes can survive in a variety of breeding environments, including those with normal (pH), acidic, and basic conditions.

The conductance and salinity of *Aedes* mosquito breeding areas may also have an impact on the number of these insects [129]. The breeding waters in this investigation showed a wide range of conductance readings, indicating the availability of inorganic dissolved solutes that may feed *Aedes* larvae. Since the salinity of every water sample taken from the breeding habitats was zero, it was clear that these were freshwater sources. Nevertheless, there was no significant relationship found between the number of *Aedes* larvae and conductance or salinity. The results of this study contradict prior research that suggests salinity and conductance are generally significant for the development of larval mosquitoes [130, 131. 132].

There are various studies on how pH affects the development of *Aedes* mosquitoes. However, we know very little about this effect. We only know the range of pH in which *Aedes* larvae can be found, but not how specific pH levels impact their development. More research is needed to understand how pH affects different stages of *Aedes* larvae. It has been shown that *Aedes* mosquitoes can breed in a wide range of pH levels, depending on their habitats. However, it was found that there was no significant correlation between the number of *Aedes* larvae and either pH or dissolved oxygen. They can also readily adapt to and experience a wide range of pH levels. It is therefore difficult to manage *Aedes* larvae in their habitats using dissolved oxygen, pH, and conductivity.

Nutritional factors

Different species of mosquitoes have different life cycles and environments for their larvae. *Ae. aegypti* originally flourished in tree holes, but populations have adapted to man-made container habitats; they frequently form in planters, tires, food containers, and flowerpots as temporary water sources [133]. Animal and leaf debris are frequently mixed together in these settings. High relative nitrogen concentration is necessary for *Ae. aegypti* to evolve to its best potential, according to a prior study [134]. *Ae. aegypti* appears to have unique nutritional needs; it seems to thrive in surroundings high in carbohydrates but requires a minimum amount of protein to utilise these nutrients [135]. This is probably similar to what occurs in their natural environments, where they can always obtain carbohydrates from plant-based sources and are also provided with nutrients from bacteria that live alongside animal waste. An autogenous female mosquito normally need blood meals from vertebrates later in life in order for the protein needed for egg formation to occur. One important area of study for the development of alternative larval control techniques could be the nutritional requirements of mosquito larvae. Overall, the best life history features were shown by *Ae. aegypti* larvae fed a medium-low protein diet. This medium-low protein diet, however, might not be the best option for larvae because growth is influenced by a variety of factors other than protein and carbohydrate intake, and various protein-to-carbohydrate dietary ratios have not yet been evaluated. It is unclear what the larval *Ae. aegypti* needs to eat, which could have an impact on physiological and developmental parameters and be crucial information for vector management. Development rates and mortality have been found to be correlated with dietary availability and nutritional quality (Figure 3).

It is unclear how the makeup of the larval diet affects fitness metrics including growth period, adult body mass, and nutrient storage. For practical purposes, such as developing rearing regimens that increase experimental consistency and mass rearing efficiency, it is crucial to understand the distinct roles that various nutrient classes play in controlling larval growth. An understanding of the metabolic requirements and pathways involved in the developmental processes of juvenile mosquitoes can be gained by examining larval feeding and its effects on growth and maturity. *Ae. aegypti* larvae that receive insufficient nourishment during their larval stages may experience delayed or unsuccessful development [136] or develop adults with insufficient nutritional reserves [137]. As a result, these mosquitoes frequently need several blood meals prior to reproducing, which increases their ability to transmit the virus to hosts and increases their interaction with vectors. For healthy growth, mosquito larvae require a variety of nutrients, such as carbohydrates, nucleotides, sterols, polyunsaturated fatty acids, vitamins, and the fourteen essential amino acids [136, 138-140]. Moreover, *Ae. aegypti* depend on gut microbiota to absorb nutrients required for growth and development, according to recent studies by Coon *et al.* [141]. To be more precise, *E. coli* uses cytochrome bd oxidases to facilitate aerobic respiration, which aids in inducing larval moulting. *Ae. aegypti* larvae with defective cytochrome bd oxidases exhibit stunted development [141].

It appears that mosquito larvae have evolved to withstand times of food scarcity and benefit from intermittent food inputs in containers [142]. Research has indicated that their food mostly consists of solid casein, in addition to cholesterol, RNA, inorganic salts, and eight B vitamins, including biotin, folic acid, thiamine, pyridoxine, riboflavin, niacinamide, calcium pantothenate, and choline chloride. Other nutrients such as sucrose, carnitine, glutathione, l-cystine, inositol, and p-aminobenzoic acid were deemed superfluous, according to the study [142, 143].Other studies suggest that several vitamins and essential amino acids are crucial for healthy larval development. Specifically, thiamine, riboflavin, nicotinic acid, pantothenic acid, biotin, and essential amino acids such as l-valine, l-leucine, l-isoleucine, l-phenylalanine, l-methionine, l-histidine, l-arginine, l-tryptophan, l-threonine, and l-lysine are necessary [136]. Additionally, the amino acid l-cystine, along with vitamins like pyridoxine and folic acid, is vital for successful pupation. Other important nutrients for normal growth and development include l-proline, l-hydroxyproline, l-serine, vitamin B12, choline chloride, and essential glutathione. A deficiency in vitamin B12 can slow down the pupation process, while p-aminobenzoic acid may delay ecdysis [136]. According to recent findings, moderate dietary levels are frequently associated with the largest adults, shortest developmental timeframes, and best survival rates. These results imply that although populations should be able to adjust to fluctuations in food supply, food scarcity or excess can have a deleterious effect on fitness, resulting in smaller adult bodies and fewer survival rates [144]. As a result, the diet of mosquito adults and larvae may have distinct effects on their life history features. Both the amount and quality of food available during the larval stage and the adult stage have a significant impact on body size and survival rates, and they also jointly affect fertility. This emphasises the significance of taking into account both larval and adult settings when examining factors that affect mosquito fitness and performance, with substantial implications for our understanding of mosquito population dynamics and vectorial capacity [145]. The next stage of this study will look into the molecular and biochemical processes that affect *Ae. aegypti* larval development. Determining the essential triggers and conditions for vital processes like larval growth and pupation in this significant disease vector would require analysing hormonal and nutritional signalling responses to several feeding regimens [135].

Detritus Type

Studies by Barrera (1996) [5] and Daugherty *et al.* (2000) [146] have demonstrated how mosquito larvae can be impacted by differences in food quality among various forms of detritus. Their results show that when larvae are given animal detritus instead of plant detritus, they develop faster and grow to larger adult bodies. Furthermore, compared to larvae that feed on slowly decomposing plant debris, those that consume rapidly decaying plant detritus also show faster development and larger adult sizes [147, 148].

Geographic variations in the amount and makeup of plant and animal communities along gradients of urbanisation and climate can result in a variety of detritus kinds [149, 150]. This data shows that different detritus types impact not only mosquito performance and population growth but also competitive outcomes among these species. Therefore, in order to determine whether detritus significantly influences the distribution of *Aedes* species in the field, it is imperative to quantify geographic variations in detritus types and conduct additional studies on their chemical and microbial properties [151].

Competition

Larvae of both *Ae. albopictus* and *Ae. aegypti* can be found together in a variety of artificial and natural container environments, with varying sizes and nutrient availability [152, 153]. Mosquito larvae in these habitats fight for few food supplies, and have no report of competition for space. Higher larval densities in these environments are generally associated with slower adult development durations, lower survival rates, smaller adult body sizes, and shorter lifespans [43, 78, 79, 154, 155]. Variations in these life cycle characteristics may therefore have an impact on both vectorial capability and vector competence, or the power to spread infections (Figure 3) [4, 78, 79]. The magnitude of the relationship between larval density and mosquito life-history traits are vary based upon the initial larval density and mosquito species present [5, 78, 79, 156].

Often interspecific (in between different species) competition in the larval environment is asymmetrical resulting in a population reduction or complete exclusion of the inferior competitor [9, 40, 157, 158]. The consequences of interspecific competition can be influenced by biotic (such as predators, types of detritus, and host availability) as well as abiotic factors (such as temperature, relative humidity, and water chemistry). These effects could potentially change the competitive advantages, lessen asymmetry, or make cohabitation easier [9, 159-162]. These conditions-dependent competitions can impact adult life-history features and species distributions, which in turn can impact the dynamics of disease transmission [159, 163]. Nevertheless, little is known about the biotic and abiotic variables influencing condition-specific competition [159, 160, 164]. Our understanding of how condition-specific competition results in either competitive exclusion or coexistence is hampered by this information gap. *Ae. albopictus* and *Aedes japonicus* can play very different roles in the spread of illness depending on competition, predation, and parasitism [165]. Depending on the invader's vector effectiveness, the dynamics of disease transmission may change when an invasive mosquito species outcompetes a native one [166]. For example, the *Anopheles stephensi* population may drop as a result of interspecific competition between *Ae. aegypti* and *Anopheles stephensi*.

In their individual growth, each species' larvae grew more quickly, and when given plenty of food, the females grew larger, indicating intraspecific (in between same species) competition. *Ae. albopictus* was able to outcompete *Aedes cretinus* while they both evolved in the same area with scarce food resources. In particular, compared to when they matured in isolation, *Ae. albopictus* larvae developed in 1.3-2.4 days less time during competition, while *Ae. cretinus* larvae developed in 0.9-1.4 days less time. In circumstances of reduced food supply and lower population densities, *Ae. albopictus* females became larger due to interspecific competition. This implies that *Ae. albopictus* is a better rival, especially in situations where there are few larval food sources. *Ae. aegypti* exhibited more pronounced negative repercussions from high population densities and, to some degree, from interactions with *Ae. albopictus*, especially when food supplies were restricted. On the other hand, *Ae. albopictus* exhibited better population performance in a range of environmental circumstances. Its ability to adapt to changing ecological conditions and its advantage over *Ae. aegypti* may help it succeed in its invasion of places like Réunion Island [167]. Additional studies revealed that *Ae. aegypti* larvae dominated *Aedes* species collections and were most prevalent in savannahs, settlements, and wooded areas [168-170]. Whereas according to research, larvae growing in water that had previously been occupied by other larvae experienced negative consequences on their overall size and weight, developmental time, and adult longevity. This implies that environmental changes or chemicals secreted into the body are important factors in intraspecific competition. That being said, the indicated component of intraspecific competition does not result in disparate expressions of competition costs between genders. More generally, Mueller's two main theories are supported by the effects of environmental changes, especially those caused by conspecifics' secretions (1997). Firstly, it is not sufficient to reduce intraspecific rivalry to the division of food resources. Secondly, the changes in the environment brought about by high population densities are not permanent, since the build-up of materials secreted by larvae probably plays a role in the slow deterioration of the ecosystem under dense population settings [171]. The findings from this laboratory experiment suggest that container size can influence the competitive outcomes between *Ae. albopictus* and *Ae. aegypti*. To use container size as an indicator of competition under specific conditions, field studies should investigate the various types and sizes of containers present in regions where the distributions of these two species intersect. Future research will focus on how container size impacts oviposition by gravid females and will also explore the effects of altering specific parameters of container size and shape (e.g., modifying surface area while keeping volume constant) on competition between *Ae. aegypti* and *Ae. albopictus*. Such studies will be instrumental in elucidating the mechanistic relationship between container shape and size and their influence on condition-specific competition [172]. Prior *in vitro* investigations suggest that *Ae. albopictus* poses a significant threat to other species of mosquitoes. Larvae from *Ae. aegypti* [165, 178], *Ae. japonicus* [173], *Ae. triseriatus* [174, 175], *Ae. koreicus*, and *Culex pipiens* [159, 176] have all been shown to be severely hampered in their growth and ability to survive by *Ae. albopictus* larvae. On the other hand, *Ae. albopictus* larvae are not significantly affected by the presence of *Cx. pipiens* larvae. The higher efficiency with which *Ae. albopictus* converts food into biomass is assumed to be the cause of this asymmetrical competition [176]. Moreover, it has been demonstrated that the kinds of food resources that are accessible affect the strength of competitive interactions [176]. *Ae. albopictus* and *Cx. pipiens* can share receptacles filled with water to serve as larval homes in natural environments. While *Cx. pipiens* is more likely to reproduce in bigger bodies of water, *Ae. albopictus* typically prefers smaller natural and artificial containers for oviposition [176, 177]. However, both species are able to live in moderately sized spaces. *Ae. albopictus* and *Cx. pipiens* coexisted in 67% of the larval habitats that were seen, especially in tires, drums, buckets, and catch basins, according to entomological investigations carried out in northern Italy in the summers of 1996 and 1997 [176]. As a result, a mix of niche divergence and interspecific competition involving both temporal and spatial aspects may have an impact on these two species' ability to cohabit [40, 178]. Interspecific competition between *Cx. pipiens* and *Ae. albopictus* is common in temperate areas, and increased mosquito concentrations brought on by warmer temperatures exacerbate the rivalry. Furthermore, drier weather patterns could result in more mosquito species' nesting locations overlapping, which would raise the possibility of competition.

Habitat

Generally both species of *Aedes* mosquitoes prefer artificial containers to breed than natural containers; but the breeding preference ratios (BPR) were different place to place and time to time. According to some authors water drums were breeding places for both species in developing countries [179]. Other reports indicated that used tires constituted more than 50% of *Ae. aegypti* breeding places [180], 45% of *Ae. aegypti* and *Ae. albopictus* [181], and 26.5% of *Ae. aegypti* and *Ae.albopictus* [182]. However, the highest number of breeding places in the Barombong, Galesong, Marina, and Bira were water drums [183]. Other studies reported the percentage of water drums being used as breeding places for *Ae. aegypti* was 19.3% [184], 19.7% [185], and 31.8% [183]. A study found that for both *Ae. aegypti* and *Ae. albopictus*, the larval abundance in artificial containers (90.57%) was higher than in natural containers (9.43%); tires were the preferred container type for *Ae. aegypti* breeding (17.82%), but the most preferred form of container for *Ae. albopictus* is found to be plastic cups (28.00%) [186]. It was also reported, for both *Aedes* species, there was a higher relative larval abundance in dark-colored containers compared to light-colored containers; for *Ae. aegypti* (57.34%) and *Ae. albopictus* (61.32%), a notably high relative abundance was noted with an increase in shade level [186]. Other study indicated that the *Aedes* mosquitoes preferred earthen pots both in the indoors and outdoors and discarded tires, plant pots and old vehicles/boats lying outdoors for laying eggs in Berhampur, Odisha. Discarded tires and old vehicles/boats were the best preferred breeding sites as the water remains undisturbed in them [187]. The BPR was found to be maximum in a plastic container (1.33) followed by an earthen pot (0.98) in Bhawanipatna, Odisha. No *Aedes* larvae could be collected from metal drums and plants axils [188].

Furthermore, as compared to rural areas, urban areas promote faster larval and pupal development and a higher larval-to-adult survival rate; the findings of current research demonstrated that urbanization has a major impact on the ecology of *Ae. albopictus*; the altered environment in the urbanizing and urbanized area made *Ae. albopictus's* growth and development more conducive [189]. Study carried out in Burkina Faso discovered that suburban regions had a higher abundance of *Ae. aegypti* larvae than urban areas did [170, 190]. In contrast, *Ae. aegypti* and *Ae. albopictus* larvae are frequently observed coexisting in the same containers in Western African cities; these containers are mostly man-made and include old tires, abandoned containers, tin cans, jars, and water storage tanks [170, 191-196]. Additionally, tires' interior conditions of decreased light and humidity make them especially alluring to *Aedes* mosquitoes [170, 197]. According to a recent study conducted in Yaoundé, plant debris inside breeding containers was positively correlated with the presence of *Ae. aegypti* and *Ae. albopictus* larvae [196].
Mosquitoes breed in a variety of habitats, including forests, mountains, plains, deserts, tropical forests, salt marshes, and tidal zones [198, 199] predicted that *Ae. aegypti* and *Ae. albopictus* would expand their range to various levels of changing climate.

Larval Density

The larval density dependent competition in mosquito is also associated with delayed maturity [200-204] and increased juvenile mortality [205-209]. The significant factors like temperature, diet, density, and their two way interactions are important to explain development rate variation of the larval stages of *Ae. aegypti* mosquitoes. These factors as well as two and three way interactions are also significantly associated with the developmental rate from hatch to emergence. The developmental time also was heteroskedastic with the highest variation occurring at the extremes of diet and density conditions. All three factors significantly impacted survival curves of experimental larvae that died during development. The habitat's temperature has significant impact on juvenile mortality than diet or larval density.

Conclusion

The existence of *Aedes* larvae is affected by various environmental and biological factors. Temperature influences developmental velocity, whereas water quality determines survival rates. Nutritional parameters and detritus type affect growth and pupation success, with more abundant organic matter enhancing results. Competition among larvae may reduce resources, delaying growth and increasing mortality rates. Hormonal modulation is crucial in metamorphosis and the time of development. These elements collectively influence the duration, survival, and metamorphosis of the larval stage into adulthood. Comprehending these influences is essential for efficient vector control techniques.

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