

**SPECIES COMPOSITION AND PRODUCTIVITY
OF MOSQUITOES IN RELATION TO PHYSICO-
CHEMICAL CONDITIONS, TENERAL
RESERVES, INSECTICIDE SUSCEPTIBILITY
AND COMMUNITY KNOWLEDGE ON
MOSQUITO BORNE DISEASES IN NIGER,
NIGERIA**

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UNIVERSITI SAINS MALAYSIA

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NIGERIA**

by

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for the degree of
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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AHC	Adult Holding Cage
<i>Ae.</i>	<i>Aedes</i>
<i>An.</i>	<i>Anopheles</i>
<i>Cx.</i>	<i>Culex</i>
CDC	Centre for Diseases Control
CO1	Cytochrome Oxidase Subunit 1
DNA	Deoxyribonucleic Acid
DMR	Duncan Multiple Range
EDTA	Ethylenediaminetetraacetic acid
GPS	Geographical Positioning Station
ITS2	Internal Transcribed Spacer. 2
KDT	Knock Down Time
LGA	Local Government Area
LHC	Larval Holding Cage
MBD	Mosquito-Borne Diseases
PCR	Polymerase Chain Reaction
PR	Potential Resistance
SE	Standard Error
SPSS	Statistical Package for Social Sciences
USM	Universiti Sains Malaysia
WHO	World Health Organization

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**KOMPOSISI SPESIES DAN PRODUKTIVITI NYAMUK BERKAITAN
DENGAN KEADAAN FISIKO-KIMIA DAN SIMPANAN TENERAL,
KERENTANAN INSEKTISID DAN PENGETAHUAN MASYARAKAT
TERHADAP PENYAKIT BAWAAN NYAMUK DI NIGER, NIGERIA**

ABSTRAK

Komposisi spesies dalam produktiviti larva nyamuk dinilai di tiga habitat pembiakan terpilih iaitu, selokan, paya dan tadahan air besar yang terletak di daerah Bosso, Katcha, Lapai dan Shiroro di negeri Niger, Nigeria. Reka bentuk kajian lapangan yang melibatkan bekas terbuka dan tertutup telah digunakan, sampel nyamuk diambil menggunakan teknik celup dan goncangan serta dikenal pasti secara morfologi. Sebanyak tujuh spesies iaitu, *Aedes aegypti*, *Ae. dorsalis*; *Anopheles gambiae*, *An. coustani*, *Culex quinquefasciatus*, *Cx. nigripalpus*, *Cx. salinarius* dikenal pasti. Hasil kajian menunjukkan, tadahan air besar sebagai habitat paling produktif dengan pelbagai jenis spesies yang ditemui pada kadar tinggi diikuti oleh selokan dan paya. Musim hujan (Jun-Ogos) adalah puncak penghasilan semua peringkat nyamuk sepanjang masa kajian, sementara bulan November adalah paling sedikit (musim kering). Sampel air dari setiap habitat pembiakan untuk analisis fisiko-kimia dikumpul menggunakan botol gelap berkapasiti 500ml, dan parameter seperti suhu, pH, kekonduksian, jumlah keliatan air, oksigen terlarut, permintaan oksigen biokimia, nitrat, fosfat, kalsium, sulfur, kalium, klorin, dan kealkalian bersama-sama dengan parameter meteorologi seperti hujan, kelembapan relatif dan suhu persekitaran yang dikumpul dari stesen bacaan telah disiasat mengikut kaedah standard bagi setiap parameter. Kualiti keadaan fisiko-kimia dan persekitaran didapati memberi impak positif dengan mempengaruhi perkembangan dan pengeluaran nyamuk justeru

menjadikan habitat menjadi lebih produktif. 60 larva nyamuk hidup larva peringkat empat (L4) telah disiasat untuk rizab teneral berikut prosedur standard. Anthron, vanilin, dan reagen biru marak coomassie digunakan dalam mencari nilai piawai dalam glukosa anhidrat, minyak kacang soya, dan darah untuk komponen simpanan (gula, glikogen, lipid, dan protein). Nilai ketumpatan optic dibaca dan dikira pada 625 dan 595nm menggunakan fotometer spektrum untuk rizab nutrien dalam nyamuk manakala, 30 sayap (kiri dan kanan) dari habitat pembiakan dalam setiap kajian LGA diukur untuk panjang sayap dan digunakan sebagai indeks untuk kecergasan vektor. Rizab teneral didapati mempengaruhi kecergasan vector bagi peringkat kehidupan melalui panjang sayap populasi nyamuk yang dikaji. Untuk mengenal pasti spesies nyamuk *Anopheles* yang tepat yang bertanggungjawab untuk penyakit nyamuk biasa iaitu malaria, pengenalanpastian molekular telah dijalankan ke atas 100 *Anophelines* betina dewasa menggunakan DNA COX1 dan ITS2 mitokondria dan ribosom melalui teknik PCR. Dua spesies adik-beradik yang berbeza telah disahkan iaitu; *An. gambiae* ss (Pyrethophorus) dan *An. rufipes* populasi (Neocillia) ditemui dengan *An. gambiae* s.s sebagai spesies adik beradik majoriti dalam kompleks *An. gambiae* dan telah diedarkan di semua habitat pembiakan LGA yang dikaji bagaimanapun, *An. rufipes* dalam *An. rufipes* kumpulan (Neocillia) telah dikesan sebagai spesies adik-beradik dalam badan air besar dan longkang Booso LGA. Bioassay insektisid kemudiannya dilakukan ke atas 1000 betina dewasa *An. gambiae* s.s dan *Cx. quinquefasciatus* populasi sebagai nyamuk yang paling banyak dan produktif kepada bahan kimia iaitu, permethrin, propoxur, dan malathion (iaitu, piretroid, karbida, dan organofosfat) mengikut standard prosedur operasi bioassay botol CDC ke atas betina berumur 3 hari, nyamuk yang diberi gula dalam 1 jam dan selepas pendedahan mortaliti selepas 24 jam dan nilai knockdown untuk KDT50 telah dikira pada 30 minit. Ramuan insektisid kimia

yang diuji didapati rentan kepada malathion terhadap dua nyamuk berbeza dalam semua LGA kajian kecuali *Cx. quinquefasciatus* di Lapai, berpotensi rintang terhadap permethrin yang direkodkan terhadap nyamuk yang dikumpul daripada semua LGA kajian, di dua lokasi LGA kajian untuk propoxur dan satu lokasi LGA terhadap malathion. Sebagai asas untuk mengetahui tentang pengetahuan masyarakat mengenai nyamuk dan status kerentanannya terhadap racun serangga yang biasa digunakan untuk mengawal penularan penyakit, sejumlah 967 responden melengkapkan soal selidik mengenai sikap dan amalan pengetahuan (KAP) dan kesedaran mengenai program kawalan nyamuk (MCP), yang kebanyakannya berumur antara 20 hingga 50 tahun ke atas, kebanyakan responden berasal dari komuniti tempatan di kawasan kajian dengan hanya sedikit yang berpendidikan. Walaupun terdapat banyak kempen pendidikan dijalankan mengenai bahaya yang disebabkan oleh nyamuk, responden masih kurang kesedaran atau mengabaikan pengetahuan yang diperolehi mengenai perlindungan diri mereka, terhadap nyamuk, oleh itu penyakit yang disebabkan oleh nyamuk terus menghantui kawasan kajian. Secara umum, kajian ini memberikan data asas untuk kajian lanjutan mengenai nyamuk dan serangga akuatik lain untuk pengurusan vektor yang berkesan.

**SPECIES COMPOSITION AND PRODUCTIVITY OF MOSQUITOES IN
RELATION TO PHYSICO-CHEMICAL CONDITIONS, TENERAL
RESERVES, INSECTICIDE SUSCEPTIBILITY AND COMMUNITY
KNOWLEDGE ON MOSQUITO BORNE DISEASES IN NIGER, NIGERIA**

ABSTRACT

Species compositions in mosquito larval abundance and productivity were assessed in three selected breeding habitats namely, gutters, swamps, and large water bodies located in Bosso, Katcha, Lapai, and Shiroro local government areas (LGAs) of Niger State, Nigeria. The experimental field design that involved opened and closed cage studies were employed, mosquitoes were sampled by dipping and agitation techniques. A total of seven species namely, *Aedes aegypti*, *Ae. dorsalis*, *Anopheles gambiae*, *An. coustani*, *Culex quinquefasciatus*, *Cx. nigripalpus*, and *Cx. salinarius* were morphologically identified. The findings revealed, large water bodies as the most productive habitats with the varying number of all the species encountered that aggregated at a high quantity followed by gutters and least was the swamps. The rainy season (June -August) was the peak of abundance and productions of all the stages of mosquitoes studied in the seasons, while November was the least (dry season). Water samples from each breeding habitat for physico-chemical analysis were collected using 500ml capacity dark bottles, and parameters such as temperature, pH, conductivity, total water hardness, dissolve oxygen, biochemical oxygen demands, nitrate, phosphate, calcium, sulphur, potassium, chlorine, and alkalinity together with meteorological parameters such as rainfall, relative humidity and environmental temperature collected from reading station were investigated following standard

methods for each parameter. The quality of physico-chemical and environmental conditions was found to have positive impacts by influencing the development and production of mosquitoes hence, making the habitats to be more productive. 60 live mosquito larval stage four (L4) were investigated for the teneral reserves following standard procedures. Anthrone, vanillin, and coomassie brilliant blue reagents were used in finding the standard values in anhydrous glucose, soya bean oil, and blood for the reserve components (sugar, glycogen, lipid, and protein). The optical density values were read and calculated at 625 and 595nm using a spectrum photometer for nutrient reserves in mosquitoes while, 30 wings (left and right) from breeding habitats in each of the study LGA were measured for the wing length and used as an index for vectorial fitness. Teneral reserves were found to influence the vectorial fitness of life stage through the wing lengths of the studied mosquito populations. To identify the exact species of *Anopheles* mosquitoes that are responsible for the common mosquito disease i.e., malaria, molecular identification was conducted on 100 adults female *Anophelines* using DNAs COX1 and ITS2 of mitochondria and ribosomes through PCR techniques. Two different sibling species were confirmed viz; *An. gambiae* ss (Pyrethophorus) and *An. rufipes* (Neocillia) population were found with *An. gambiae* s.s as majority sibling species in *An. gambiae* complex and were distributed in all the breeding habitats of the studied LGAs however, *An. rufipes* in *An. rufipes* (Neocillia) group was detected as sibling species in large water bodies and gutters of Booso LGA. Insecticide bioassays were then performed on 1000 adult females *An. gambiae* s.s and *Cx. quinquefasciatus* populations as the most abundance and productive mosquitoes to chemical ingredients i.e., permethrin, propoxur, and malathion (i.e., pyrethroids, carbimide, and organophosphates) according to the CDC bottle bioassay standard operating procedures on 3 days old female, sugar-fed mosquitoes in 1 hour and post-

exposure mortality after 24 hours and knockdown values for KDT_{50} were calculated at 30 minutes. Tested chemical insecticide ingredients were found susceptible to malathion against the two different mosquitoes in all the study LGAs except for *Cx. quinquefasciatus* in Lapai, potentially resistant to permethrin recorded against mosquitoes collected from all study LGAs, in two locations of the study LGAs to propoxur and one location LGA to malathion. As bases to have an insight regarding community knowledge on mosquitoes, disease, sign/symptoms, breeding habitats, and their susceptibility status to commonly used insecticides towards control of disease transmissions, a total of 1,350 questionnaires were distributed to respondents, only 967 completed the questionnaire on knowledge attitude and practice (KAP) and awareness on mosquito control program (MCP), comprising mostly aged between 18-20 and above 50 years old, most of the respondents were from the local communities of the study areas only a few were educated. Despite massive educational campaigns on the danger posed by mosquitoes, respondents still had poor or neglected the knowledge acquired on their protection, against mosquitoes, hence mosquito-borne diseases continue to devastate the areas. In general, this study provides baseline data for further studies on mosquito and other aquatic insect fauna for effective vector management.

CHAPTER 1

INTRODUCTION

1.1 Research Background

The consequences of diseases spread in the world are crucial, and because of these diseases effects, decision-making, are seriously considered for developing adaptive convinced habits to prevent harm and protect health and existence (Raude et al., 2018). These diseases are caused by parasitic pathogens, several of which are transmitted by insect vectors. Mosquitoes are the most widespread vectors of insect-borne disease in most cases, transmitting the commonest disease which is malaria (Ikpeama et al., 2017). Mosquito species of the *Anopheles*, *Aedes* and *Culex* genera have a range of main vector species of protozoan, virus, and nematode pathogens, and malaria, yellow fever, dengue fever, chikungunya fever, and other mosquito diseases are among the most prevalent worldwide (Gooch, 2017; Ikpeama et al., 2017; Akorli et al., 2019).

Mosquito-borne diseases have been the world's leading health threat for decades, and at least 500 to 700 million people are thought to be at risk, according to estimates, and remained major public health issues, causing substantial morbidity and mortality around the world (Gooch, 2017; Akorli et al., 2019). The diseases of mosquitoes are water-related because their life cycles start from water as aquatic stages (eggs, larvae, and pupae) and final metamorphosize to adults as terrestrial insects, and mosquitoes' population densities depend on how successful growth and development of these aquatic stages which in return give an epidemiological estimate of their population (Garba & Olayemi, 2015). It is estimated that around 10% of the global diseases are responsible by mosquito vectors, making them the most important cause

of ill health and death across the world (Madzlan et al., 2018). One of the most important mosquito breeding places for mosquito-borne diseases that are indispensable medically and contribute to the current worldwide public health concern is Africa (Makanda et al., 2019).

Anthropogenic deeds such dumping of organic waste food, creation artificial places for water accumulation, bad behavioural manners (unsanitary conditions) in most African countries are to blame for the proliferation of mosquito breeding grounds, which has resulted in a high population density and, as a result, a variety of mosquito diseases around the world (Khan & Khan; 2018; Makanda et al., 2019). The presence of many water bodies in Nigeria can provide breeding grounds for mosquitoes, thus expanding their distribution in all geographical ranges, and modifications of environment and season also take part in a role in the spread of mosquito species and the parasites they carry (Udoidung et al., 2020). Diseases that are caused by mosquitoes remain Nigeria's most serious public health issue, wreaking havoc on the development of the country's economic development, estimating the country more than 1% of growth domestic products (GDP) per year (Ibrahim et al., 2022). The economic cost of these diseases in Nigeria has recently been reported to be over \$ 346.9 million per year in terms of care costs, prevention, and lost man-hours (Barde et al., 2019).

The high density of mosquito vectors, especially in some communities in Niger State, is due to the availability of water bodies for domestic and agricultural activities such as rice cultivation, irrigation, and food/commercial fishing (Ikeh et al., 2017). These practices, however, cause environmental contamination, such as the proliferation of clogged gutters and the formation of water channels that result in water pools, which are harmful to people's health since they serve as breeding habitats for

several mosquito species that are accountable for the feast of mosquito diseases. Although the occurrence and abundance of mosquito larvae and pupae vary by species, habitat, and season, which is closely linked to the accessibility of appropriate larval breeding habitats (Ikeh et al., 2017; Akorli et al., 2019). Many species prefer vegetation-rich habitats for breeding, while others prefer open, sunny pools, and some mosquitoes prefer breeding in tree holes for their reproduction, however not many species reproduce in tree holes or the axils of plant leaves (Garbar & Olayemi., 2015; Selvan et al., 2015).

Flooding and high water currents, on the other hand, have been linked to the death of mosquito larvae and pupae, particularly those of the species *Anopheles*, due to a decline in oxygen stress, which causes physical injury to the larvae. (Garba & Olayemi, 2015). Mosquito disease vectors and recurring outbreaks have recently become a cause of concern in tropical and subtropical regions of the world, and some of these diseases have become renowned in many countries, most notably Africa, causing millions of cases each year (Salam et al., 2018). The spread of these diseases necessitated the introduction of intensive and immediate control measures, especially against parasites, however the meaningful result was not fully achieved because of the resistance abilities by the parasites. According to Omalu et al., (2015), these widespread of drug-resistant abilities by these parasites has shifted attention to anti-vector interventions, especially against the adult stage, but this has failed to produce the desired results against mosquito diseases, particularly malaria, which is a global health issue (Ramos, 2020).

Larval control measures are suggested and intended to minimize mosquito-borne diseases by preventing mosquito vector transmission and, as a result, human vector pathogen interaction. This is also beneficial because mosquito larvae and pupae

are generally concentrated, relatively immobile, and easily available, as far as the foods require for their growth and development are accessible at the position they leave in the breeding habitats and unlike adults, mosquito larvae cannot alter their habitat to escape control activities (Dom et al., 2019). Therefore, a fundamental management strategy against major mosquito-borne diseases most especially the immature stages has been to seek out mosquito vectors to interrupt disease transmission (Niang et al., 2018).

1.2 The rationale of the study

Mosquitoes remain the primary carriers of mosquito-borne diseases (Dida et al., 2015; Sofizadeh et al., 2018; Ortega-Morales et al., 2019), these vectors are common in tropical areas like Nigeria due to the rich species diversity and potential, as well as the abundance of ideal breeding sites that promote rapid immature development and high survival (Madzlan et al., 2018). The richness and dispersion of adult mosquitoes, as well as the presence and density of other vector species, including immature stages, had previously been recorded to be dependent on larval habitats (Dida et al., 2018). According to Olayemi et al., (2014), Garba & Olayemi (2015), Gowelo et al., (2018), the vast mosquito population is influenced by a variety of breeding habitats, including drains, rain ponds, swamps, large water bodies, streams, tires, dirty empty wet containers, and several others.

Physico-chemical parameters and other factors such as those of environmental have also been stated to be major limiting factors in larval habitats, as they have a significant impact on the availability of food and regulate osmotic/ionic equilibrium for optimum metabolic and physiological activities required for immature growth, and thus have an impact on mosquito larval species relative distribution (Afolabi &

Aladesanmi, 2018). For the establishment of creative, integrated, and biologically sound vector management strategies, basic knowledge of mosquito vector biology, ecology, and genetics is essential.

Understanding nuisance mosquitoes, potential sibling species, larval ecology and productivity, and other factors such as climate, seasonal variation, availability of microhabitats for breeding, and anthropogenic related factors, as well as the sanitary conditions of the ecosystems associated with many water bodies, is critical. Morphological identification, for example, can be time-consuming when dealing with multiple sibling species and can lead to misidentification of species that have never been seen before (Carter et al., 2019). Sibling species cannot be characterized by exterior morphological traits, (Barde et al., 2019), hence molecular taxonomy is required to gain trustworthy information about disturbing mosquito vector species' identification most especially those that causes malaria and filarial fevers (*Anopheles* and *Culex* mosquitoes) (Weeraratne et al., 2018).

Furthermore, molecular methods show variations in genotypes, or the ultimate degree of variation represented by an individual's DNA sequences and unaffected by environmental factors (Wisdom et al., 2019). It is possible to accurately assess mosquito knowledge, larval ecology, diversity, distribution, productivity, and a validated technique for identifying sibling species. Monitoring larvae in their aquatic surroundings before they emerge, which is the most efficient strategy for regulating vector populations, is a major aspect of risk assessment and the development of effective control strategies.

Thus, the rationale behind the study is to relate the larval productivity and its cues with the behaviour of exact mosquito species of public health significance. So that awareness and sound knowledge on how quality larval habitats and production rates of well identified mosquitoes which influence their vectorial capacity will be established. The findings of this study will impact knowledge on the accurate identification of mosquito vectors and their productivity, which will aid in the development and implementation of an integrated mosquito disease control strategy. It will also track and assess mosquito susceptibility to insecticides in Nigeria, specifically in Niger State, where the infections occurred, and give evidence and lessons that can be shared with the other project nations and the rest of the world.

1.3 General aim of the study

The study aims to gain a better understanding of mosquito species identification in their breeding habitats, development, and how seasonal variation, Physical and chemical characteristics in their breeding habitats affect their distribution and productivity.

1.3.1 Specific objectives

- i. To determine species composition and relative abundance of mosquito vectors from the selected breeding habitats,
- ii. To determine the quantity of larvae, pupae and the adult mosquito productions in seasons from the study areas,

- iii. To assess the relationship between mosquito species abundance and variation in larval habitat physico-chemical and meteorological conditions,
- iv. To evaluate the influence of larval habitats on the teneral reserves and the wing lengths of the emerging mosquitoes for vectorial capacity,
- v. To molecularly identify and assess the insecticide susceptibility status in mosquito vector populations, and
- vi. To understand community knowledge status on mosquito-borne diseases (MBDs) and awareness on the impact of the mosquito-control program (MCP).

CHAPTER 2

LITERATURE REVIEW

2.1 Description of mosquitoes

Mosquitoes are tiny, two-winged, long-legged biting insects that get their name from the Spanish or Portuguese word "mosquito" (Kaur & Singh, 2017). They are members of the kingdom *Animalia*, Phylum, *Arthropoda*, Class *Insecta*, Order *Diptera*, Suborder *Nematocera*, infra order, *Culicomorpha*, Superfamily *Culiciodae*, and Family *Culicidae* (Harbach, 2016). Mosquitoes of medical interest are members of the *Culicidae* family, which includes 3556 valid species from 112 genera divided into two subfamilies: *Culicinae*, and *Anophelinae*, (Sofizadeh et al., 2018). *Culicinae* (with thirty genera), *Anophelinae* (with three genera).

The largest and most diverse subfamily, *Culicinae*, is further divided into several tribes: *Aedini*, *Culicini*, *Culisetini*, *Mansoniini*, *Ficalbiini*, and several others. *Aedes* and *Armigeres* are among the genera that make up the *Aedini* tribe. Mosquitoes can be found far beyond the Arctic Circle in tropical and temperate areas, but not in Antarctica. Mosquitoes can be found in caves and mines at elevations ranging from 6,000 metres above sea level in hilly areas to 1,250 metres below sea level in low-lying areas (Sofizadeh et al., 2018). Mosquitoes such as *Anopheles*, *Aedes*, and *Culex* are the most prevalent carriers of deadly diseases such as malaria, yellow fever, dengue fever, and filarial fever (Dyer, 2015; Gooch, 2017; Braack et al., 2018).

2.2 Life cycle of the mosquitoes

The mosquito goes through a completed life cycle, and passes through four different stages: egg, larva, pupa, and adult, the first three occurred mostly in water

(Bova et al., 2016). A female mosquito can lay her eggs between 100 and 300 per oviposition, depending on the temperature (Mereta et al., 2013; Day, 2016). The eggs are laid in an appropriate breeding habitat, which is usually a swamp or humid area and several other places in an aquatic setting. *Anopheles* species, for example, lay their eggs on the water's surface, and the larva hatch after around 2-3 days (Gardner et al., 2018). The development of larvae is divided into four phases, also called instars comprising of L1, L2, L3 and L4 respectively. The larvae molt after each larval stage, losing their skins to make room for new development (the larvae feed on microorganisms and organic matter in the water). The larvae transform into pupae during the fourth molt (the entire period of maturation from larvae to pupae takes 4-10 days most especially *Anopheles* species) (Day, 2016).

In around 2-4 days, the pupae mature into adult mosquitoes, and the mosquito's full life cycle takes between 7 and 20 days, depending on the species and the ambient temperature of the breeding habitat (usually a swamp or humid environment) (for example, *Culex tarsalis*, a common mosquito in California in the US) has of life cycle of about 20days (Bova et al., 2016). Few days after emerging of the adult mosquitoes from the pupal stage, then go on the hunt for blood (required to produce eggs) after which it goes to a suitable breeding place and lays her eggs. Female adult mosquitoes' chances of survival are determined by temperature and humidity, as well as their ability to obtain a blood meal and maintain established eggs in their reproductive organ while avoiding host defenses (Souza et al., 2019; Yan et al., 2021). The summary of the differences on the life cycles of the three major genera mosquitoes is presented in **(Table 2.1)**.

Table 2.1 A summary on the differences in the life cycles of the three major genera mosquitoes with complete metamorphosis

Life stage	<i>Anopheles</i>	<i>Aedes</i>	<i>Culex</i>
Eggs	Eggs are Laid singly, boast-shaped, No. of eggs (29-147), Resistance period 3 weeks, Duration of egg stage 2-3 days.	Eggs laid singly, ovoid shaped and long, No. of eggs 140 in 16 settings, Resistance period, 6-8 months, Duration of egg stage 2 days.	Eggs in rafts, oval-shaped, No. of eggs 200-300, Resistance period, can't stand drying, Duration of eggs stage 1-2 days.
Larva	Rest parallel to water surface, Duration of larval stage 4-14 days, No. of larvae produce depends on eggs survival, Resistance period in water is very short.	Suspended at an angle to water surface, Duration of larval stage 6-8 days, No. of larvae produce depends on eggs survival, Resistance period in water is short.	Rest parallel to water surface, Siphon long and narrow, Duration of larval stage 5-6 days, No. of larvae produce depends on eggs survival, Resistance period in water is short.
Pupa	Comma-shaped, Duration of pupal stage 1-2 days, No. of pupae produce depends on larval survival.	Comma-shaped, Duration of pupal stage 1-2 days, No. of pupae produce depends on larval survival.	Comma-shaped, Duration of pupal stage 1-2 days, No. of pupae produce depends on larval survival.
Adult	At rest, body is inclined angle to water surface, Duration of adult stage from pupal stage is 2-4 days, No. of adult produce depends on the pupal survival.	At rest, body exhibits a hunch back, Duration of adult stage from pupal stage is 2-3 days, No. of adult produce depends on the pupal survival.	At rest, body exhibits a hunch back, Duration of adult stage from pupal stage is 2-7 days, No. of adult produce depends on the pupal survival.

2.3 Diversity, species composition, and relative abundance of mosquitoes

Not only are entomologists interested in the structure and function of the insects, but also those involved in various environmental projects, have long been fascinated by the diversity, composition, and relative abundance of insects and their habitats (Bashirb et al., 2016; Edith et al., 2017). Mosquitoes are diverse and associated with aquatic ecosystems, and the properties of lentic water bodies play a key role in deciding which mosquito species can successful live and breed in such lentic habitats. Human activities and behaviour (such as creations of artificial water bodies) have continued to cause additional breeding habitats, and renewed variance in mosquito

species' presence and proliferation, despite their ability to reproduce anywhere (Olayemi et al., 2014; Garba & Olayemi, 2015; Bashir et al., 2016).

Mosquito immature stages can be found in a range of aquatic habitats, with their composition influenced by the quantity and structure of aquatic water sources. These conditions influence mosquitoes' oviposition sites, limiting their ability to reproduce and diversify (Aisha & Aneesh, 2014; Edith et al., 2017). Several factors (biotic and abiotic), such as the absence of predators and competitors, land cover, vegetation characteristics, and physico-chemical properties, in combination with human and animal population distributions, can influence the occurrence and richness of larvae and adults' spreading from breeding sites, and adult mosquitoes' abundance in various habitats (Bashar et al., 2016; Sayyad et al., 2022). Additionally, factors such as movement of water, elevation, water conditions (polluted, fresh, e.t.c), vegetation, water temperature, source of water, and several others, for example, the high mosquito density of *Culex* may be due to the availability of various breeding sites created by suitable rain and humidity with optimum temperature coupled with its adaptable nature to breed in a variety of habitats (Tripathi & Gupta, 2017; Karryawasam & Wegiriya, 2022).

Therefore, suitable breeding habitat for some mosquitoes may be undesirable for others, and environmental and climatic factors influence mosquito larvae distribution and abundance (Joseph et al., 2013; Seah et al., 2021). Habitat choice is dictated by gravid females' ability to differentiate habitats where their offspring's fitness is optimized, and the complicated aspect of exogenous and endogenous factors, given the variability of natural surroundings, is fitness (Gardner et al., 2018). Thus, under optimal conditions, habitat colonization would follow a trend in which average fitness disparities are identical across a range of environments where females can make

oviposition decisions for abundance. Adult females' oviposition preferences and the ability of mosquito larvae to survive in both biotic and abiotic environmental varies in each aquatic habitat regulate mosquito larval abundance and dissemination (Idris et al., 2014; Allgood & Yee, 2017; Amini et al., 2020).

According to Bashar et al., (2016), these parameters can affect a mosquito's oviposition behavior in a variety of ways, such as insects avoiding ovipositing where exact competitors are present while being drawn to areas where other mosquitoes' larvae are present. Additionally, aquatic vegetation can have a substantial influence on mosquito reproduction and abundance. The health and survival of the individual mosquito in the breeding habitats are significantly affected by the condition of the larval environment during critical developmental stages of larvae and pupae must be confined in a natural or artificial container. Larval habitat quality is affected by changes in resource levels, chemical composition, desiccation risk, and population composition over time and space, and is heavily influenced by habitat location (Fitzgerald & Livdahl, 2019).

Bashar et al., (2016), in their study on species composition and habitat characterization of mosquito larvae in semi-urban areas of Dhaka, Bangladesh, discovered a difference in mosquito abundance depending on habitat types. *Ae. aegypti* was most common in tires and less in cemented tanks, while *Ae. albopictus* was less common in tires, and *An. barbirostris* and *An. peditaeniatus* are subdominant species that prefers rice fields and ponds as breeding grounds *Cx. quinquefasciatus* preferred deep water depths, whereas *Cx. tritaeniorhynchus* preferred shallow water depths while, *Ae. aegypti* and *Ae. albopictus* prefers shallow water depths or eggs lying (Dida et al., 2018).

According to Egwu et al., (2018), mosquitoes have greater adaptability, but their existence in each type of breeding is attributable to the female mosquito's oviposition habits, which are the primary determinant of larvae abundance. Female *Anopheles gambiae* species, for example, preferentially choose and use oviposition environments with low nutrient status and high oxygen levels, as opposed to ground pools, which were previously established. Suleman & Ilahi, (2013), in their investigation on mosquito species composition and relative abundance in Swat, Pakistan, reported 21 species of mosquitoes, both adults and juvenile stages, in five genera in various breeding habitats among the mosquitoes, *An. quadrimaculatus*, *An. annular*, *An. splendidus*, *An. fluviatili*, *An. stehensi*, *An. palidus*, *Cx. quinquefasciatus*, *Cx. pseudovishnui*, *Cx. bitaeniorhynchus*, *Cx. theileri*, *Cx. mimeticus*, and *Cx. perexiguus* were the most abundant. Dida et al., (2018), in their reports, found mosquitoes of the *An. funestus* group in swamps and puddles covered in short grass, while mosquitoes of the *An. pharoensis*, *An. azamiae*, *An. christyi*, *An. maculipalpis*, while *An. gambiae* s.l. mosquitoes were found breeding in pools of other areas of the Mara River of Kenya.

IA & Ayeni, (2015), research work on the relative abundance and composition of endophilic mosquitoes in the Federal University of Technology Akure Hostels in Ondo State, Nigeria, and found that the abundance of *Culicine* and *Anopheline* mosquitoes was due to their ability to survive in a variety of breeding habitats, accordingly, *An. gambiae* larvae like clear, fresh seepage water in partially shady or sunny ponds, whereas *Ae. aegypti* breed in clogged drainage, discarded cans, openly broken cesspits, and storage containers as favored breeding habitats for larvae.

According to Mavian et al., (2019), many species exhibit high compositions in densely populated urban areas due to anthropogenic activities, breeding habitats such

as drainage and gutters were created and found influencing the abundance of mosquitoes, and species found include *Cx. fascianus*, *Cx. quinquefasciatus*, *Cx. gelidus*, *Cx. univittatus*, *Cx. sinensi*, and others. Thoughtful mosquito breeding ecology is required for any vector control strategies to be effective, including the types and preferences for larval habitats, the spatial and temporal distribution of breeding sites, as well as the physical, biological, and chemical characteristics of the habitats (Egwu et al., 2018).

2.4 Larval habitat preference, oviposition, and productivity of mosquitoes

Animals' survival and reproductive success in varied ecosystems are dependent on how efficiently they choose where to shelter, feed, and breed, therefore many species have evolved the capacity to recognize and select the best available habitats to optimize their fitness. (Gardner et al., 2018; Mathias et al., 2020). Some mosquito species may find water breeding habitats to be advantageous, while others may find them to be troublesome. Many species, on the other hand, prefer habitat with plants, while others prefer open, sunny ponds and a few species prefer tree holes or plant leaf axils to reproduce in although most species prefer permanent breeding sites, particularly natural habitats in urban, semi-urban, and peri-urban environments, temporary breeding sites are more prevalent for some species (Selvan et al., 2015; Tripath & Gupta, 2017),

Several mosquitoes oviposit at dawn and dusk, and certain intrinsic factors in larval habitats make certain habitats conducive to oviposition, larval growth, and development while others are not (Day, 2016). Environmental factors like temperature, rainfall, relative humidity, and wind all influence when gravid females fly and where they oviposit. Gravid females rely on visual and olfactory cues to seek prospective

oviposition sites while in flight, and the hot, moist, humid, and tranquil conditions encourage mosquito flying and oviposition (Day, 2016). Female mosquito oviposition decisions are a critical driver of immature stage distributions in circumstances where immature stages are limited to the habitat in which they hatch. The number of natural enemies and the number of larvae and pupae in an aquatic habitat can impact both adult oviposition behavior and progeny efficiency (Wasserberg et al., 2013; Barreaux et al., 2018). The variety and quantity of organic detritus (e.g., senescent plant material, invertebrate corpses) present in the aquatic breeding habitats affects immature mosquito development and adult oviposition behaviour. Mosquito larvae eat detritus-colonizing aquatic microorganisms (bacteria and protists) as well as minute trash particles.

Bacteria floating in the water column were filtered by mosquito larvae and, feed on leaf surfaces when leaves decompose due to soluble chemical leaching and breakdown by detritivores such as macro invertebrates and microorganisms, which combine with the nutrients such as sugar, lipid, and proteins in the water breeding habitats to boost their growth and development (Barreaux et al., 2018). Thus, the right proportions of these microbes could influence the larval abundance and productive rate (Dida, 2018). Several studies revealed that mosquitoes used different means to detect their oviposition and preference site due to the nutrient quality of the breeding habitats. For example, gravid female *Anophelinae* may use visual and olfactory cues to differentiate between water bodies and find appropriate breeding sites for oviposition (Akpan et al., 2018; Akpan et al., 2019).

Egwu et al., (2018), discovered a high prevalence of *Cx. quinquefasciatus* and *An. gambiae* in all dirty environments, including gutters and domestic runoffs, and it may be due to the female species' preference for these breeding habitats for

oviposition. *Aedes aegypti* for example preference for the breeding habitat was not only for the site in question, but also for the water quality, depth, light, and vegetation that served as sources of food and shelter suitable for their survival and growth, and light had little effect on the abundance (Dida, 2018), While *Aedes* mosquitoes lay their eggs and larvae in peri-domestic water storage containers, rainwater-holding objects such as a wide variety of discarded materials, and natural water retaining structures such as tree holes and plant axils, some containers produce large numbers of some contaminants' larvae/pupae (Paul et al., 2018).

In comparison to larger habitats, smaller habitats are more likely to contain fewer mosquito species, sustain smaller populations, and have higher rates of extinction. In studies conducted in Amazonian Peru. Carrasco-Escobar et al., (2019), discovered that the area has both endo and exophage mosquitoes and that their production rate is related to river levels, which rise significantly during the rainy season, providing female mosquitoes with numerous water bodies suitable for oviposition. Detritus, or the microorganisms that decay it, release chemicals that can be hazardous to mosquitos at high concentrations, and some species (e.g., plant secondary compounds) may be more sensitive to these chemicals than others.

Detritus and its related compounds affect adult mosquito oviposition behavior, and gravid females may use these chemical cues as markers of larval habitat preference and appropriateness for their offspring (Allgood & Yee, 2017). Furthermore, mosquito survival rates may be influenced by the chemistry of aquatic habitats' water. Because the female mosquito favors oviposition sites based on a mix of visual and chemical cues, the water quality of the breeding site is a critical element for the female mosquito to oviposit” (Panigrahi et al., 2014).

2.5 The role of physico-chemical characteristics of mosquito breeding habitats

Water is a very important and limitless resource used by a wide range of biodiversity, and water quality is a term used to assess the chemical, physical, and biological characteristics of water. Thus, the physical and chemical properties of a water body are important in terms of ecology (Seiyaboh et al., 2016). Adult mosquitoes' fitness can be linked to the nutritional and physico-chemical conditions in which larvae mature (Nikookar et al., 2017; Amarasinghe & Menike, 2022). These conditions can have a direct or indirect effect on mosquito larval distribution, density, and growth, as well as vectorial ability, thus, larval breeding habitats is the vital component for mosquito life cycle and can strappingly influence the distribution and abundance of mosquito-borne vectors (Afolabi & Aladesanmi, 2018).

Temperature, total suspended solute, total dissolved solids, and electrical conductivity appear to be among physical and chemical components that account for high mosquito density (Aminuwa et al., 2018). A high percentage of free ammonia, organic carbon, nitrate, and a high concentration of accessible salts with a slightly alkaline pH also chemical components that account for high mosquito density (Nikookar et al., 2017; Salihu et al., 2017), these properties, have been found to affect larval development and survival (Onchuru et al., 2016; Madzlan et al., 2018). Physical environmental factors such as favorable temperature, rain, and high relative humidity, on the other hand, play an important role in the growth of immature stages in breeding habitats (Wang et al., 2020; Nagy et al., 2021).

Although the physico-chemical properties of water bodies are complex and determined, ecological studies of the vector rely heavily on their composition and fauna. Amini et al., (2020), found that physical and chemical factors such as nitrates,

sulphate, calcium, potassium, and several others in mosquito breeding sites have a distinct impact on the growth and development of mosquito immature stages and variation on these factors can affect growth, development, and distribution of the mosquitoes (Razali et al., 2022). *Anopheles* mosquitoes, for example, have been discovered to breed in freshwater environments. *Anopheles* mosquito larvae and pupae have been found to survive in clear water with ideal pH, temperature, and nutrient conditions, such as fresh composition (Hamza et al., 2017). Grassy ditches along the banks of streams and rivers, as well as *Anopheles* species larval fatalities due to a drop in small, ephemeral rain pools, have been connected to high water levels in salt marshes, mangrove swamps, rice fields, and flooding (Madzlan et al., 2018).

The key physico-chemical parameters impacting mosquito species and aquatic invertebrates in their breeding habitats are water level, temperature, dissolved oxygen (DO), acidity (measured as pH), and unionized ammonia (NH₃) (Sultana et al., 2017). The water level varies greatly depending on water supply and water management measures, ranging from 2.5 to 15.0 cm, making it an inappropriate habitat for animals that demand deeper waters (Emidi et al., 2017). To function correctly, most species require a specified temperature range and since mosquito immature stages are poikilothermic, their behavior is heavily influenced by the temperature of the water they live in. Aside from nutrition, the temperature is the most important factor in mosquito larvae development and growth (Mereta et al., 2013; Annabel et al., 2021).

In general, higher water temperatures speed up the growth of aquatic stages but reduce the size of emerging adults, and above-average temperatures often result in fewer adults being born due to increased mortality (Garba & Olayemi, 2015). The strength of an acidic or basic character of a solution at a given temperature is known as pH. pH values between 0 and 7 are decreasingly acidic, whereas those between 7

and 14 are becoming increasingly alkaline, pH 7.0 is neutral a (Garba & Olayemi, 2015), and water with a near-neutral pH of 6.8-7.2 is preferred for mosquito breeding by many species (Thomas et al., 2016). The growth of mosquito eggs, larvae, and pupae is slowed when pH levels are outside of this range (Thomas et al., 2016; Sultana et al., 2017).

While several *Anopheline* species are ground pool breeders, they are for the first instar larvae to emerge. The productivity of an aquatic environment is measured in DO, many chemical compounds' oxidation-reduction states are affected by dissolved oxygen (DO) in water. It is fantastic for the self-purification of water bodies, and low dissolved oxygen (DO) requirement for an aquatic environment is an indirect reflection of lower total dissolved solute (TDS) concentrations, which can diminish transparency and produce oxygen deprivation at higher concentrations (Ishadi et al., 2014; Dom et al., 2019). The decrease in DO levels induces anaerobic conditions in the water, which harms the aquatic biota.

Many mosquito species prefer environments with low oxygen stress, which causes physical harm to the larvae with vegetation, whereas others breed in open sunlight ponds, according to diagnostic and scientific studies (Olayemi et al., 2014). The ability of water to carry an electrical current (conductivity), indicating the physical existence of dissolved compounds in water. The primary cause of ions in water is the dissociation of naturally occurring inorganic compounds. Heavy metal ions emitted by contaminants, on the other hand, may cause conductivity to rise (Devi et al., 2015; Emidi et al., 2017). Potassium is a highly reactive alkali metal that does not exist naturally as a free metal. Mosquito larvae are negatively correlated with potassium concentration, implying that mosquito larvae prefer less alkaline water.

The potassium content of freshwater can be influenced by soil dust, this potassium is commonly assessed in lake water when an evaluation is made since it is an important component of many artificial fertilizer formulas (Salihu et al., 2017). Fluoride is an inorganic ion found in water due to its presence in the earth's crust and human discharges into the atmosphere, fluoride has been identified as a toxic ion, and its existence in water bodies in trace amounts has been linked to substantial mortality of aquatic flora and fauna (Hamza et al., 2017). Salinity is an indicator of dissolved salts in water that is strongest during low flow times and decreases as the water level drops. For aquatic plants and animals, the right salt concentration is critical and any organism that is exposed to salinity outside of its natural range will experience stress or even death (Devi et al., 2015; Thomas et al., 2016).

Depending on the tolerance range of different organisms, changes in the physico-chemical properties of these water bodies may create conditions that are either advantageous or unfavourable to breeding success. Mosquito breeding sites and physico-chemical characteristics must thus be studied to effectively control mosquito-borne diseases (Hamza et al., 2017).

2.6 The influence of meteorological factors and season on the larval habitat productivity

Rainfall, relative humidity, and temperature (including temperature range) all have an impact on mosquito development and survival, as well as mosquito density and oviposition rate, all of which have been connected to vector competence for mosquitoes (Shil & Balasubramanian, 2020; Wang et al., 2020). According to Seah et al., (2021), female oviposition behavior, as well as their time-based space distribution, which is primarily dependent on the environment and local climate (meteorology) in

which they occur, affects species abundance in any breeding habitat type, with female mosquitoes looking for conditions that are conducive to progeny survival. These data and their relationship with mosquito abundance will help assess parasite activity levels and, as a result, disease risk (Bashar & Tuno, 2014).

Tropical areas, such as Nigeria, have the best combination of rainfall, temperature, and humidity, allowing mosquitoes to breed and survive (Msugh-Ter et al., 2017). Meteorological circumstances have an impact on mosquito metabolism, oviposition activity, and female egg production as a result (Serpa et al., 2013). Temperature is believed to be the key fixed factor leading mosquito development rate among meteorological features, to the exclusion of other known important factors such as diet and density (Wang et al., 2020). Temperature affects a variety of life-history properties in both mosquitoes and parasites, making it a difficult element in vector-borne illness transmission (Caminade et al., 2014; Christiansen-Jucht et al., 2015).

According to Aminuwa et al., (2018) temperatures during these two life stages (Larval and adult stages) can interact to affect important life-history traits such as longevity. Effects are typically studied during larval development (influencing rate of development) or adult life (influencing life span), but it has been shown that temperatures during these two life stages can interact to affect important life-history traits such as longevity. According to Wang et al., (2020), the ideal temperature for mosquito egg formation and hatching is 30°C. It is also believed that the higher the temperature, the faster the gonotrophic cycle of mosquitoes and vice-verse. Temperatures between 50°C and 40°C, as well as humidity levels between 55 and 80%, are ideal for the example malaria parasite to complete their life cycle (Wang et al., 2020).

Temperature impacts the duration of mosquito larvae development and the rate of expansion of malaria parasites within the vector, even though rainfall reduces the number of mosquito vector breeding places (Kibret et al., 2019). In Africa, larval development can take up to 45 days at 16 degrees Celsius, compared to just 10 days at 30 degrees Celsius (limiting the number of mosquito generations and exposing the larvae to predators) (reducing the number of mosquito generations and putting the larvae at greater risk of predators). Temperatures above 30°C, on the other hand, have been thought to be harmful to mosquito growth because they affect the length of the aquatic stage of the life cycle of the mosquito (Msugh-Ter et al., 2017). Temperature is important for pupae survival, which means that minor temperature changes will affect mosquito populations only when they have reached the critical pupae level.

Temperature can be considered a minor factor affecting mosquito population regeneration since this stage lasts just a few days out of the approximately 15 days it takes to completely develop from egg to adult (Wang et al., 2020). Adult feeding patterns are influenced by humidity in particular, excessive relative humidity has been shown to slow mosquito behavior, causing them to remain stagnant in their breeding, biting, and resting areas. According to Simon-Oke & Olofintoye, (2015), mosquito vectors' life spans are shortened when relative humidity falls below 60%. However, they have confirmed that mosquitoes prefer high relative humidity and cool shade for breeding (Msugh-Ter et al., 2017).

Rainfall supplies mosquito breeding places and ensures an appropriate relative humidity of at least 50-60 %, allowing mosquitoes to survive longer. Rainfall expands mosquito breeding grounds, and mosquito vector disease cases surge in many tropical places during the rainy season. Furthermore, because mosquitoes must survive long enough for parasites to complete their development within them, environmental factors

affecting their longevity can influence sickness incidence, according to the study. (Sallam et al., 2017). Mosquitoes survive longer in the rainy season than in the dry season because of the favorable humidity caused by the rains, making them more likely to acquire infections during blood meals and hence porous owing to ovipositional activities (Idris et al., 2014).

Mosquito reproduction, human-biting behavior, and survival are all enhanced during the rainy season, and mosquito larvae concentrations in larval environments increased throughout the rainy season and declined during the dry season, while peak densities differed by habitat type. Seasonal impacts on *Anophele* mosquito sporozoite infection and entomological inoculation rates have been found in a similar way (Omalu et al., 2015). Throughout the rainy season, there is a lot of rain, which leads to a lot of mosquitoes' breeding areas, as well as a lot of humidity, which is good for adult mosquito existence and spreading (Idris et al., 2014). *Anopheles gambiae*, according to Dida et al., (2018), prefers to breed in temporary sunlight ground pools, swamps formed by rainfall, and thus the population density of the species is matched to the precipitation release, which accounts for the larval and pupal stages' growth.

Oneeb et al., (2016), found that month-wise relative abundance of *Anophele* species was highest in July and lowest in December in their report on the seasonal distribution of mosquitoes and their interaction with meteorological factors. Mosquitoes are more plentiful during the rainy season, especially in July, and then again at the beginning of the post-monsoon season, in September and October. Mosquito abundance fell during the late monsoon season as rainfall and temperature decreased, with the lowest abundance in December, and on the rise again as the dry season draws to a close, this time from March to June, due to increasing rainfall (Seah et al., 2021).

2.7 The impact of teneral reserve in the development of wings for vectorial capacity in mosquito

The dietary environment larvae encounter has a significant impact on female fitness-related features such as body growth, teneral metabolic and reserves fecundity, flight, formation of new tissues and organs, blood meal intake, and insect utilization (Kang et al., 2017; Sasmita et al., 2019). The accumulation of resources during the larval stages of mosquitoes determines the energy reserves in adults. Energy reserves can be considered a life-history trait because they are linked to a mosquito's durability and fecundity. Mosquito body sizes (pupal weight, adult weight, wing length, and so on) are also controlled by larval food resources, hence larval habitat food resources play an important role in mosquito life cycle and abundance (Mohan et al., 2017; Sultana et al., 2021).

According to Kang et al., (2017), Ukubuiwe et al., (2018), most adult life features, such as survival, flight, vitellogenesis, and longevity, are dependent on the accumulation, distribution, and use of teneral reserve (lipid, glycogen, glucose, and protein) during juvenile development. The mobilization of teneral reserve components throughout mosquito life stages (first, second, third, and fourth larval stages, pupae, and adults) is important because these teneral components are needed for a variety of immature and post-immature life activities (Ukubuiwe et al., 2018; Zirbel & Alto, 2018). Lack of adequate nutrition during mosquito larval development may result in delayed or failed development, as well as the output of adults with inadequate nutritional stores, making pathogen transmission impossible (Nascimento et al., 2021).

The higher the protein, glycogen, sugar, and lipid reserves during emergence for a specific mosquito species, the greater the fertility and lifespan, and hence adds to the insect's health. Because little, starving mosquito larvae have lower energy stores