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College of Medical, Veterinary and Life Sciences,

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# Aquatic Ecology of the Malaria Vector, *Anopheles funestus* in South-Eastern Tanzania

Submitted in fulfilment of the requirements for the degreeof

Doctor of Philosophy

By

**Najat Feruzi Kahamba**

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*“He is the One who made the earth a cradle for you, and made in its roadways for you to move, and sent down water from the sky. By that water, We brought forth with it pairs of different vegetation."*

*~ Quran 20:53*

*“By time, indeed, mankind is in loss, except for those who have believed and done righteous deeds and advised each other to truth and advised each other to patience.”*

*~ Quran 103:1-3*

*“He gives wisdom to whom He wills, and whoever has been given wisdom has certainly been given much good. And none will remember except those of understanding.”*

*~ Quran 2:269*

*“No white is superior to black, and no black is superior to white, none has the superiority over another except by good actions”*

*~ Muhammad saw*

*“Love is a guiding light and source of strength. It keeps us going through the toughest times, giving us the courage to face challenges and the resilience to overcome them. In the warmth of love, we find the energy to persevere and the inspiration to achieve our goals.”*

*~ Najat Kahamba*

*“If you enter this world knowing you are loved and you leave this world knowing the same, then everything that happens in between can be dealt with.”*

*~ Michael Jackson*

*“To give someone a piece of your heart, is worth more than all the wealth in the world.”*

*~ Michael Jackson*

# Summary

When I was in primary school, I learned that female Anopheline mosquitoes are responsible for malaria transmission. Then, just few years ago, I learned that the *Anopheles funestus* species stands out as a significant contributor to malaria transmission, particularly in East and Southern African countries. This sparked my curiosity about why this specific mosquito species is not targeted in malaria control efforts. I learned that *An. funestus* is notoriously difficult to manage for several reasons: it is challenging to keep in laboratory settings, it has a long lifespan and high resilience compared to other mosquitoes like *Anopheles arabiensis*, and its ecology and survival strategies are not very well understood. These complexities make controlling this mosquito especially difficult. Given these challenges, my PhD research focused on one aspect of enhancing our understanding of the aquatic ecology of *An. funestus*. The primary goal of my PhD project was to investigate the aquatic ecology of *An. funestus* and to explore community perspectives on Larval Source Management (LSM) to improve malaria control strategies in Tanzania.

To achieve this goal, I had the following specific objectives: 1) To conduct a literature review on the ecology of *An. funestus*; with the goal of highlighting knowledge gaps and identifying how current understanding can be used to improve malaria control in areas where *An. funestus* is the dominant vector, 2) To investigate the use of aquatic habitats by *An. funestus* larvae during the dry season and test how this is associated with environmental and land use factors, 3) To assess the seasonal variation in aquatic habitat availability and use by the malaria vector *An. funestus*, 4) To assess the nutritional reserve, survival, and insecticide susceptibility status of *An. funestus* adults emerging from different aquatic habitats, 5) To assess the seasonal variation in habitat use (both larvae and adult) by *An. funestus*, 6) To explore the societal uses of the main water bodies inhabited by this vector and the implications for Larval Source Management. All of PhD objectives were conducted in south-eastern Tanzania, and each objective is addressed in a separate chapter.

The first objective (Chapter 2) was to identify critical knowledge gaps and explore strategies to leverage existing understanding for enhanced malaria control. Here I highlighted unique ecological traits and vulnerabilities of *An. funestus* that, if exploited, could significantly reduce malaria transmission in areas where it is dominant. Key knowledge gaps identified include limited knowledge of the aquatic ecology, the specific ecological conditions that favour *An. funestus* proliferation, and the species' behavioural adaptations that may influence its response to control measures. These gaps shaped the subsequent research presented in Chapters 3 to 7, focusing on detailed ecological observations of the aquatic ecology of this vector to improve targeted intervention strategies.

The second objective (Chapter 3) involved utilizing satellite imagery, field surveys, and geospatial modelling to map and characterize the habitats of *An. funestus*. Here I identified environmental and land use factors influencing the distribution and use of aquatic habitats by *An. funestus* in 18 villages during the dry season. Results indicated that *An. funestus* larvae predominantly occupied river streams and ground pools, with their presence associated with clear water, shading, and forested areas. These findings were used to develop a habitat suitability model, providing critical insights for targeted vector control efforts during the dry season.

The third objective (chapter 4) building from the second objective, here I investigated whether the pattern of larval habitat availability and use shifted between the dry and wet season. To test this, I conducted another cross-sectional surveys of *An. funestus* larvae during both wet and dry seasons in five villages in southeastern Tanzania. Results showed that while *An. funestus* predominantly occupies permanent or semi-permanent aquatic habitats in both seasons, there is a higher diversity and number of habitats used during the wet season compared to the dry season. However, a greater proportion of available habitats were inhabited by *An. funestus* in the dry season. These findings highlight the need for flexible and adaptive LSM strategies that target permanent habitats during dry months and accommodate a diverse range of habitats in wet seasons.

Building on results of larval habitat use (Chapter 3 & 4), the fourth objective (chapter 5) aimed to test if adult *An. funestus* fitness and ability to contribute to transmission varies in response to larval habitat type. This was done by assessing the survival, insecticide susceptibility, body size and energetic reserves of adult *An. funestus* emerging from various aquatic habitats across seasons. The results here indicated a significant variation in some mosquito fitness traits between larval habitat types. For instance, mosquitoes from rice field habitats had higher nutritional reserves than ground pools and river streams, although this did not correspond to increased survival rates. In contrast, ground pools produced mosquitoes with better survival rates and higher insecticide resistance. The findings suggest that habitats like ground pools, which foster greater mosquito fitness and resistance, should be prioritized in LSM strategies to effectively reduce malaria transmission.

In the fifth objective (chapter 6) I tested for fine-scale longitudinal variation in the abundance and habitat use by *An. funestus* larvae and adults across a full year in two southeastern Tanzanian villages. Aims were to describe the pattern of seasonality in adult and larval dynamics, and test whether it varied between aquatic habitat (larvae) or house (adult) type. This comprehensive approach revealed significant temporal variability in *An. funestus* populations. Ground pools emerged as the most significant habitat for both larval presence and abundance, showing pronounced seasonal peaks during certain months. In contrast, other habitats like river streams, ditches, dug pits, spring-fed wells, and rice fields exhibited different seasonal dynamics, often with reduced larval densities compared to ground pools. For the adult mosquito, densities were significantly different across house types, with notable seasonal fluctuations. This indicates that effective control measures must consider the timing and type of habitats to maximize their impact.

Collectively, data I collected to address objectives 1-5 can provided a useful guidance on the times during the year and types of aquatic habitats would be most useful to prioritize LSM for control of *An. funestus*. However, this is only part of the story, as ability to implement any kind of LSM is fundamentally dependent on community acceptability and support. To explore this, my sixth objective investigated the societal uses of water bodies inhabited by malaria vectors in the communities in southeastern Tanzania where entomological surveillance was conducted.  I engaged with local communities through interviews and focus group discussions and explored how community daily practices would impact LSM efforts. This chapter revealed that, more than 90% of aquatic habitats were used by community for different purposes such as domestic chores, agriculture, livestock watering, and fishing. Focus group discussions indicated community are ready to implement LSM, with a preference for larviciding and habitat manipulation over habitat removal. Community concerns centred on the safety of larvicides for animal and human health. Here I highlighted the importance of engaging community to ensure the interventions are practical, acceptable, and sustainable.

In conclusion, my PhD research represents the first comprehensive investigation of the aquatic ecology of *An. funestus*. This foundational work provides critical insights for developing more effective malaria control interventions. By thoroughly mapping habitat utilization, assessing seasonal variations, and examining mosquito fitness and resistance profiles, this research offers a new insight on targeted vector control strategies. Additionally, by integrating ecological findings with community engagement and addressing socio-economic factors influencing Larval Source Management (LSM), this thesis presents a holistic approach to malaria control.

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# Acknowledgements

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# Author’s declaration

I, Najat Feruzi Kahamba, declare that this thesis titled, “Aquatic Ecology of Malaria Vectors, *Anopheles funestus* in Southeastern Tanzania,” and the work presented within it are entirely my own, except where otherwise stated.

I confirm that:

- This work was started and completed entirely while I was pursuing a research degree of Doctor of Philosophy at the University of Glasgow.
- I have received primary supervision and continuous support from Professor Heather Ferguson and Professor Fredros Okumu throughout my research.
- Specific chapters I have benefited the supervision from Dr. Luca Nelli, Dr. Francesco Baldini and Dr. Marceline Finda.
- I have recognized the contributions of co-authors, field and laboratory technicians, and volunteers as named in my published chapters.
- Where I have quoted from the published and unpublished work of others, the source has been properly acknowledged. With the exception of such quotations, this thesis is entirely my own work.
- I further declare that no part of this work has been submitted as part of any other degree or any other qualification at this University or any other institution.

# List of abbreviations

| | |
|---|---|
| **ACT:** | Artemisinin-based Combination Therapy |
| **ANOVA:** | Analysis of Variance |
| **ATSBs:** | Attractive Targeted Sugar Baits |
| **Bti:** | *Bacillus thuringiensis subspecies israelensis* |
| **Bs:** | *Bacillus sphaericus* |
| **CDC:** | Centre for Disease Control and Prevention |
| **CDC-LT:** | Centre for Disease Control Light Trap |
| **CI:** | Confidence Interval |
| **DDT:** | Dichlorodiphenyltrichloroethane |
| **DEM:** | Digital Elevation Model |
| **EIR:** | Entomological Inoculation Rate |
| **ESA:** | European Space Agency |
| **FANG:** | *Funestus* Angola |
| **FUMOZ:** | *Funestus* Mozambique |
| **GHSL:** | Global Human Settlement Layer |
| **GMEP:** | Global Malaria Eradication Program |
| **GPS:** | Global Positioning System |
| **HLC:** | Human Landing Catches |
| **IRGs:** | Insect Growth Regulators |
| **IRS:** | Indoor Residual Spraying |
| **ITNs:** | Insecticidal Treated Nets |
| **IVM:** | Integrated Vector Management |
| **LLINs:** | Long-Lasting Insecticidal Nets |
| **GLMM:** | Generalized Linear Mixed Models |
| **GAMM** | Generalized Additive Mixed Model |
| **LSM:** | Larval Source Management |
| **M:** | Molecular Type M |
| **MCMC:** | Markov Chain Monte Carlo |

| | |
|---|---|
| **MDA:** | Mass Drug Administration |
| **ML:** | Machine Learning |
| **MOH:** | Ministry of Health |
| **MW:** | Molecular Type MW |
| **NDVI:** | Normalized Difference Vegetation Index |
| **NDWI:** | Normalized Difference Water Index |
| **PBO:** | Piperonyl Butoxide |
| **PCR:** | Polymerase Chain Reaction |
| **PMI:** | President’s Malaria Initiative |
| **RBM:** | Roll Back Malaria |
| **RDT:** | Rapid Diagnostic Test |
| **ROC:** | Receiver Operating Characteristic |
| **RTSS/AS01 (RTSS):** | Malaria Vaccine |
| **s.l.:** | *Sensu Lato* |
| **s.s.:** | *Sensu Stricto* |
| **SSA:** | Sub-Saharan Africa |
| **SVM:** | Support Vector Machine |
| **UAVs:** | Unmanned Aerial Vehicles |
| **UNDP:** | United Nations Development Programme |
| **UNICEF:** | United Nations International Children's Emergency Fund |
| **VCM:** | Vector Control Management |
| **W:** | Molecular Type W |
| **WHO:** | World Health Organization |

# Dedication

*Dedicating this work to my parents, my daddy Feruzi Hassan Kahamba and my mom Rukia Nuru. You have always been my supporters and advisers since when I was a child, guiding me through every step of my life. To my dad, whose wisdom and encouragement have been a constant source of strength, and to my mom, who has always been there for me, offering her unconditional love and support.*

*This achievement is a testament to your influence, invaluable lessons, and belief in me.*

*And say "Lord, show Mercy to my parents as they nurtured me when I was small"*

# Chapter 1: General introduction

## 1.1 Malaria history and current global burden

Malaria remains one of the most significant vector-borne diseases affecting humans globally. The understanding of malaria transmission and control has evolved considerably since Sir Ronald Ross discovered that *Anopheles* mosquitoes are responsible for transmitting the disease in 1897 [1,2]. Early malaria control strategies focused on environmental management to reduce aquatic habitats used by mosquito larvae, and the use of insecticides such as Paris Green in larval habitats [3,4]. The Global Malaria Eradication Program (GMEP) launched by the World Health Organization (WHO) in 1955, primarily relied on a switch to targeting adult mosquito vectors by spraying DDT and other chemicals inside houses and treating people using synthetic antimalarial drugs [5,6]. While this program succeeded in eradicating malaria in some regions, it ultimately failed in tropical Africa due to logistical challenges, emerging insecticide resistance, and lack of cooperation [7].

The introduction of chloroquine in the 1960s significantly reduced malaria cases, but the eventual development of chloroquine resistance, along with other challenges such as inadequate healthcare infrastructure, socio-economic barriers, political instability, and limited access to effective treatment and prevention methods, led to a resurgence of the disease [8-11]. Renewed efforts in the 1990s and early 2000s, including initiatives like Roll Back Malaria (1998), the Global Fund (2002), and the President's Malaria Initiative (2005), contributed to a significant reduction malaria transmission [12,13]. Between 2000 and 2015, for example, malaria incidence decreased by 27% worldwide and by nearly 40% across Africa, showing the impact of these interventions [14]. However, despite these achievements, malaria remain a major public health challenge.

According to the recent World Malaria Report, there were still approximately 247 million malaria cases and 619,000 malaria-related deaths worldwide in 2022 [15]. The disease disproportionately affects children under five, who account for most malaria-related mortality [15]. Sub-Saharan Africa bears the highest impact, with 95% of all malaria morbidity and mortality occurring in this region. Tanzania, in particular, ranks among the top ten countries with the highest malaria burden in Africa [15].

## 1.2 Malaria parasite life cycle and pathology

Malaria has been difficult to eliminate, in part because of the complexity of its transmission which relies on the interaction between *Plasmodium sp*. parasites, *Anopheles* mosquito vectors, human hosts, and the environment [16]. Eradication of malaria will require consideration of all these four components. Malaria transmission starts when an infected female *Anopheles* mosquito bites a person [17,18]. The mosquito injects the *Plasmodium sp*. parasite in the form of sporozoites which migrate directly to the liver. In the liver cells, the sporozoites undergo rounds of multiple division to develop into asexual and then sexual transmission stages (gametocytes) in the blood system. Mosquitoes are infected when they feed on the blood of an infected person. Gametocytes imbibed during blood feeding will undergo multiple stages of development inside mosquito vectors (gametocyte, ookinete, oocyst) before reaching the transmissible sporozoite form. Sporozoites migrate into the salivary glands of mosquito vectors where they will be injected into the next person they bite (Fig. 1.1) [17,18].

Malaria pathology manifests in a variety of symptoms and consequences. The disease often begins with fever, headache, and joint aches, progressing to more severe issues such as anaemia, cerebral malaria, and respiratory distress. Cerebral malaria can cause seizures and coma, while severe anaemia results from the destruction of red blood cells [19]. Malaria also impacts immunity, making individuals more susceptible to other infections and complicating co-morbid conditions [20].

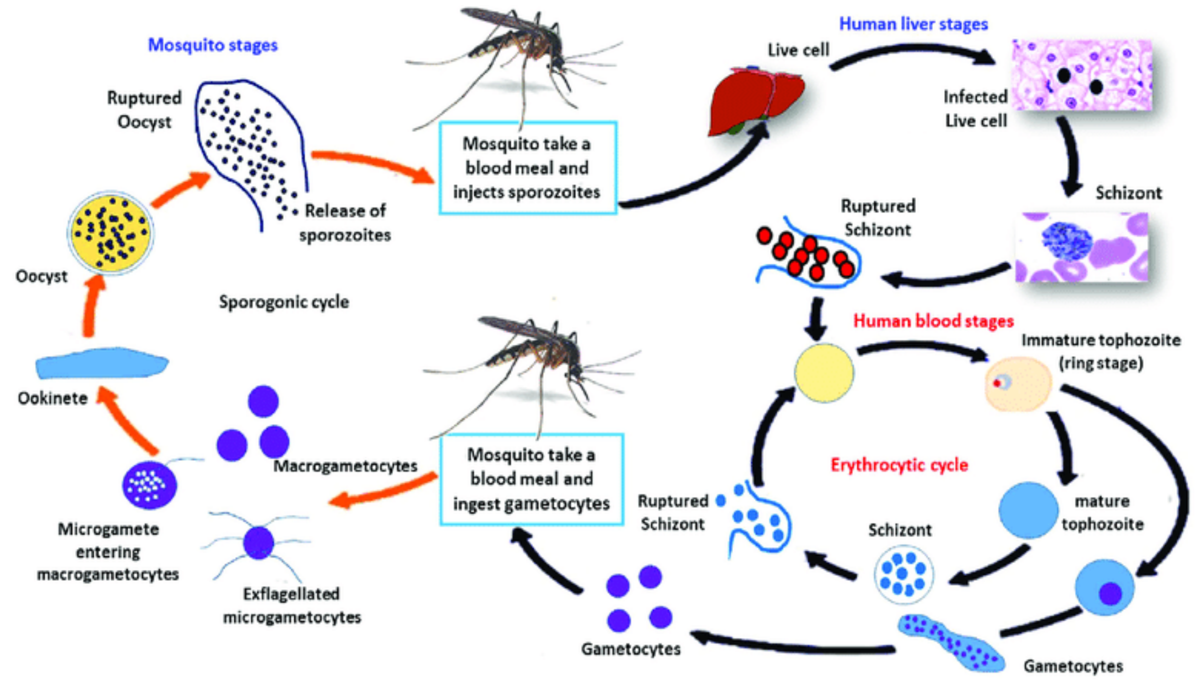

**Figure 1.1:** Sexual and asexual life cycle of plasmodium parasite. This image is adapted from [21]

## 1.3 Mosquito life cycle

The life cycle of malaria vectors and other mosquitoes consists of four stages i.e., egg, larvae, pupa, and adult. First, *Anopheles* females lay eggs in aquatic habitats including river streams, ground pools, spring fed, swarms, rice paddies, hoofprints [22-24]. The time needed for eggs to hatch into larvae, and for larvae to develop into pupae, is highly depending on the temperatures (development is slower at low temperatures) [25,26]. Adults usually emerge from the pupal stage at dusk; with both male and females feeding on sugar (nectar) for energy shortly after emergence [27,28]. Mating occurs within 1-2 days of emergence [27,29], after which females will seek a host to acquire a blood meal as needed for egg development. Once a blood meal is acquired, the female mosquito's eggs develop over a period of two to three days. After maturation, the female lays the eggs in suitable aquatic habitats, thus beginning the cycle anew (Fig. 1.2).

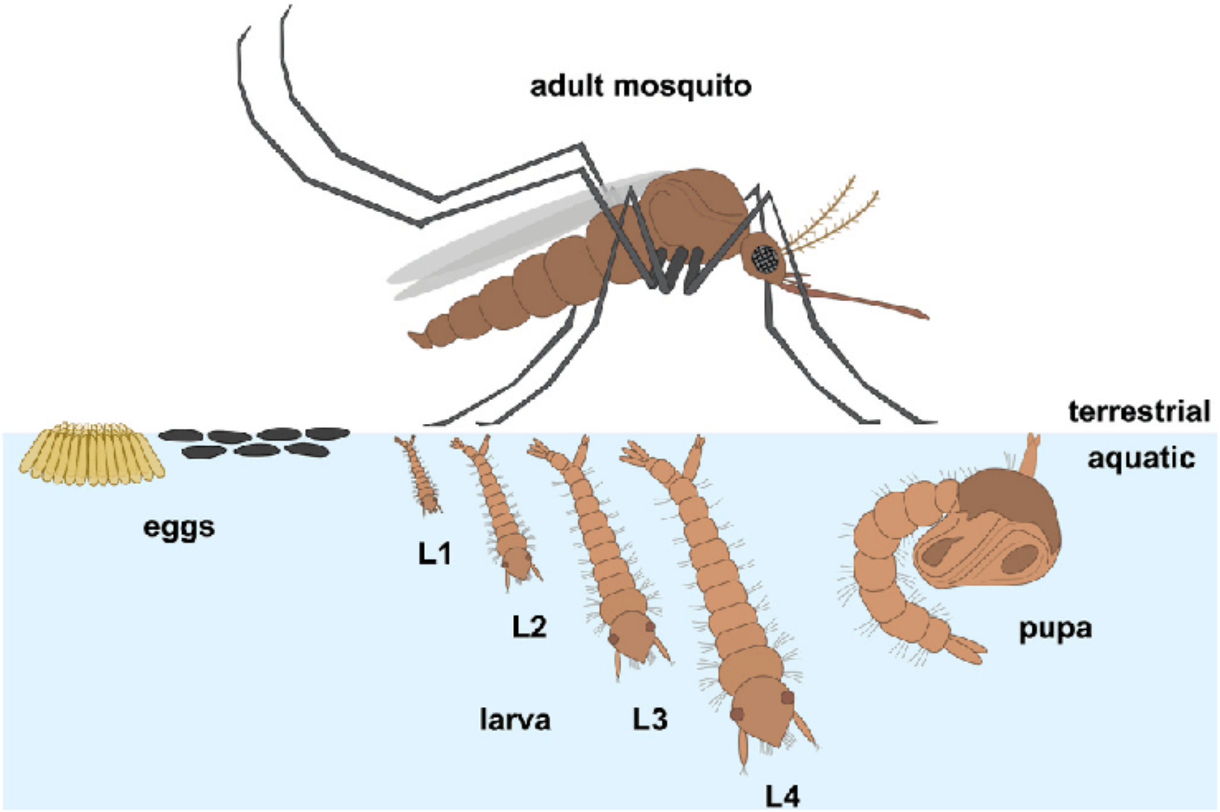

**Figure 1.2:** Mosquito life cycle. This image is adapted from [30]

## 1.4 Malaria Control

Effective malaria control can be achieved through three primary routes: drugs, vaccines, and vector control.

### 1.4.1 Drugs

Antimalarial drugs are crucial for treating and preventing malaria. They work by targeting the parasite at different stages of its life cycle. Some drug-based interventions, such as seasonal chemoprophylaxis and mass drug administration (MDA), have proven to be very effective in certain settings. However, the parasite's ability to develop resistance to these drugs poses a significant challenge [20]. Continuous monitoring and development of new drugs are essential to maintain the effectiveness of drug-based interventions.

### 1.4.2 Vaccines

There is now a malaria vaccine available, providing a new tool in the fight against the disease. The RTS, S/AS01 (RTS, S) malaria vaccine has shown partial protection against *Plasmodium falciparum* in young children [31]. While it represents a significant breakthrough, the vaccine does not provide complete immunity: its efficacy is limited, and it requires multiple doses to maintain protection [32,33]. Ongoing research aims to improve the efficacy and duration of malaria vaccines, making them a more reliable component of malaria control strategies [32].

### 1.4.3 Vector control interventions

Currently, the World Health Organization (WHO) recommends two core vector control strategies for malaria control: Indoor Residual Spraying (IRS) and Insecticidal Treated Nets (ITNs). Additionally, Larval Source Management (LSM) is recommended as supplementary methods to enhance the effectiveness of these core interventions [15].

i. *Insecticide Treated Nets (ITNs)*

These are bed nets treated with insecticides such as permethrin and deltamethrin that provide both a physical barrier to deter mosquitoes, and a chemical to repel or kill them. These nets are used while sleeping, protecting individuals from mosquito bites during peak mosquito hours biting hours (typically concentrated between 10pm-4am in African malaria vectors [34]. ITNs have played a crucial role in reducing malaria transmission, especially when widely distributed and consistently used. The use of ITNs dates back to the 1980s, with significant advancements and promotion occurring in the 1990s [5,35].

The Roll Back Malaria initiative, launched in 1998 by WHO, UNICEF, UNDP, and the World Bank, significantly boosted the use of ITNs as a key malaria control strategy [12,13,36]. The development of long-lasting insecticidal nets (LLINs), which do not require frequent retreatment, further enhanced their effectiveness and ease of use [37].

Production and distribution of ITNs are large-scale efforts involving various stakeholders. These nets are distributed globally, often through large health organizations and government programs. For example, the Global Fund and the President's Malaria Initiative (PMI) have been instrumental in financing and distributing millions of ITNs in malaria-endemic regions [38]. These coordinated efforts have significantly increased access to ITNs.

According to the WHO, more than 2.9 billion ITNs were supplied globally between 2004 and 2022, with 2.5 billion (86%) distributed in sub-Saharan Africa [15,38]. The widespread use of ITNs has significantly reduced malaria incidence. Studies have shown that ITNs have contributed to a 50% reduction in malaria cases in sub-Saharan Africa [39,40]. Additionally, UNICEF reports that the use of LLINs has been associated with a significant decrease in malaria-related deaths among children under five. The success of ITNs underscores the importance of continuous distribution and proper usage to maintain their effectiveness in malaria prevention [40]. Efforts to improve the durability and insecticide resistance of ITNs are ongoing to ensure they remain a vital tool in the fight against malaria [41,42].

### ii. *Indoor Residual Spraying (IRS)*

This intervention involves spraying the interior walls of homes with long-lasting insecticides to kill mosquitoes that come into contact with these surfaces. This method targets mosquitoes that rest indoors after blood feeding and has been highly effective in reducing malaria transmission across Africa [43,44]. Indoor residual spraying has been a cornerstone of malaria control efforts since the Global Malaria Eradication Campaign of the 1950s and 1960s [5,6]. Its effectiveness in reducing malaria incidence was demonstrated in various regions, leading to its continued use as a key intervention [15]. In more recent times, IRS has played a significant role in countries like South Africa [45], Zambia [43,46], and Ethiopia [47], contributing to substantial declines in malaria cases and deaths.

Indoor residual spraying provides community-wide protection by reducing the lifespan of mosquitoes and decreasing their capacity to transmit malaria. The use of long-lasting insecticides ensures that the protective effect of IRS can last for several months, making it a cost-effective strategy when implemented correctly. Studies have shown that IRS, when combined with other interventions such as ITNs, can lead to a significant reduction in malaria transmission [45,48]. For example, in Zambia, IRS contributed to a 62% reduction in malaria incidence between 2000 and 2015 [49]. IRS not only protects individuals within treated homes but also reduces the overall mosquito population, providing indirect protection to the wider community [49].

The effectiveness of both ITNs and IRS in malaria control is increasingly challenged by several factors. The widespread development of insecticide resistance among major vector species significantly undermines these interventions [50,51]. Additionally, ecological shifts in malaria vector species composition [52] and behavioural adaptations such as increased outdoor or early evening biting [53,54] further complicate control efforts. Proper usage and maintenance of ITNs are critical, as frequent washing and exposure to harsh conditions can degrade their effectiveness [55]. Ensuring high coverage and distribution of ITNs also poses logistical and funding challenges [15]. Similarly, IRS faces operational challenges including high costs, the need for substantial resources, and community acceptance issues [56]. A study in Dielmo, Senegal, demonstrated increased insecticide resistance in malaria vectors due to chemical usage in vector control [57,58]. Consequently, despite the significant successes of ITNs and IRS, there is a critical need for supplementary methods to address vectors that exhibit physiological and behavioural resistance [59,60]. These supplementary methods, such as Larval Source Management (LSM) and community engagement, are essential to enhance the overall effectiveness of malaria control strategies.

## 1.5 Larval source management as supplementary method

Larval source management (LSM) focuses on targeting mosquitoes during their larval stages in aquatic habitats [61]. The LSM approach includes: (i) habitat modification (eliminating potential aquatic habitats by either permanent change of land use and filling/ coverage of water bodies) [61], (ii) habitat manipulation (reducing larval habitats through temporary changes to the aquatic environment such as flushing, clearing the vegetation and expose the habitats to sunlight), (iii) biological control (introduction to natural enemies such as predators, parasites or other pathogens such as *Bacillus thuringiensis subspecies israelensis* (bti) and (iv) larviciding (application of chemical insecticides or pathogens to aquatic habitats) [61].

Larval Source Management has been deprioritized in many African countries despite its success in some localities [62,63]. Historically, LSM was an important part of the first Global Malaria Eradication Program in Africa. However, with the advent of ITNs and IRS, the focus shifted, leading to a decline in the prioritization of LSM. Effective LSM is a promising approach because it targets mosquitoes when they are confined in small pools, and approximately two-thirds of the mosquito lifecycle is spent in the aquatic stage [64]. However, the success of this approach depends on the types of larval habitats used and is only recommended as a supplementary method by the World Health Organization in cases where larval habitats are “few, fixed and easily findable” [65,66].

In some countries like Brazil in 1940s, the implementation of LSM contributed significantly to substantial reductions in transmission [67–69]. There is renewed interest in deploying LSM to tackle residual malaria transmission in African countries [61,70]. However, this will require a more thorough understanding of the larval ecology of the vector species responsible for residual transmission [63,68,71]; with a particular emphasis on identification of what types of habitats are used, their distribution in time and space, and what habitats contribute most to transmission in terms of the number and fitness of adult females they produce.

### 1.5.1 Community engagement and larval source management

Although understanding which aquatic habitats are used by mosquito larvae and when they are utilized is vital for planning LSM, this knowledge alone is not sufficient for effective implementation. Several other considerations, including logistics and financing, are required, with the most important factor being the acceptability of LSM types by local communities [72-74].

Engaging the community and understanding their perspectives are essential components of successful LSM. Community involvement can enhance the identification and management of larval habitats, ensuring that interventions are practical, culturally acceptable, and sustainable [75,76]. By integrating local knowledge and participation, LSM programs can be tailored to the specific ecological and social contexts of the target areas, thereby increasing their effectiveness and sustainability.

For example, in Tanzania, local community members were trained to identify and treat potential larval habitats, significantly contributing to the success of LSM interventions [73]. In another instance, in Kenya, local health workers were engaged to map and manage larval sites, ensuring that interventions were carried out efficiently and met the community's needs. This approach not only helps in identifying and managing oviposition sites but also fosters community ownership and cooperation, which are critical for the long-term success of malaria control efforts.

## 1.6 African malaria vector species and their distribution

Globally, over 100 Anopheline mosquito species can transmit malaria parasites, but they differ in importance for transmitting malaria [77]. In Africa, the primary vectors are *Anopheles arabiensis, Anopheles coluzzii*, and *Anopheles gambiae* from the *Gambiae* complex, and *Anopheles funestus* from the *Funestus* subgroup [78]. These species are the most significant in transmitting malaria parasites, primarily *Plasmodium falciparum* and *Plasmodium vivax* [79]. The importance of these vectors is typically measured through sporozoite infection rates and the Entomological Inoculation Rate (EIR), which indicates the frequency at which individuals are bitten by infectious mosquitoes [80].

Despite being outnumbered by *An. arabiensis* and *An. gambiae, An. funestus* remains a major contributor to malaria transmission in endemic regions [81-83]. Historical data from the 1920s-30s in South Africa showed sporozoite infection rates in *An. funestus* of up to 27% [84,85]; much higher than often reported for *An. arabiensis* and *An. gambiae* s.l in the same regions, which typically ranged below 10% [86-88]. In West Africa, sporozoite rates in *An. funestus* vary significantly, with 50% in Burkina Faso [89] and 3.3% in Senegal [90]. In East Africa, studies reported sporozoite rates of 1.71% in 2008 and 2.2% in 2012 [91]. Recent studies indicate that over 85% of malaria transmission in certain areas is attributable to *An. funestus*, underscoring its importance and highlighting its higher transmission potential compared to other vectors in the same areas [81].

*Anopheles gambiae s.l.* and *An. funestus s.l.* are both species complexes, consisting of multiple closely related species that are often indistinguishable morphologically. These complexes exhibit differences in larval ecology, host choice, biting behavior, and transmission potential. *An. gambiae* is found widely across sub-Saharan Africa, preferring temporary, sunlit water bodies and exhibiting a strong preference for human hosts, with peak nocturnal biting activity around midnight [92,93]. In contrast, *An. funestus* is associated with more permanent, shaded water bodies, adapts in host choice but often prefers humans, and tends to bite indoors throughout the night, contributing to its high transmission potential [92].

## 1.7 Research Focus

In contrast to other major African malaria vectors, such as *An. gambiae* s.l., knowledge of the ecology and fundamental biology of *An. funestus* s.l. is sparse [92]. Investigation of the ecology of *An. funestus s.l.* is particularly warranted in settings where it is responsible for the bulk of transmission. This is the case in southern Tanzania where recent studies show this vector accounts for 90% of overall residual malaria transmission [81]. In Tanzania, most of the knowledge about *An. funestus* s.l. comes from standard adult vector surveillance, which has measured its distribution, density, and sporozoite infection rates [81,94,95]. These studies have confirmed the major role of this vector in malaria transmission, particularly in the southwestern region, where the formerly dominant *An. gambiae* has almost disappeared due to the widespread use of ITNs [96].

This thesis encompasses a series of studies to investigate the determinants of *An. funestus* larval ecology and habitat use, the impact of larval habitat uses on adult fitness and transmission capacity, and seasonal variation in larval and adult habitat use. Additionally, these biological data are complemented with qualitative research on community perspectives on LSM deployments within my study area as needed to inform the feasibility of LSM. Previous studies in the area have generated valuable insights into the mating, resting, biting, and oviposition behaviours of adult *An. funestus* sl populations in Tanzania [97–99]. However, there is relatively little knowledge on their larval ecology and seasonal dynamics. Surveillance studies in Dar es Salaam and southeastern Tanzania have elucidated the distribution of *An. funestus* larvae, and some physical and chemical characteristics of their aquatic habitats [100,101]. This has provided an understanding of the range of larval habitats used by *An. funestus sl,* but less is known about the importance of different types of habitats to population maintenance, particularly during the dry season when aquatic habitat availability may be limited.

Successful implementation of LSM for *An. funestus* requires more than a basic understanding of the characteristics and types of habitats used for larval development. While it may be operationally and financially impossible to target all *An. funestus* larval habitats, identification of the most important habitat types could facilitate implementation of a more targeted and thus cost effective LSM program [102]. Larval habitats of greatest importance may not be the ones that produce most of the larvae, but rather the habitats that produce mosquitoes with the highest fitness (i.e., highest survival and reproductive capacity) which may be most epidemiologically important [103]. Given adult mosquito fitness is often negatively correlated with larval density due to resource competition [104], it is possible that low density larval habitats may produce adults with the highest survival and reproductive potential [105]. Additionally, there may be variation in insecticide resistance among mosquitoes developing in different larval habitats [106]. This variation can arise from factors such as differential exposure to pollutants or varying genetic makeup of mosquito populations. Considering insecticide resistance is crucial when deciding which habitats to target for vector control, as it may be more effective to target habitats that produce high-fitness and highly resistant mosquitoes, rather than just the most productive ones. This approach ensures that vector control efforts are not only efficient but also sustainable in the long term.

Similarly, our current understanding of the ecology of adult *An. funestus* has not yet fully explored how the seasonal dynamics of this species may vary between habitats in response to changes in aquatic habitat availability and/or the suitability of different house types. Different types of aquatic habitats become available and disappear throughout the year due to seasonal variations in rainfall. Additionally, the quality of these habitats for larvae can vary seasonally due to environmental conditions and mosquito density [107]. This seasonal variability in habitat availability and quality highlights the importance of comparing habitat use across different times of the year.

Moreover, the suitability of different house types for mosquito resting and feeding may also vary seasonally. Factors such as microclimate, including indoor humidity and temperature, can differ significantly between house types due to variations in wall materials and construction styles. These differences can cause mosquitoes to shift between house types during different seasons, seeking optimal resting and feeding conditions. For example, during warmer periods, mosquitoes may prefer houses that offer cooler microclimates, whereas in cooler periods, they may favour warmer environments.

While studies have described the basic seasonal dynamics of adult *An. funestus* and their associations with house types [99,108], the potential for seasonal dynamics to vary between habitats remains underexplored. Understanding these seasonal shifts is crucial as the proximity of the nearest larval habitats and household characteristics are key factors influencing the indoor abundance of *An. funestus* [109]. Several studies have reported a positive association between the abundance of indoor resting and host-seeking mosquitoes and the distance to the nearest aquatic habitats. Houses close to these habitats typically have higher indoor densities of mosquitoes [99]. Other key determinants of indoor mosquito densities include household characteristics such as the presence of livestock, the number of people living in the house, and the presence of eaves [81,110].

To address these gaps, my thesis has investigated the larval and adult ecology of *An. funestus* populations in southern Tanzania, with an emphasis on understanding how their use and abundance in different habitat types (aquatic larval sites and houses) varies across seasons. The importance of community engagement was recognized and incorporated into this research when it was observed that aquatic habitats were also utilized by local communities. By studying community attitudes, preferences, and practices related to LSM, the research aimed to integrate local knowledge and participation into malaria control strategies. Engaging the community was crucial for comprehending the usage of larval habitats and the potential implications for LSM deployment. Community involvement enhances the identification and management of larval habitats, ensuring that interventions are practical, culturally acceptable, and sustainable [70]. This integrated approach aims to develop a more comprehensive and effective malaria control strategy tailored to the local ecological and community contexts.

## 1.8 Overarching Objective

This thesis aimed to investigate the aquatic and adult ecology of the dominant malaria vector, *An. funestus*, which is a major vector for residual malaria transmission in Tanzania and other parts of Africa. The overall goal was to fill key knowledge gaps on the seasonal dynamics and habitat utilization of this vector, and community perceptions toward Larval source Management (LSM), which is crucial for optimizing vector control interventions. The information used in this study was collected through a combination of literature reviews, field surveys, laboratory experiments, quantitative and qualitative analysis conducted in Tanzania and University of Glasgow.

### 1.8.1 Specific Objectives

1. To conduct a literature review on the ecology of *An. funestus*; with the goal of highlighting knowledge gaps and identifying how current understanding can be used to improve malaria control in areas where *An. funestus* is the dominant vector. This objective is addressed in **Chapter 2**, which summarizes current knowledge on *An. funestus* ecology, behaviour, and control strategies, and identifies knowledge gaps and potential areas for further research. Some key gaps identified are addressed in this thesis, while others remain open questions for future work on *An. funestus*. This study has now been published [92].

2. To investigate the use of aquatic habitats by *An. funestus* larvae during the dry season in south-eastern Tanzania and how this is associated with environmental and land use factors. This objective is addressed in **Chapter 3**, which uses satellite imagery, field surveys, and geospatial modelling to map and characterize dry season habitats of *An. funestus*. Building on the gaps identified in the aquatic ecology of *An. funestus* from the review chapter, this chapter investigates the influence of habitat characteristics, land cover, and human population densities on the distribution of these habitats. The methods employed include systematic survey of water bodies for mosquito larvae, characterization of physio-chemical parameters, and the use of a generalized linear model to assess the presence of *An. funestus* larvae in relation to habitat characteristics, land use, and human population densities. This chapter developed a habitat suitability model for *An. funestus*, providing crucial insights for targeted vector control strategies, particularly during the dry season. Results of this study have now been published [22].

3. To assess the seasonal variation in aquatic habitat availability and use by larvae of the malaria vector *An. funestus*. This objective is addressed in **Chapter 4,** which investigates how *An. funestus* utilizes different aquatic habitats across wet and dry seasons. While Chapter 3 focused on habitat use by *An. funestus* during the dry season, it left unanswered whether these patterns remained

constant or shifted during the wet season with the increased abundance and diversity of aquatic habitats. To address this question, a follow-up cross-sectional survey was conducted, mapping and characterizing potential aquatic habitats, monitoring larval presence and abundance, and determining the most utilized habitats in both seasons. This study included surveys of numerous habitats and villages from the dry season study, aiming to test the seasonal consistency of habitat use by *An. funestus*.

4. To assess the survival, insecticide susceptibility status, and fitness of *An. funestus* adults emerging from different aquatic habitats and in different seasons. This objective is addressed in **Chapter 5**. While Chapters 3 and 4 focused on identifying the most frequently used and productive aquatic habitats for *An. funestus* larvae, questions remained about the epidemiological importance of adult mosquitoes emerging from these habitats. Specifically, whether these adults exhibit similar fitness and resistance to insecticides. To address this, Chapter 5 extends the research by conducting laboratory and field tests to evaluate the survival rates and insecticide resistance of adults and analysing their nutritional content. This comprehensive approach aims to determine if the habitats identified in earlier chapters produce adults with varying fitness and resistance profiles, thus providing deeper insights into their potential impact on malaria transmission.

5. To assess the seasonal variation in habitats use (both larvae and adult) by the dominant malaria vector, *An. funestus*, in south-eastern Tanzania. This objective is addressed in **Chapter 6**, which involves monitoring mosquito larvae densities in various habitat types and adult mosquito densities in different house types throughout the year. Building on the findings from Chapters 3 and 4, this chapter provided a comprehensive understanding of habitats (for both larval and adult) affected the mosquito densities. To address this, the study monitored mosquito larvae densities in diverse aquatic habitats and tracked adult mosquito densities in various house types throughout the year. The goal was to identify

seasonal trends and the factors influencing habitat selection for both larvae and adults.

6. To explore the societal uses of the main water bodies inhabited by malaria vectors and their implications for Larval Source Management (LSM). This objective is addressed in **Chapter 7**, which investigates how local communities use aquatic habitats and how these uses affect LSM strategies. Building upon observations made during entomological larval surveys in previous chapters, this chapter shifts focus to the socio-economic dimension. It examines how the local utilization of water bodies, which serve as habitats for malaria vectors, can influence the effectiveness of LSM strategies. To achieve this, I engaged with local communities through interviews and focus group discussions, exploring various local activities on mosquito aquatic sites. This approach aimed to identify the potential conflicts and synergies between community water use practices and LSM efforts. This study is under revision in Malaria journal.

**Chapter 8** provides a general discussion of key results from all chapters and how they fit together to address the overall objective of the PhD, providing an overview of general limitations, challenges, arising questions, and the suggestions for future work. Here I have also provided a policy brief drawn from this thesis to be used by National Malaria Control Program (NMCP) and policy makers in Tanzania, here it has provided the general policy implication of the major finding generated from this thesis.

# Chapter 2: Using ecological observations to improve malaria control in areas where *Anopheles funestus* is the dominant vector

This thesis started with conducting a detailed literature review to explore what is known and what are gaps in our understanding about *An. funestus*. This work is Published in Malaria Journal 2021: https://doi.org/10.1186/s12936-022-04198-3

## Abstract

The most important malaria vectors in sub-Saharan Africa are *Anopheles gambiae, Anopheles arabiensis, Anopheles funestus,* and *Anopheles coluzzii*. Of these, *An. funestus* presently dominates in many settings in east and southern Africa. While research on this vector species has been impeded by difficulties in creating laboratory colonies, available evidence suggests it has certain ecological vulnerabilities that could be strategically exploited to greatly reduce malaria transmission in areas where it dominates. This chapter reviews current knowledge on the major life-history traits of *An. funestus*, its aquatic and adult stages, and its responsiveness to key interventions. Aims were to outline plausible strategies for reducing malaria transmission by this species group and sustaining the gains over the medium to long term.

Both male and female *An. funestus* rest indoors and the females frequently feed on humans indoors, although moderate to high degrees of zoophagy can occur in areas with large livestock populations. There are also a few reports of outdoor biting by the species, highlighting a broader range of behavioural phenotypes that can be considered when designing new interventions to improve vector control. In comparison to other African malaria vectors, *An. funestus* distinctively prefers permanent and semi-permanent aquatic habitats, including river streams, ponds, swamps, and spring-fed pools. The species is therefore well-adapted to sustain its populations even during dry months and can support year-round malaria transmission.

These ecological features suggest that highly effective control of *An. funestus* could be achieved primarily through strategic combinations of species-targeted larval source management and high-quality insecticide-based methods targeting adult mosquitoes. If done consistently, such an integrated strategy has the potential to drastically reduce local populations of *An. funestus* and significantly reduce malaria transmission in areas where this vector species dominates. To sustain the gains, control programmes should be complemented with gradual environmental improvements such as house modification, as well as continuous engagements of the resident communities and other stakeholders.

## 2.1 Background

For the past twenty years, there has been increased international focus on improving malaria control and accelerating efforts towards elimination [111]. Significant progress was made until 2015, mainly due to the scale-up of effective vector control interventions including insecticide-treated nets (ITNs) and indoor residual spraying (IRS). Universal coverage of these interventions coupled with effective case management contributed most of the gains [112]. Yet the impact of these interventions appears to be flattening in sub-Saharan Africa, where malaria accounts for 95% of cases and 96% of deaths [111]. Further progress with these existing core vector control interventions is now limited by various mosquito adaptations notably resistance to public health insecticides and behavioural adaptations [94,113]. Other challenges include low levels of funding for malaria and general weaknesses in the health systems.

In addition to the constraints generated by evolutionary adaptations and socio-economic factors, the impact of vector control is hindered by ecological heterogeneity in how vectors, parasites, and human hosts interact with one another and the environment [16]. For instance, different vector species require different ecological conditions to complete vital life cycle processes such as oviposition, larval development, mating, and blood-feeding. Specifically, vector species may vary in their use and preference of sugar sources, hosts, larval habitats, or resting sites [64].

Unfortunately, such species-specific differences are rarely considered when implementing vector control, with the two core interventions of IRS and ITNs being similarly recommended for all the major African vector species and across most settings [111]. This "one size fits all" approach may simplify the deployment and scale-up vector control programmes, but it is erroneous to assume that all vector species are vulnerable and respond similarly to these and other interventions [71]. For example, indoor interventions such as ITNs and IRS are very effective against mosquitoes that mostly bite humans indoors and rest indoors but are less effective against exophilic and zoophagic species [114,115]. Given the increasing recognition of the role of outdoor-biting, outdoor-resting and zoophagic species in maintaining residual transmission [114], it is important that interventions target all relevant ecological and behavioural adaptations of key vector species [71].

The major malaria vectors in sub-Saharan Africa (SSA) include *Anopheles coluzzii, Anopheles gambiae sensu stricto (s.s.), Anopheles funestus s.s.,* and *Anopheles arabiensis*, but several others also play secondary role in specific localities [77]. These vector species differ in bionomics, vectorial capacities, and contribution to overall transmission, resulting in varying stability of malaria transmission across geographies [116]. The importance of *An. funestus s.s.* (hereafter is referred to simply as *An. funestus)* as a dominant malaria vector has been documented in many east and southern African countries [81,91,117-120]. In locations such as south-eastern Tanzania [81,95], and in some districts in northern Tanzania around Lake Victoria [121], this species is responsible for 85-97% of all malaria transmission events. In addition to having relatively high sporozoite prevalence and high vectorial capacity, *An. funestus* has also been shown to be highly resistant to insecticides [121], have long adult survival [122], and be more anthropophilic [82] than co-existing vector species in several settings. Consequently, *An. funestus* may have amongst the highest vectorial capacity of all African vector species.

The disproportionate role of *An. funestus* reflects the basic Pareto distribution, with most of the transmission coming from this species even in areas where it has relatively lower abundance in the overall vector community [123]. The dominance of *An. funestus* as a vector suggests that prioritizing the species for control may yield significant suppression or even local elimination of transmission [81]. More targeted strategies against *An. funestus* would require an improved understanding of the biology and ecology of the species, which remains challenging and relatively neglected due to the complexities of studying this species in the laboratory and in the wild [124,125]. Together with the difficulties in creating laboratory colonies of the species, the above constraints have led to major knowledge gaps. These gaps are often bridged in intervention or modelling studies by assuming that information from other African vectors, for example *An. gambiae sensu lato* (*s.l.*), are broadly transferrable to *An. funestus*.

This article challenges this assumption of generalizability with other African vector species by synthesizing the existing knowledge on the life history, behaviour, and ecology (larval and adult) of *An. funestus*. The article highlights key knowledge gaps in the current understanding of this species and highlights areas of its ecology that may generate differential responsiveness to key interventions. Based on these insights, plausible strategies are presented for significantly disrupting malaria transmission in areas where *An. funestus* dominates through the implementation of combined interventions tailored to its ecology.

## 2.2 Distribution and importance of *Anopheles funestus* in the east and southern Africa

The *An. funestus* group consists of at least 11 known species whose distribution extends across sub-Saharan Africa [77]. The members of this group include *Anopheles funestus* (*s.s.*), *Anopheles vaneedeni*, *Anopheles parensis*, *Anopheles aruni*, *Anopheles confusus*, *Anopheles rivulorum*, *Anopheles fuscivenosus*, *Anopheles leesoni*, and *Anopheles brucei* [126,127]. Additional species recently included are *An. funestus*-like, which were identified in Malawi [128] and *An. rivulorum*-like, identified in Cameroon [127,129]. Other studies from different locations suggest a further subdivision of *An. funestus* into three geographically distinct molecular types (M, W, MW), with the M-type found in eastern Africa, W in western and central Africa and MW present in southern Africa [130]. However, more than one molecular form has been reported in some locations [130]. For example, all three types have been found in Malawi, both M and MW-types in Tanzania, and the M and W-type in Kenya [130]. Furthermore, recently two more types have been described: Y from Malawi and Z from four locations of Angola, Malawi, Ghana, and Zambia [131].

The sibling species in the *An. funestus* group appear to have different biology and roles in malaria transmission. They are also morphologically similar at the adult stage, making differentiation requiring molecular identification [132]. However highly skilled taxonomists can separate species based on immature aquatic stage morphology [133,134]. Given the limited capacity for molecular identification in many settings, many members in the group can easily be misidentified [126], potentially leading to the potential role of other species within the *funestus* group being misunderstood.

However, to date *An. funestus s.s* remains the most significant vector in this group. Data from east Africa, where *An. funestus* is now highly resistant to common public health insecticides [135], indicates very high sporozoite infection rates compared to other *Anopheles* vector species [81,121]. In these locations, it is evidently responsible for most of the transmission as measured by entomological inoculation rates (EIR). Higher sporozoite prevalence than *An. arabiensis* has also been reported in Zambia [136], Malawi [13], and the islands of Madagascar [137]. Beyond East and southern Africa, *An. funestus* is also an important vector in Central and West Africa. In west African countries such as Ghana [138], Côte d'Ivoire [139], and Benin [140], *An. funestus* has been reported alongside other species such as *An. gambiae* and *An. coluzzii*. Table 1 provides examples of selected studies from different African countries, where the species has been investigated, and its importance in malaria transmission described. These studies broadly show that *An. funestus* typically has among the highest infections rates amongst malaria vector species (Table 2.1).

Most other species in the *An. funestus* group are not known to be malaria vectors. However, for malaria transmission, *An. rivulorum* has been incriminated in some locations in Tanzania and Kenya [81,141,142]. In South Africa, both *An. vaneedeni* and *An.*

*parensis* have been shown to contribute to residual malaria transmission [143]. Another study in Kenya did not provide evidence of *An. parensis* supporting transmission, although this species was commonly found resting indoors, it was mainly fed on cows and uninfected with malaria parasites [144]. In South Africa, indoor densities of *An. parensis* outnumbered *An. funestus* following extended IRS campaigns [143] and thus, the role of the former species in sustaining residual malaria transmission needs to be determined. Another member of *An. funestus* group previously incriminated in transmission was *An. leesoni* in eastern Tanzania [145]. Overall, there has been very limited investigations of these other sibling species and their involvement in malaria transmission, and they are rarely identified or screened during routine entomological surveillance.

**Table 2.1:** Examples of some studies in Africa showing the role of different *Anopheles* species in malaria transmission

| SN | Country | Year | Dominant vector[s] | Other *Anopheles* | Sporozoite prevalence | EIR contribution | Feeding habits | Human blood index | Resistance status and mechanism of resistance detected | Ref |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Tanzania | 2021 | *An. funestus* | *An. arabiensis*<br>*An. parensis,*<br>*An. rivulorum,*<br>*An. gambiae s.s* | Not reported | *An. funestus s.l* (96.47%)<br>*An. gambiae s.l* (3.53%) | *An. funestus:* endophilic<br>*An. arabiensis* exophilic | Not reported | *An. funestus*: resistant to pyrethroids<br>*L1014S-Kdr mutation detected in An. gambiae s.s.* | 121 |
| 2. | Tanzania | 2018 | *An. funestus* | *An. arabiensis*<br>*An. coustani* | *An. funestus* (0.205%)<br>*An. arabiensis* (0%) | *An. funestus* (100%)<br>*An. arabiensis* (0%) | Not reported | Not reported | *An. arabiensis* confirmed resistance toward pyrethroid | 95 |
| 3. | Zambia | 2017 | *An. funestus* | *An. leesoni*<br>*An. gambiae s.s* | *An. funestus* (2.7 %)<br>*An. gambiae s.s* (3.1 %) | *An. funestus* (87.03%)<br>*An. gambiae s.s* (19.97%) | Not reported | *An. funestus* (3.2%)<br>*An. gambiae s.s,* (25.7%) | Not reported | 136 |
| 4. | Tanzania | 2017 | *An. funestus* | *An. arabiensis*<br>*An. leesoni*<br>*An. rivulorum*<br>*An. pharoensis*<br>*An. squamosus*<br>*An. ziemanni*<br>*An. wellcomei* | *An. arabiensis,* (0.0002%)<br>*An funestus,* (0.0053%) | *An. funestus* (86.21%)<br>*An. arabiensis* (13.79%) | *An. funestus* anthropophagic | *An. funestus* (100%)<br>*An. leesoni* (100%)<br>*An. arabiensis* (73.4%) | *An. funestus* resistance to deltamethrin, permethrin, lambda cyhalothrin and DDT confirmed<br>Susceptible to Pirimiphos-methyl, malathion and dieldrin | 81 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **4.** | Kenya | 2011 | *An. funestus s.l.* | *An. gambiae s.s*<br>*An. arabiensis* | *An. funestus* (0.0057%)<br>*An. gambiae* (0.0043%) | *An funestus s.l* 63.6%<br>*An. gambiae s.l* 36.3% | Not reported | *An funestus s.l* (94.1 %)<br>*An. gambiae* (83.9 %) | Not reported | 146 |
| **5.** | Madagascar | 2010 | *An. funestus* | *An. gambiae*<br>*An. mascarensis* | *An. funestus* (1.58%)<br>*An. gambiae* s.l. (0.48%)<br>An. mascarensis (0.75%) | *An. funestus* (77.3 %)<br>*An. gambiae* (6.47%)<br>*An. mascarensis* (16.19%) | *An. funestus* Anthropophagic<br>*An. gambiae* Anthropophagic | Not reported | Not reported | 137 |
| **6.** | Kenya | 2017 | *An. funestus* | *An. arabiensis*<br>*An. gambiae s.s*<br>*An. coustani*<br>*An. pharoensis* | *An. funestus* (1.8%)<br>*An. arabiensis* (0.16%) | *An. funestus* (63.6%)<br>*An. arabiensis* (36.3%) | *An. funestus* anthropophagic<br>*An. arabiensis* exophilic and zoophagic | *An. funestus* (60%)<br>*An. arabiensis* (2.5%)<br>*An. gambiae* (50%) | Not reported | 147 |
| **7** | Benin | 2019 | *An. arabiensis* | *An. funestus s.s*<br>*An. coluzzii*<br>*An. gambiae s.s*<br>*An. ziemani*<br>*An. pharaonis* | *An. funestus* (0.048%)<br>*An. gambiae* s.l (0.017%)<br>*An. nilli* (0.0125%) | *An. funestus* (5.86 %)<br>*An. gambiae* s.l (82.2%)<br>*An. nilli* (11.9%) | Not reported | *An. gambiae* s.l (91.3%) | Not reported | 140 |
| **8.** | Rwanda | 2018 | *An. gambiae* | *An. funestus*<br>*An. ziemanni*<br>*An. coustani* | *An. gambiae* s.l (2.79%) | *An. gambiae* s.l (100%) | *An. gambiae* s.l endophily | Not reported | Not reported | 148 |
| **9.** | Ethiopia | 2017 | *An. arabiensis* | *An.funestus* s.l,<br>*An. demeilloni*<br>*An. cinereus,* | *An. arabiensis* (3%) | *An. arabiensis* (100%)<br>*An. demeilloni* (0%) | Not reported | Not reported | Not reported | 149 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | *An. pharoensis,* | *An. demeilloni,* (0%) | | | | | |
| **10.** | Ethiopia | 2017 | *An. arabiensis* | *An. funestus s.l.*<br>*An. coluzi*<br>*An. pharoensis* | *An. funestus* (2.3%)<br>*An. arabiensis* (4.1%)<br>*An. pharoensis* (4.5%) | *An. funestus* (22.6%)<br>*An. arabiensis* (61.5%)<br>*An. pharoensis* (15.7%) | *An. funestus* Anthropophagic<br>*An. arabiensis* Anthropophagic | *An. funestus* s.l. (87.2%)<br>*An. arabiensis* (82.4%) | Not reported | 150 |
| **11.** | Côte d'Ivoire | 2015 | *An. gambiae* | *An. funestus*<br>*An. nilli*<br>*An. pharoensis*<br>*An. coustani*<br>*An. ziemanni*<br>*An. wellcomei*<br>*An. brohieri* | *An. funestus* (1.3%)<br>*An. gambiae* (2.5%) | *An. funestus* (7.85%)<br>*An. gambiae* (92.15%) | Not reported | Not reported | Not reported | 139 |
| **13.** | Ghana | 2012 | *An. gambiae s.s* | *An. arabiensis,*<br>*An. funestus*<br>*An. pharoensis* | *An. gambiae s.s.* (1.52%)<br>*An. funestus* (0%) | *An. gambiae* s.s. (100%)<br>*An. funestus* (0%) | Not reported | *An. gambiae* s.s. (66.67%) | Not reported | 138 |
| **14.** | Chad | 2009 | *An. arabiensis* | *An. pharoensi*<br>*An. funestus*<br>*An. ziemann* | *An. arabiensis* (1.4%)<br>*An. funestus* (1.4%)<br>*An. pharoensi* (0.8%) | *An. arabiensis* (84.5%)<br>*An. pharoensis* (12.2%)<br>*An. funestus* (2.5%)<br>An. ziemanni (0.8%) | *An. arabiensis,* endophagic<br>*An. funestus* endophagic | *An. funestus* (90.6%)<br>*An. pharoensis* (71.4%)<br>*An. arabiensis* (63.9%) | Not reported | 151 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | *An. ziemann* (0.5%) | | | | | |
| **15.** | Cameroon | 2005 | *An. gambiae* | *An. moucheti*<br>*An. funestus* | *An. gambiae* (15.3%)<br>*An. moucheti* (3.4%)<br>*An. funestus* (17.0%) | *An. gambiae* (84%)<br>*An. moucheti* (11%)<br>*An. funestus* (5%) | Not reported | Not reported | Not reported | 152 |
| **16.** | Nigeria | 2010 | *An. gambiae s.s* | *An. melas*<br>*An. nilli* | *An. gambiae s.s (42.5%)*<br>*An. melas (57.5%)*<br>*An. nili (0%)* | *An. gambiae s.s (83%)*<br>*An. melas (17%)*<br>*An. nili (0%)* | Not reported | *An. gambiae s.s (63.3%)*<br>*An. melas (73.8%)*<br>*An. nili (0%)* | Not reported | 153 |

*N.B These papers were randomly selected as examples to show the reported importance of An. funestus in malaria transmission in different settings in Africa. The search was done intentionally to provide examples of reported importance in different setting.*

## 2.3 Larval ecology of *Anopheles funestus*

Even though there has only been a small number of studies that specifically focused on the larval ecology of *An. funestus* [23,24,154,155], there are several field investigations that have revealed that *An. funestus* larvae can co-exist with other malaria vectors [156]. In early work done in the 1930s, *An. funestus* was observed to oviposit in clear permanent water bodies including swamps, streams, ditches and ponds [101]. Aquatic habitats containing their larvae were characterized as being shaded by hanging trees, bushes, or emergent vegetation [101]. Another early study from Malindi in the east coast of Kenya reported the rare occurrence of *An. funestus* in wells and domestic water containers around houses [157].

A distinct feature of *An. funestus* larval ecology is that this species is reported to occupy larger and more permanent or semi-permanent water bodies than other malaria vectors [23]; often characterized with emergent or floating vegetation. These habitats generally do not have direct sunlight exposure [23]. *Anopheles funestus* is indeed rarely found in completely open waters or in small sunlit puddles [61], contrary to other African vector species, such as *Anopheles arabiensis* and *An. gambiae*, which frequently use small or temporary habitats such as water-filled footprints [159,160]. The differential use of larval habitats has been associated with distinct seasonality in malaria transmission, with *An. gambiae s.l.* driving the large transmission peaks occurring in the rainy season, while An. *funestus* being more able to sustain high levels of malaria transmission throughout the year [81]. Indeed, field observations in eastern Africa have shown that the adult population of this species often peak start of the dry season [81,161].

The permanent larval habitats used by *An. funestus* include slow-moving water along the edges of rivers, especially in tributaries found on high altitudes [101,162]. In Tanzania, Nambunga et al. [101] categorized larval habitats used by *An. funestus* into 3 types: i) small ponds and spring-fed wells found at low altitudes (150-200 m), ii) slow-moving waters along rivers and streams at higher altitudes (above 300 m) and iii) large open ponds that maintain water for most of the year in both low and high-altitude areas. The most prolific of these habitats were the rivers and streams [101]. Elsewhere in east Africa, *An. funestus* has also been observed breeding in lakeshore pools during periods of low water [163], while in west Africa this species has mostly been described as breeding in river tributaries [164] (Fig. 2.1). These larval habitat descriptions are mostly specific to *An. funestus*. However, other sibling species such as *An. rivulorum, An. leesoni,* and *An. parensis* have been observed to share aquatic habitats with *An. funestus* [132], though there can be differences in their level of tolerance to salinity [165]. Consequently, larval source management (LSM) targeted *An. funestus* could potentially also impact other secondary vector species in this group.

The overall survival and development of *Anopheles* larvae are influenced by several biotic and abiotic factors including the availability of nutrients, larval densities, and predation [166]. For instance, mosquito larvae developing in crowded habitats often have reduced body size, as well as reduced lipid, glycogen, and protein contents due to increased intra-specific competition for resources [167]. Larval development is also very sensitive to climatic conditions; with varying sensitivity to temperatures and rainfall [105] as well as salinity [165]. In particular, *An. funestus* larvae tend to be more sensitive to fluctuations in water temperatures than other vector species [168], which partly explains why the species often occupies larger perennial habitats with less microclimatic fluctuations than small temporary habitats [105,168]. The optimum temperature for *An. funestus* larval development is 27 °C, however survival declines when temperature approach 32 °C and lower to 18 °C [25]. Rainfall tends to refill habitats and perpetuates vector populations whereas the cumulative lag (two weeks) rainfall increases survival [25]. However, excessive downpours and flooding can destroy larval habitats and flush out larvae, eggs, and pupae [125].

**Figure 2.1:** Examples of common aquatic habitat types for *An. funestus* in Kenya, Cameroon, and southern Tanzania. Pictures were adapted from published articles by Kweka et al. [159] and Nambunga et al. [101].

## 2.4 Adult ecology of *Anopheles funestus*: behaviour, important life-history traits, and survival strategies

The behaviours of adult *Anopheles* have a direct impact on their vectorial capacity, a measure that describes the transmission potential of a vector in terms of its abundance, survival, ability to transmit pathogens and rate of feeding on humans [169]. Vector species that adapted to specialize on humans are more efficient transmitters of human malaria than those with opportunistic or generalist feeding behaviours [170]. *Anopheles funestus* is usually highly endophilic (refers to a tendency of indoor resting) and anthropophilic (refers to a tendency of feeding on humans), giving rise to its high vectorial capacity amongst African vectors [82]. The proportion of blood meals that mosquitoes obtain from humans as opposed to other vertebrates, i.e., the human blood index (HBI) is often reported to be higher in *An. funestus* and *An. gambiae s.s.* than in other African malaria vectors [54]. This explains their competency as vectors of malaria, and the stability of malaria in tropical Africa where these species are present [116,171].

With regard to their blood-feeding and resting habits, *An. funestus* is often assumed to be most similar to *An. gambiae s.s.* in being highly endophilic [172]. However, there are accounts of *An. funestu*s biting outdoors [161], resting outdoors [68], and being attracted to cattle [173,174]. Modest levels of zoophagy have been documented in some cattle-keeping communities [170]. As molecular identification was not performed to confirm species identity in some previous literature, other morphologically cryptic species within the *An. funestus s.l.* might be responsible for these reports of exophily and zoophily. Consequently, the existence and potential importance of outdoor biting in this species may have been underexplored and may need to be updated. For example, *An. rivulorum* is a species that is morphologically similar to *An. funestus*, but more associated with exophilic and endophilic behaviours [141]. However, a recent study, after molecular characterization, it was confirmed that *An. funestus* were attracted to both humans and cattle [174]; suggesting that some degree of zoophagy may occur in this species [173].

*Anopheles funestus,* like other *Anopheles* species, mates in aerial swarms. In comparison to *An. gambiae s.l.* the swarms of *An. funestus* tend to be smaller and more difficult to locate [175,176]. *Anopheles funestus* is refractory to mating in confined spaces, and instead appears to require large open spaces to mate [64,177]. In Tanzania [175] and Mozambique [178], where *An. funestus* swarms have been characterized, males were observed to congregate close to human dwellings inside villages, unlike swarms of *An. arabiensis* that are generally found at the edges of the village. While *An. funestus* is thought to primarily mate outdoors, new evidence indicates that significant proportions of mating in both *An. funestus* and *An. arabiensis* can occur inside homes [179], corroborating previous observations of *An. gambiae s.l.* mating inside experimental huts in west Africa [180]. While the ecological significance of such indoor mating remains to be elucidated, the observation of large densities of male *An. funestus* resting inside houses suggests it might be a common occurrence [179]. Furthermore, because of the apparent high degree of eurygamy (a reproductive strategy where mating occurs between individuals from different populations or groups), inducing mating in the laboratory is very difficult. As a result, there have been relatively few successful efforts to colonize *An. funestus*, with just two well-established colonies in existence from Angola (FANG) [181] and Mozambique (FUMOZ) [182] and FUTAZ (Hape et al unpublished). Given the complexity associated with mosquito mating behaviours, further research should be conducted to address this challenge [124]. There are currently ongoing attempts in Tanzania towards these objectives, though this has initially focused on assessing key fitness and survival parameters of *An. funestus* [124,125].

The survival of adult female mosquitoes is a crucial determinant for their vector capacity since the mosquito must survive for at least 10-12 days to be able to transmit malaria parasites [64]. Unfortunately, direct measurement of adult mosquito survival in the field is difficult, and only a small number of methods are available to estimate it through indirect measures such as mark-recapture or ovarian dissection [64]. Such reliability of estimates can vary depending on factors such as variations in the technical skill of the personnel and the widespread use of insecticidal interventions such as ITNs in the field. Nonetheless, the limited amount of available evidence suggests that *An. funestus* has greater adult survival than other malaria vectors such as *An. arabiensis* [178,183]. In Tanzania, the daily survival probabilities of this species estimated before wide-scale ITNs use were consistently greater than 80% [96]. More recent estimates of age structure based on parity dissections suggest *An. funestus* survival is greater than *An. arabiensis* in some settings [184]. This greater longevity of *An. funestus* in combination with its anthropophilic behaviours provide multiple opportunities for this vector to become infected and transmit malaria.

Lastly, changes in climatic conditions may also have a substantial influence on the survival and longevity of *An. funestus*. For instance, very low and high temperatures influence their development and survival [185]. Unfortunately, there has been little research examining the direct effect of temperature on *An. funestus* life-history characteristics.

## 2.5 Exploiting the ecology of *Anopheles funestus* to improve malaria control in areas where the species dominates.

### 2.5.1 Larval source management (LSM)

There are four main strategies for LSM; 1) habitat modification which refers to alterations made to the environments to limit vector breeding, 2) habitat manipulation, which refers to repeated activities that remove the larvae, such as flushing streams, 3) larviciding, which refers to regular application of insecticides to water bodies where mosquitoes breed, and 4) biological control, which refers to the introduction of natural predators such as larvivorous fish into aquatic habitats. The suitability of each approach depends on the local ecology of the main malaria vector, as well as environmental conditions. For example, the temporary, small, and scattered larval habitats of *An. gambiae s.s.* could perhaps be simply dried up, covered, or removed (i.e., habitats modification). On the other hand, the larger, more permanent habitats used by *An. funestus* (e.g., large ponds and streams) may be suitable for direct environmental modification and manipulation.

There may however be some notable challenges for the control of *An. funestus* in aquatic habitats. For example, the spring-fed pools used by the species may also be a source of clean water for local communities. Thus, removal of these habitats would not be appropriate. Instead, specific larvicides that pose no safety risk for humans and animals may be considered. Fortunately, it has been shown that, the use of bio larvicide formulations, for example *Bacillus thrungiensis* var. *israeliensis (Bti), Bacillus sphaericus (Bs)* and some insect growth regulators (IRGs) such as pyriproxyfen, are effective in controlling malaria vectors [186]. These strategies can be cost-effective, feasible, widely accepted by communities, and are safe for use even in domestic water sources and non-target organisms [187]. However, their applications for large habitats such as river streams may need additional investigations.

Current WHO guidelines indicate that larviciding is most appropriate where larval habitats are “fixed, few, and findable” (FFF), and less feasible where habitats are abundant and scattered [65]. While the term “FFF” is often considered finite, it may be better to define them on gradients. This would allow for the determination of the degree to which larval source management may be applicable in different settings. For instance, the findability of habitats, including small or more temporary types could be significantly enhanced by using satellite imagery or unmanned aerial vehicles (UAVs), which enable greater visibility and operational efficiencies [188]. However, the use of UAVs comes with certain limitations. In densely forested areas, UAVs may have difficulty in accurately identifying larval habitats due to obstruction by tree canopies. Additionally, local legislation in certain countries can restrict the use of UAVs, posing challenges for their deployment in mosquito control efforts. There are also issues of maintaining continuous control, as UAVs require operational planning, including battery management, airspace permissions, and trained personnel to operate the equipment effectively [189,190]. A significant advantage for LSM for *An. funestus* is its reliance on permanent and large aquatic habitats, which are often less numerous than those of other vector species and can persist even in dry seasons [65]. Once identified and characterized, the unique characteristics of these habitats make them potentially easier to target by LSM even in rural areas than the more numerous or expansive habitats of other vector species such as *An. arabiensis*. The relative scarcity and ecological uniqueness of *An. funestus* larval habitats therefore offers excellent opportunities for targeted control. In Tanzania, Nambunga et al. showed that after initial surveys to characterize aquatic water bodies, *An. funestus* habitats in rural settings can fit the description of fixed, few, and findable [101]. In Mexico, where the malaria vector, *Anopheles pseudopunctipennis* also breeds along river streams like *An. funestus*, mosquito densities were significantly reduced after implementing an LSM programme involving clearing the vegetation on the sides of the river to expose mosquitoes to sunlight [191]. Controlling *An. funestus* using such an approach will require defining a comprehensive implementation strategy that integrates community participation to provide the effective workforce needed to operationalize the initiative with maximum impact.

Larval source management was historically one of the most effective malaria control methods but has since been deprioritized in Africa, where methods that target adults, namely ITNs and IRS are now preferred. This was because LSM was considered impractical in African settings due to the abundance of small and temporary larval habitats typically occupied by *An. gambiae s.l.* Such habitats can be difficult to comprehensively locate, characterize and treat promptly. Moreover, the Ross-Macdonald model had further emphasized the significance of reducing adult survival as a more effective approach than reducing vector population size [192]. However, Fillinger & Lindsay have argued against this paradigm by showing the significance and success of LSM [62]. Some of the best-known examples of historic successes with LSM include the elimination of *An. gambiae* from Brazil and Wadi Haifa, Egypt in the mid-20th century, both of which depended primarily on comprehensive LSM programmes [193]. In recent years, there have been renewed interests in LSM as a supplementary control tool, and many African countries are now including it in their malaria elimination agendas [62]. For example, In Tanzania, following the successful demonstration of LSM impact in urban areas in the mid-2000s [194]), this approach is being promoted in both rural and urban councils to enhance other vector control efforts [194,195].

The strategic advantage of LSM over IRS and ITNs is that it controls mosquitoes at source [196], and can effectively reduce the population densities of malaria vectors in several settings [62]. LSM could therefore be effective even in areas where mosquitoes are resistant to insecticides used to control adults, or where the adult vector populations are adapted to bite outdoors and/ or on non-human hosts. Effective targeting of habitats used by *An. funestus* is likely to provide a long-term and cost-effective solution, especially if done alongside an adulticide campaign.

Despite the high potential of LSM in malaria elimination, this approach has some limitations. Larviciding, for example, is currently only recommended in areas where larval habitats are 'few,' 'fixed,' and 'findable'; often limiting its practical applicability to just the dry season since rainfall creates abundant cryptic habitats that may be difficult to treat [65]. Additionally, habitat modification and manipulation may be unacceptable in certain areas where communities rely on the same habitats for domestic needs (Kahamba *et al*, unpublished).

### 2.5.2 Targeting adult *Anopheles funestus* using insecticide-treated nets and indoor residual spraying

Insecticide-treated nets (ITNs) and indoor residual spraying (IRS) have been major contributors to malaria control since 2000 [112]. Both strategies are increasingly threatened by factors such as insecticide resistance, which affect *An. funestus* as well as other malaria vectors. Studies in Zambia and Tanzania have shown that *An. funestus* populations can survive exposure to pyrethroids at doses up to ten-fold higher than the standard dosages recommended by WHO [197]. Both studies also indicated that resistance levels in *An. funestus* may be stronger than in the other major vector, such as *An. arabiensis,* in the same locations [197]. Another study in Uganda also showed that *An. funestus* populations were fully resistant to pyrethroids but susceptible to carbamates [198]. It has also been reported in Cameroon that the species is resistant to a range of insecticide classes, including pyrethroids [199]. Resistance in *An. funestus* populations has also been described in west African countries such as Burkina Faso against dieldrin and Benin against DDT [200–202].

Despite there being fewer studies on insecticide resistance in *An. funestus* than in *An. gambiae s.l.* [198,203], a majority of the pyrethroid resistance in this species appears to be of metabolic origin, where the expression of key enzymes such as cytochrome P450 mixed-function oxygenase or glutathione transferases (GSTs) increase to detoxify pyrethroids and organochlorides such as DDT [204,205]. Despite there being significant geographic gaps and relatively limited data on resistance in *An. funestus*, available information indicates that this vector is extremely resistant to pyrethroids except when co-formulated with a PBO synergist [206,207]. However, it is less resistant to non-pyrethroids such as carbamates and organophosphate than other vector species [135]. The species can also develop multiple resistance mechanisms and may be more resistant than other malaria vectors [135].

Sustaining the public health impact of ITNs and IRS in areas where *An. funestus* dominates will require improved formulations of existing insecticides or the use of new insecticide classes against which vectors are still susceptible. While these requirements for better insecticide strategies are also needed for other vector species [208], the higher resistance levels in *An. funestus* suggests greater urgency. A range of new vector control tools have recently become available or are under development with the aim of overcoming resistance in malaria vectors. This includes nets incorporating the synergist, piperonyl butoxide (PBO), and nets with multiple actives including non-pyrethroids which may yield greater benefits if deployed at scale in areas of pyrethroid resistance [209,210]. In line with current WHO guidelines on PBO nets, most of the east and southern Africa region already have moderate to strong resistance and would qualify for PBO net distribution [211]. Unfortunately, the majority of these new products have so far been evaluated against only *An. gambiae s.l,* thus there is need to understand how they might affect *An. funestus*. However, in northern Tanzania districts where *An. funestus* is the dominant malaria vector, ITNs with multiple actives have recently demonstrated superior performance over pyrethroid-only ITNs, clearly illustrating the potential of such innovations [212].

Similarly, the efficacy of IRS for *An. funestus* control could be improved through the use of longer lasting formulations based on non-pyrethroid insecticides. Unlike ITNs, which are primarily dependent on pyrethroids, IRS campaigns have largely phased out pyrethroids and are now done using either carbamates, organophosphates, or neonicotinoids [213]. IRS impact depends on consistent application of high-quality insecticides, with spraying done at preferably twice yearly, and repeated for several years until malaria transmission intensities drop below locally acceptable thresholds [211]. IRS has been particularly effective against indoor resting malaria vectors including *An. funestus* [214], with the highest impact for malaria control occurring in rural Africa. For instance, early evidence from Tanzania indicated that after a period of spraying in Pare and Taveta regions, IRS effectively eliminated local populations of *An. funestus* with no re-colonization for at least eight years [215]. This sustained impact was achieved because of the highly endophilic behaviour of *An. funestus,* coupled with the scarcity and dispersed nature of suitable larval habitats which slowed local re-colonization once the vector populations started dwindling. Similarly, evidence from southern Africa where IRS with DDT was widely implemented indicates this approach successfully contained transmission by *An. funestus* over five decades [113,205]. When the programme transitioned to pyrethroids instead of DDT between 1997 and 1999, populations of *An. funestus* carrying pyrethroid-resistance reinvaded the areas causing new malaria epidemics in 2000 and prompting the reinstatement of DDT [216,217].

Taken together, this evidence suggests that a consistent programme of adulticiding with carefully selected insecticides against which the vector is susceptible could dramatically crash malaria transmission in areas where *An. funestus* is dominant.

Based on this hypothesis, a simplified approach for high-quality and high-coverage IRS or other forms of adulticiding would have a disproportionately impact and perhaps result in reducing *An. funestus* populations in a given area. The impacts would be amplified if the intervention targeting adults is accompanied by an effective LSM programme that targets the right kind of aquatic habitats, hence reducing the likelihood of re-colonization of the areas and sustaining the gains.

Other than insecticide resistance, another important concern regarding IRS is that it can be logistically difficult and expensive to implement in large scale. In fact, while the number of countries adopting IRS has increased since 2000, the number of people protected has stagnated [43,48], as the countries adopt more targeted and small-scale operations. Other challenges include the high quantities of insecticides necessary, the need for large spray teams that are well-trained, challenges with disposal of unused pesticides and pesticide wastes and the need to remove household belongings during spraying. It is important, therefore, that future efforts should target improved formats for delivering IRS or its equivalents in ways that do not compromise the public health value.

### 2.5.4 Other interventions with potential against *Anopheles funestus* adults

In addition to the proposed strategic use of IRS, ITNs, and LSM, vector control against *An. funestus* could benefit from additional interventions targeting adults during different life-history stages or behaviours. To be most efficacious, selection of the complementary interventions must be informed by basic understanding of the natural attributes of the vector species. One example could be the use of attractive targeted sugar baits (ATSBs), which kill mosquitoes during sugar feeding. This intervention has the benefit of being usable both indoors and outdoors and being able to target both male and female mosquitoes [179]. Recent field observations of *An. funestus* males occurring at high frequencies indoors suggest that males could be readily targetable by ATSBs or other indoor approaches [218].

Other options that could effectively reduce exposure to *An. funestus* are house improvements such as house screening [219] and eave-based interventions, which target mosquitoes when entering houses through the eave spaces. In particular, the eave-based interventions may include insecticide-treated eave ribbons [220], eave baffles [221] and eave tubes [222]. These interventions have the additional advantage of being less cumbersome than IRS and requiring far lower quantities of insecticides. Importantly, because the eave spaces are distally removed from human contact, a much wider range of insecticide classes could be used on these interventions; preferably those which have no cross-resistance with pyrethroids. Such house-based approaches are anticipated to be particularly effective against *An. funestus* given its highly endophilic and endophagic nature.

There are also non-insecticidal interventions that may be effective for *An. funestus* control. For example, mass deployment of odour-baited traps on Rusinga Island in western Kenya resulted in more than 40% reduction in malaria incidence, primarily by targeting *An. funestus* [107]. Mathematical simulations suggest that odour baited traps used alongside ITNs could significantly improve control and potentially lead to local elimination in multiple settings across Africa [223,224].

It has been proposed that genetically modified mosquitoes carrying the gene drive technology could also eventually be an alternative to broadly address current challenges with vector control. However, current gene drive developments for malaria control are primarily focused on *An. gambiae s.s.* [225,226] and have no immediate applications in areas dominated by *An. funestus*. However, recent work has suggested that certain types of gene drives, which employ homology-directed repairs to ensure their proliferation in the genomes may be suitable for use in *An. funestus* [227]. Along with further advancements in genetic technology, a deeper knowledge of the mating behaviour and gene flow trajectories in this species will be critical for evaluating the potential for such genetic approaches in controlling *An. funestus*. Since the public health value of the above alternative tools has not yet been confirmed, additional research is necessary to determine their true potential and cost-effectiveness.

### 2.5.5 Community engagement to enhance the control of malaria in areas dominated by *Anopheles funestus*

To ensure the success of existing or novel interventions for *An. funestus* control, it is crucial to engage community members and other key stakeholders when planning the implementation of these interventions [228]. Early and continuous community engagement is vital in guaranteeing usability, acceptability, sustainability, and overall effectiveness of the interventions [228]. Community members generally have significant levels of knowledge and experiences, which can be valuable in ensuring success of malaria control interventions. Detailed qualitative surveys may be necessary to understand community views and the potential acceptability of any treatment or manipulation of the aquatic habitats. For best results, community engagement initiatives should go beyond simply raising awareness about a particular intervention. Instead, the initiatives should also build partnerships with the communities to create and/or improve their sense of ownership of the interventions; and to encourage their participation in the success of the interventions [229].

There are numerous documented ways to engage the communities in malaria control efforts in Africa. In southern Tanzania, Mwangungulu et al. demonstrated that community members could be relied upon to identify areas with the highest densities of malaria vectors, a useful means for low-cost community-based planning of malaria control [230]. Other studies in Tanzania and Burkina Faso have also demonstrated that community members can be relied upon to identify and spray *Anopheles* mosquito swarms with insecticides [175,231]. Additionally, household members were recruited to monitor human activities and behaviours that increase the risk of contact with malaria vectors [232].

It has been observed that important *An. funestus* habitats, such as spring-fed pools, ponds, and streams, often also serve as water sources for domestic uses, irrigation, or livestock use (Kahamba et al, pers. commun.). In this regard, local communities can be involved to integrate LSM into their daily practices. Such strategies have already been demonstrated on a small scale in rural Tanzania, where pastoralists were recruited to identify and treat aquatic mosquito habitats during the dry season [233]. A related example is where larvicides have been mixed with fertilizers so that farmers could apply these to their farms to provide the added advantage of mosquito control [234]. Such programmes could be expanded and improved by training selected members of local communities to identify and treat potential habitats for *An. funestus*.

Lastly, for community members to have meaningful involvement in malaria control efforts, they must have good awareness and understanding of the risk, burden, and severity of malaria. Improving a community's knowledge and awareness needs to go beyond merely explaining scientific knowledge to the community members. It must also consider important cultural values, experiences, practices and interests in the respective communities [228].

## 2.6 Conclusions

*Anopheles funestus* is widely distributed and accounts for a higher proportion of malaria transmission in East and South African countries. While research on this species has been limited partly due to difficulties in creating laboratory colonies, available evidence suggests it possesses several distinct ecological characteristics which may render it amenable to certain high-impact interventions approaches targeting both its immature and adult stages. Its preferred aquatic habitats tend to be few and non-temporary and may include rivers, streams, large ponds, and spring-fed pools. This species is mostly endophilic and anthropophagic though both outdoor-feeding and animal-biting populations have also been reported, especially where residents keep a lot of livestock. The existence and magnitude of these "atypical" behaviours need to be considered when designing complementary interventions. Considering the dominance and ecological distinctiveness of *An. funestus*, it is hypothesized that combining targeted larval source management and at least one method that effectively target adults (including insecticide-resistant populations) could be both operationally feasible and highly impactful. In areas where *An. funestus* is the dominant vector, the approach could cause major reductions in malaria transmission by drastically reducing the local populations of the species and limiting the likelihood of its re-colonization. For best results, the programme may be followed by gradual house screening and cultivating strong community engagement to guarantee sustainability. It should also be recognized that the broader goal of malaria elimination would require a much more expansive operation targeting all important vectors beyond *An. funestus*.

# Chapter 3: Geospatial modelling of dry season habitats of the malaria vector, *Anopheles funestus,* in south-eastern Tanzania

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**Abstract**

**Introduction:** *Anopheles funestus* is a major malaria vector in east and southern Africa, and currently dominates transmission in many parts of Tanzania. Previous research has identified its preference for specific aquatic habitats, especially those that persist in dry months. This suggests the potential for targeted control through precise habitat mapping and characterization. In this study, we investigated the influence of aquatic habitat characteristics, land cover, and human population densities on *An. funestus* larval distribution during dry seasons, and subsequently developed a habitat suitability model for this vector species in southeastern Tanzania.

**Method:** Eighteen villages in south-eastern Tanzania were surveyed during the dry season from September-December 2021. Water bodies were systematically inspected for mosquito larvae and characterized by their physio-chemical characteristics and surrounding environmental features. A generalized linear mixed model was used to assess the presence of *An. funestus* larvae as a function of the physio-chemical characteristics, land use and human population densities. The results from this model were used to generate spatially explicit predictions of habitat suitability in the study districts.

**Results:** Of 1,466 aquatic habitats surveyed, 440 were positive for *An. funestus*. River streams had the highest positivity (74%; n=322) followed by ground pools (15%; n=67). The final model had an 83% accuracy in predicting positive *An. funestus* habitats, with the most important characteristics being permanent and clear water, and shadings. In addition, there was a positive association with forested areas and a negative association with built-up areas. Human population densities were not

associated with *An. funestus* distribution.

**Conclusion:** This study underscores the crucial role of both specific aquatic habitat characteristics and other larger-scale environmental factors, notably land-cover, in determining the distribution of *An. funestus*. In this study area, the species predominantly inhabits river streams and ground pools; and prefers clear, perennial waters with and shading. The strong positive association with more pristine environments with tree covers and negative association with built-up areas underscore the importance of ecological transitions in vector distribution and malaria transmission risk. Such spatially explicit predictions could enable more precise interventions, particularly larval source management, to accelerate malaria control.

## 3.1 Introduction

Malaria control strategies have primarily focused on insecticide-treated nets (ITNs) and indoor residual spraying (IRS) to combat mosquito vector populations. However, these interventions are facing major challenges, including widespread insecticide resistance and behavioural adaptations of vector species [235,236]. In response there is need for complementary interventions, including larval source management (LSM), which is increasingly being considered by endemic countries; particularly in urban and peri-urban settings [235,237]. In contrast, LSM implementation in rural areas faces several challenges, notably logistical difficulties and existing guidelines requiring that aquatic habitats must be few, fixed, and findable [61].

Successful implementation of LSM requires a thorough understanding of the larval ecology of the target species, and especially the ability to locate their main aquatic habitats. Whether malaria transmission is seasonal or perennial, identifying the main habitats that sustain vector populations through the dry seasons would be particularly important since such habitats could be targeted to maximize control when the vector populations are lowest.

*Anopheles funestus s.s* is widely recognized as a major malaria vector in east and southern Africa [92,238]. In south-eastern Tanzania [86,87], as well as some districts in northern Tanzania [88], this species is now responsible for over 85% of malaria transmission. This dominance is due to several attributes, including the species' preference for feeding on humans indoors and resting indoors [27,239], its strong resistance to common pyrethroid insecticides [240], and its high daily survival rates [27]. Indeed, field evidence suggests that *An. funestus* can dominate malaria transmission even in areas where its densities are lower than other malaria vector species [86]. Unfortunately, in many settings, its basic biology and ecology are less well characterized compared to those of other vector species [92].

While studies focusing on *An. funestus* larval ecology are scarce, some studies show that whereas its aquatic habitats occasionally overlap with those of other mosquito species, there are certain unique attributes that underly its preferences [241,242]. Early studies in the 1930s provided valuable insights, indicating that *An. funestus* was more likely to be found in permanent water bodies such as river streams, ditches, and ponds [155,243], unlike *An. gambiae* complex mosquitoes, which generally prefer smaller and less permanent habitats [244]. A more recent study in south-eastern Tanzania found that *An. funestus* primarily oviposits in habitats along river tributaries, and in large ponds [243]. Distinctive features of these habitats, compared to those used by other malaria vectors, included clear waters, emergent vegetation, shading, depths exceeding 0.5m and permanent or semi-permanent availability [243]. Given the significance of *An. funestus* in the region, there was need to extend these efforts by conducting a detailed analyses of the importance of land cover characteristics.

This current study was therefore designed to explore how habitat characteristics, landcover types, and human population densities affect *An. funestus* distribution; then use the findings to create a habitat suitability maps for the vector species in south-eastern Tanzania.

## 3.2 Methods

### 3.2.1 Study site

The field survey was done in 18 villages in south-eastern Tanzania. These included 11 villages in Ulanga district (Chikuti, Chirombora, Ebuyu, Gombe, Ikungua, Iragua, Kichangani, Kidugalo, Lukande, Mwaya, Mzelezi), and 7 villages in Malinyi district (Itete, Mtimbira, Sofi Mission, Sofi Majiji, Kalengakelo, Kiswago, and Ipera Asilia) in south-eastern Tanzania (Fig.3.1). The area has an altitude of 250 - 650 meters above sea level, yearly mean temperatures of 20 - 33°C and annual rainfall of 1200 - 1800 mm [245]. Generally, the dry season occurs between June and November, short rains in November and December, and long rains from February to May [245]. The area has diverse land use features, including small towns, villages, savannahs, crops, irrigation, grazing lands, forests and shrublands. There is a large flood plain with numerous rice farms, bordered by Udzungwa mountains to the north and Mahenge hills to the south (Fig.3.1). *Anopheles arabiensis* and *An. funestus* are the main malaria vectors, the latter mediating most of the transmission [86,246]. The main economic activities are livestock-keeping, fishing, and crop farming [247,248].

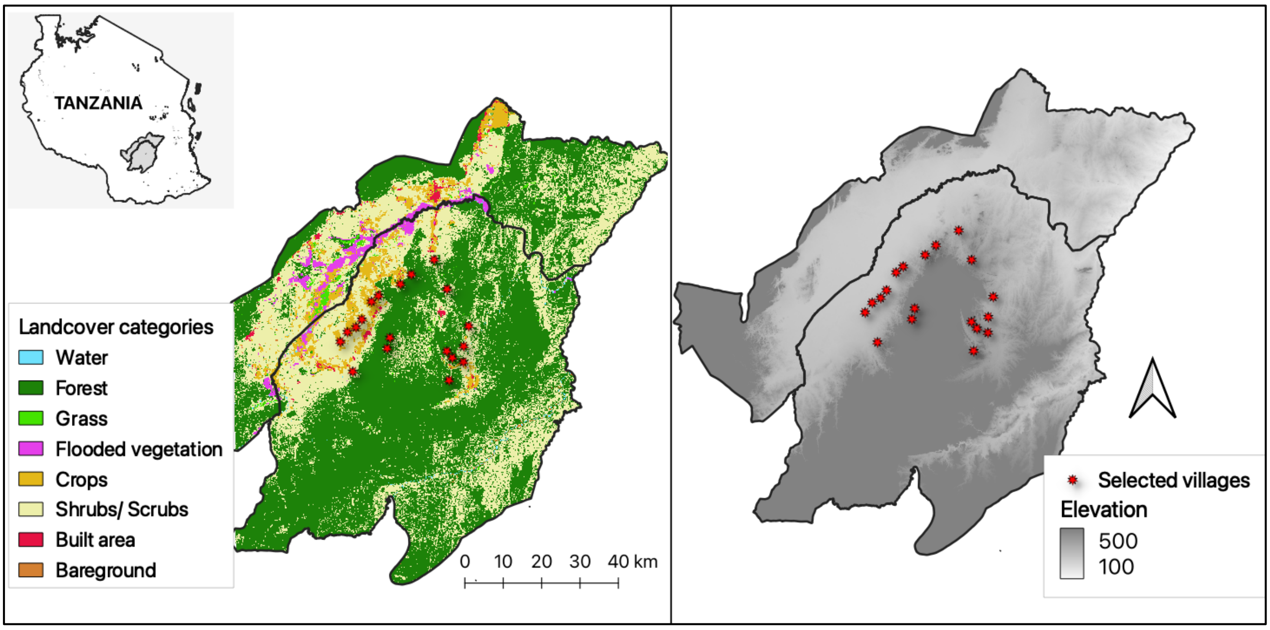

**Figure 3.1:** Study villages for dry season surveys of *An. funestus* aquatic habitats in south-eastern Tanzania.

## 3.2.2 Sampling and characterization of aquatic habitats

The habitat survey was conducted during the dry months of September to December 2021. Community members aged 18 years and above were recruited and trained to identify potential aquatic habitats, including natural and human-made water bodies; and record their physio-chemical attributes, regardless of whether larvae were present or not. The training process involved field demonstrations and practical exercises on habitat identification, which were conducted in the first two villages surveyed. These villages served as training sites, where the technicians' skills were validated by cross-referencing their observations with those of experienced scientists to ensure accuracy and consistency. After the training, the team was divided into two groups, allowing for the simultaneous surveying of two villages at a time.

To ensure comprehensive coverage of each village, a team of five people, spaced 2 meters apart, systematically surveyed the area along pre-determined transects within boundaries set by the village authorities.

The transects were designed to systematically cover each village, with routes predetermined based on consultations with village authorities to ensure comprehensive habitat coverage. Each transect traversed approximately 500 to 1000 meters in length, depending on village size. Team followed these transects at regular intervals, ensuring that no potential aquatic habitat was overlooked.

For each water body observed during the transect walks, the team recorded: i) time and date of visit, ii) GPS coordinates, iii) habitat type (classified into river streams, stagnant ground pools, marshes, wells, dug pits, brick or concrete pits, ditches, rice fields, hoofprints), iv) habitat size (surface area, m2), v) water clarity, vi) water source (rainfall accumulation or ground water), vii) water movement (stagnant, slow or fast moving), viii) water permanence (permanent, semi-permanent, or temporary), ix) water depth, x) presence and type of algal growth (brown, blue, filamentous), xi) presence of shading, xii) types and quantity of vegetation and xiii) environmental characteristics surrounding the habitats within 200 m (such as cultivation, bush areas, cattle grazing, and distance to nearest human habitations) (Table 3.1). Additionally, physio-chemical metrics, including pH, Total Dissolved Solids (TDS), and Electroconductivity (EC) were measured using a water-quality meter.

**Table 3.1:** Categories used to classify aquatic habitat types and physio-chemical characteristics.

| **Feature Category** | **Description** | **Measurement Method** |
|---|---|---|
| **Habitat Type (category)** | River streams (including stream pools formed after the evaporation), ground pools (such as marshes, swamps, and ponds), ditches (found within rice fields, beside rivers, and alongside roads), rice fields, spring-fed wells (comprising dug holes for spring water), pits | Visual observation |

| | | |
|---|---|---|
| | (including brick pits and construction pits), puddles, hoofprints, and tire tracks | |
| **Water Movement** | Slow, fast (moving), Stagnant | Visual observation, using dry stick |
| **Water clarity** | Clear, colored (non-polluted), polluted | Visual observation |
| **Water Source** | Rainwater (surface water), non-rainwater (groundwater) | Local knowledge, visual observation |
| **Water Permanence** | Permanent- have water all year and historical stability across multiple seasons,<br>Semi-permanent - ones fluctuate but mostly retain water at least six months in a year,<br>Temporary - only have water temporarily mostly occurs during wet seasons, | Local knowledge, visual observation |
| **Presence of vegetation** | None or vegetated | Visual observation |
| **Vegetation type** | None, floating, emergent | Visual observation |
| **Presence of algae** | None or present | Visual observation |
| **Algae type** | Filamentous, green, blue-green, brown | Visual observation |
| **Water Depth** | Measured in cm, < 10 cm, 10-50 cm, > 50 cm | Long stick and measuring tape |
| **Habitat Size, surface area** | Measured in square meters, < 10 $m^2$, 10-100 $m^2$, 100-250 $m^2$, > 250 $m^2$ | GPS, measuring tape |
| **Shade/canopy** | Partial, heavily/fully (shaded), none | Visual observation |
| **Surrounding Environment** | Scrub/bush, cattle grazing, cultivated fields, residential area | Visual observation |

|  |  |  |
|---|---|---|
| **Distance to the nearest house** | Measured in square meters, < 100 m, 100-500 m, > 500 m | GPS |

### 3.3.3 Larval surveys

All identified water bodies were examined for presence of mosquito larvae using either the standard 350 ml dipper (for small habitats with shallow depths) or a large 10L bucket (for larger and deeper habitats) as previously described [243] (Fig. 3.2). The number of dips performed in each aquatic habitat was determined based on its size, following a predefined protocol. For habitats smaller than 5 square meters, we performed a single dip. In habitats measuring 6 to 10 square meters, we conducted two dips, and for those between 11 to 15 square meters, three dips were done. This incremental approach continued for larger habitats, with a limit of 20 dips for any habitat exceeding 120 $m^2$. Collected larvae were identified to the genus and species group level, whenever possible, using standard taxonomic keys [249,250]. Within the *Anopheles* genus, late instars (III and IV) of the *An. funestus* group and the *An. gambiae* complex could be easily distinguished based on their morphology [250,251]. Consequently, in this paper, the term "aquatic habitats" refers to any surveyed water body, while "positive habitats" denotes those where *An. funestus* was confirmed via dipping.

**Figure 3.2:** sampling techniques, a.) standard dipping b.) dipping using 10l bucket.

### 3.3.4 Environmental covariates

A digital elevation model (DEM) with 10 m resolution [252] was used to extract data on elevation, slope, terrain, and aspect for each aquatic habitat location. Land cover data was derived from the European Space Agency (ESA) Sentinel-2 satellite imagery acquired in June 2022. This consisted of eight land cover classes and had a spatial resolution of 10 m, with and an overall accuracy of 75% [253]. The ESA imagery allowed analyses of both land-cover and land-use characteristics, such as urban areas and forestation, and helped identify small-scale landscape features and patterns crucial for understanding the local level relationships with malaria risk [254]. For each aquatic habitat, the proportion of each land cover type (water, trees, grasslands, flooded vegetation, shrubs, built-up areas, bare ground, and crops) was extracted within a 300m buffer. The choice of a 300m buffer was based on field observations and previous studies that identified this distance as an average range within which mosquito larvae are most likely to find suitable habitats near human settlements [255,256]. This range also reflects the average distance between households and larval habitats in the study area. In addition, the distance from each aquatic habitat to the nearest feature of each land-cover class was measured. Consideration of both the buffer zone and distance to habitats allowed for a more nuanced analysis of how both the immediate landscape composition and the proximity to specific land-cover types correlate with the presence of *An. funestus* larvae in aquatic habitats (Table 3.2).

Finally, human population density data within, the 300m buffer, was obtained from the Global Human Settlement Layer (GHSL), a spatial raster dataset composed of 100m x 100m cells, with each representing the number of people in that area [257].

**Table 3.2:** Candidate covariates evaluated for predicting the presence of aquatic habitats of *An. funestus* mosquitoes.

| Variable | Source and description |
|---|---|
| Village<br>Habitat type*<br>Habitat size | Physical parameter recorded for each aquatic habitat in the field |

| | |
|---|---|
| Water depth<br>Water source*<br>Watercolour*<br>Water movement*<br>Permanence of water*<br>Presence of vegetation<br>Vegetation types<br>Algae status<br>Types of algae<br>Presence of shades*<br>Surroundings environment*<br>Distance to nearby house | |
| Proportion of:<br>Tree/ forest areas*<br>shrublands<br>grassland<br>crops<br>built-up areas*<br>bare land<br>flooded vegetation | Proportion of landcover classes calculated in a 300m buffer around each aquatic habitat. from the surveyed points.<br>Data from European Space Agency (ESA) Sentinel-2 satellite imagery [253]. |
| Distance (m) from the nearest:<br>tree/forest<br>shrubland<br>grassland<br>crops<br>built-up area<br>bare land<br>flooded vegetation | Distances (m) between each aquatic habitat and the nearest patch of each landcover class.<br>Data from European Space Agency (ESA) Sentinel-2 satellite imagery [253]. |
| Elevation (m)<br>Slope (°)<br>Aspect | Derived from a 10 m resolution digital elevation model (https://earthexplorer.usgs.gov) [252]. |
| Population density | Number of people living in the 300m buffer. |

| | |
|---|---|
| | Data obtained from the Global Human Settlement Layer [257]. |

**Significant variables that were retained in the final model (detailed in result section)*

## 3.5 Statistical analysis

An initial descriptive analysis was conducted to assess the occurrence and distribution patterns of aquatic habitats occupied by *An. funestus;* and variations by type, specific location (village), and the landcover categories (**Fig. 3.2**). A generalized linear model with a binomial distribution was then used to examine the relationship between the presence of *An. funestus* larvae (absent = 0; present = 1) and a range of environmental and landscape variables (Table 3.1). Starting with a full model, including all the candidate variables, an automated backward stepwise selection was used to identify significant variables for inclusion in the final model, based on likelihood-ratio-tests (Table 3.1).

To validate performance of the model, a two-fold cross-validation process was used. The dataset was divided into a training subset (80%) and a test subset (20%). The model was trained on the training dataset and validated on the test set using *Tjur's* $R^2$ calculations and area under the curve (AUC) receiver operating characteristics (ROC), with upper limits of 1.0 for a perfectly fitting model (Fig. 3.3).

The final model was used to generate spatial predictions of the likelihood of encountering *An. funestus* larvae in aquatic habitats found in different locations. To generate these maps, a 200 m resolution grid of regular points covering the study area of Malinyi and Ulanga districts (total area = 22,777 km$^2$) was created. Covariates retained in the final logistic model, such as proportions of land-cover types, were extracted and applied to each grid to predict habitat suitability across the unsampled areas. To model how specific habitat characteristics variations might influence the suitability for *An. funestus*, we tested multiple scenarios, with varied attribute values. For instance, we created scenarios where water turbidity or habitat permanence were varied, reflecting different potential conditions.

All statistical analyses, including variable extraction, model fitting, and predictions, were performed using the R statistical program version 4.2.1, with the packages *rms, MASS, lme4* and *glmm* [258]. Preliminary data handling, and visualization were done using the software QGIS [259].

## 3.6 Interactive maps for predictions and web application

To facilitate the exploration of different suitability scenarios, we also developed an interactive map using the Leaflet and Shiny packages in R [260] This web-based tool provides a dynamic platform for viewing and adjusting predictions from our model through a user-friendly graphical user interface (GUI). The GUI is designed to be intuitive, allowing different stakeholders to interactively modify model inputs and observe the effects on the geographical suitability for *An. funestus* habitats. Users can select or alter various parameters values, then update the predictive maps to instantly visualize how these changes affect the predicted suitability.

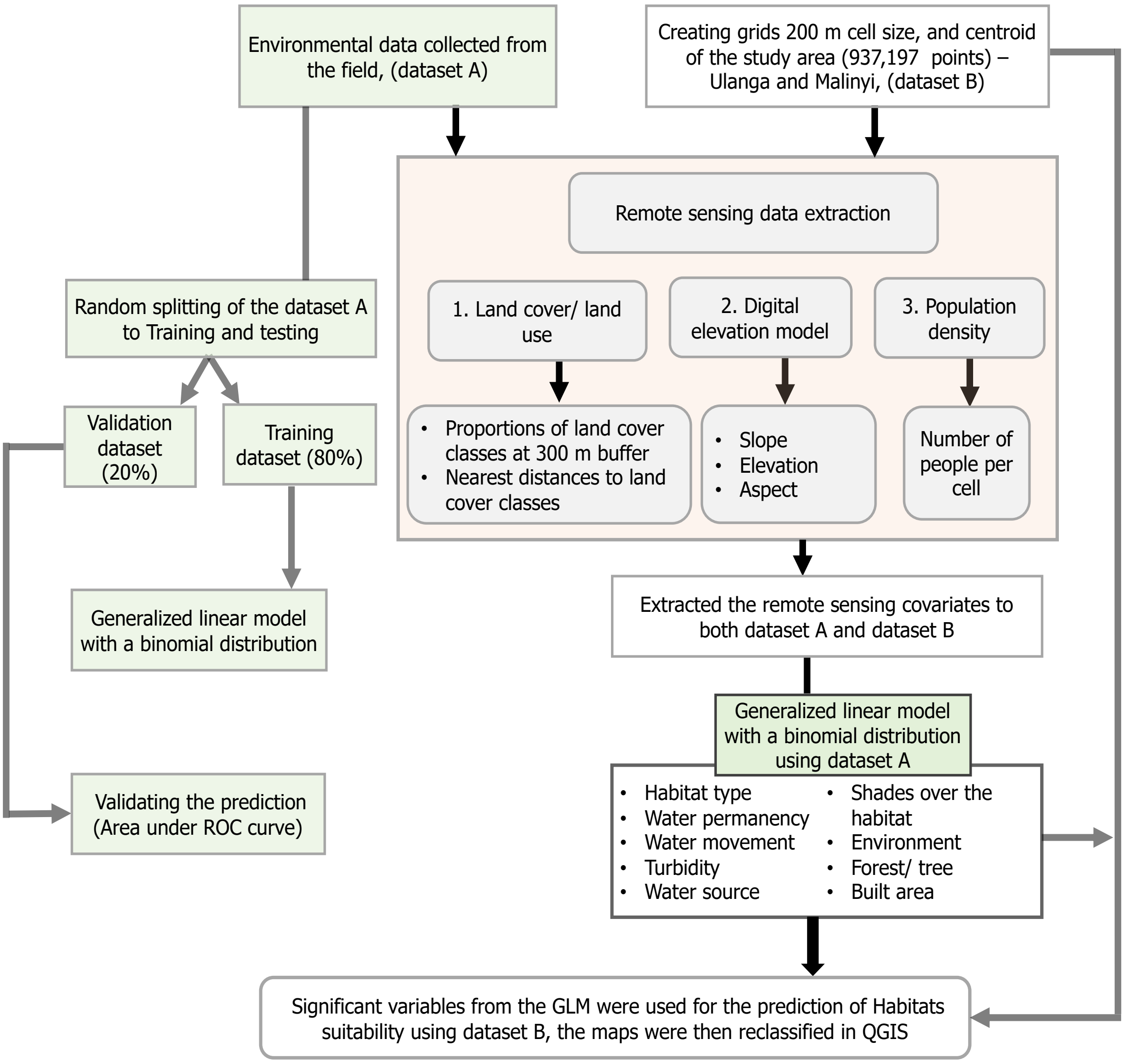

**Figure 3.3:** Flowchart showing the analysis procedures, involving two datasets. On the left-hand side, highlighted by the green boxes represent the field-collected data (dataset A), modelled using logistic regression to determine the significant variables, and validated using a two-fold cross-validation technique. On the right is a 200 m grid covering the entire study area (dataset B), which was used to perform the prediction of the habitat's suitability based on the retained significant variables.

## 3.7 Results

### 3.7.1 Descriptive analyses of *An. funestus* positivity in different habitat type

The comprehensive assessment of potential habitats included river streams, ground pools, wells, dug pits, brick and concrete pits, rice fields, and ditches (Fig. 3.4). Among the 1,466 potential aquatic habitats inspected, 440 (30%) were positive for *An. funestus* larvae. River streams were the most common water bodies observed (695 out of 1,466), and approximately three-quarters of them had *An. funestus* larvae (Table 3.3). Ground pools had the next highest positivity for *An. funestus* larvae (15% of 212 habitats), followed by wells and dug pits (4.8%), ditches (4.5%), rice fields and concrete pits (<1.2%, Table 3.3). Notably, no *An. funestus* larvae were detected in the 10 hoofprint habitats surveyed. Puddle or vehicle track habitats were not present.

**Figure 3.4:** Example of surveyed aquatic habitats that were found to harbour *An. funestus* larvae. The river stream image was captured by drone, it is approximately 2 meters in width and spanning over 100 meters in length. The ground pool is a stagnant water body with a surface area of approximately 5 square meters. The rice fields cover an area of about 0.10 hectares (1000 square meters). The ditch is a narrow water body with slow-moving water, approximately 1 meter wide and 15 meters long. The spring-fed well is a human-made pit with clear water, about 1 meter in diameter, while the dug pit (another human-made habitat) is approximately 3 meters in diameter and is used for various purposes.

**Table 3.3:** Percentage of habitats of different types that had *An. funestus* larvae. The values are averaged across villages.

| **Habitat type** | **Total counts** | **Percentage with *An. funestus* larvae** |
|---|---|---|
| **River streams*** | 695 | 74.0% |
| **Ground pools**** | 212 | 15.0% |
| **Spring-fed wells and dug pits ***** | 409 | 4.8% |
| **Brick and concrete pits***** | 49 | 0.6% |
| **Agricultural fields - Rice fields** | 27 | 1.1% |
| **Ditch** | 68 | 4.5% |
| **Hoofprint** | 10 | 0.0% |

| | | |
|---|---|---|
| **Puddle and tyre tracks** | 0 | - |
| **Total** | **1466** | **100%** |

**During the survey, river streams were divided into 50-meter-long segments and each segment was individually characterized, this class also included remnant water pools on the riverbeds and therefore, multiple segments could be part of the same river stream. **Ground pools included large or small marshes and ponds with stagnating water, sometime having vegetation. ***Spring-fed wells & dug pits as well as brick & concrete pits were all created by communities, hence can be jointed referred to as human-made pits.*

### 3.7.2 Descriptive analysis of *An. funestus* habitat types in different villages

The percentage of *An. funestus* aquatic habitats varied between villages, likely in association with local differences in habitat types. For example, in Chikuti, *An. funestus* larvae were found exclusively in river streams, accounting for 100% of positive habitats. Similarly, in Lukande and Mtimbira villages, river streams accounted for 97% and 96% of all positive habitats respectively. However, there were also villages where the river streams were present but completely lacked *An. funestus* larvae (e.g., Kichangani and Ipera Asilia). Ground pools, the habitat type with the second highest *An. funestus* positivity, accounted for 100%, 85%, and 60% of all positive habitats in Ipera Asilia, Mwaya, and Sofi Mission villages respectively. On the other hand, rice fields, ditches, and hoofprints appeared unfavourable for *An. funestus* larvae with either minimal presence or complete absence of this species (Table 3.4).

**Table 3.4:** The percentage of habitats of different types positive for *Anopheles funestus* in different villages in Ulanga and Malinyi districts during the dry season of 2021

| **Village** | **Total number of habitats** | ***An. funestus* positive habitats** | **Total number of habitats observed (and percentage positive for *An. funestus*)** | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | **River streams** | **Ground Pools** | **Human-made pits*** | **Rice fields** | **Ditches** | **Hoofprints** |
| **Chikuti** | 47 | 9 | 23 (100) | 2 (0) | 22 (0) | 0 (0) | 0 (0) | 0 (0) |
| **Chirombora** | 85 | 34 | 37 (85) | 12 (15) | 36 (0) | 0 (0) | 0 (0) | 0 (0) |
| **Ebuyu** | 88 | 44 | 44 (84) | 7 (11) | 35 (5) | 0 (0) | 0 (0) | 2 (0) |
| **Gombe** | 86 | 42 | 61 (83) | 3 (7) | 14 (2) | 1 (0) | 5 (7) | 2 (0) |
| **Ikungua** | 213 | 66 | 85 (35) | 31 (27) | 24 (7) | 21 (7) | 52 (23) | 0 (0) |
| **Ipera Asilia** | 11 | 6 | 5 (0) | 6 (100) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| **Iragua** | 64 | 9 | 28 (67) | 14 (0) | 16 (22) | 3 (0) | 3 (11) | 0 (0) |
| **Itete** | 38 | 26 | 26 (80) | 3 (8) | 9 (12) | 0 (0) | 0 (0) | 0 (0) |
| **Kalengakelo** | 119 | 30 | 66 (87) | 4 (3) | 49 (10) | 0 (0) | 0 (0) | 0 (0) |
| **Kichangani** | 57 | 0 | 33 (0) | 9 (0) | 15 (0) | 0 (0) | 0 (0) | 0 (0) |
| **Kidugalo** | 85 | 17 | 37 (82) | 2 (6) | 45 (12) | 0 (0) | 0 (0) | 1 (0) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Kiswago** | 19 | 12 | 8 (58) | 3 (25) | 8 (17) | 0 (0) | 0 (0) | 0 (0) |
| **Lukande** | 111 | 35 | 56 (97) | 13 (0) | 40 (0) | 1 (0) | 1 (3) | 0 (0) |
| **Mtimbira** | 108 | 27 | 48 (96) | 1 (0) | 53 (4) | 0 (0) | 1 (0) | 5 (0) |
| **Mwaya** | 102 | 13 | 36 (15) | 50 (85) | 14 (0) | 1 (0) | 1 (0) | 0 (0) |
| **Mzelezi** | 68 | 36 | 61 (94) | 0 (0) | 5 (6) | 0 (0) | 2 (0) | 0 (0) |
| **Sofi Majiji** | 67 | 14 | 16 (86) | 9 (14) | 42 (0) | 0 (0) | 0 (0) | 0 (0) |
| **Sofi Mission** | 98 | 25 | 25 (40) | 43 (60) | 27 (0) | 0 (0) | 3 (0) | 0 (0) |

**Human-made pits here included spring-fed wells & dug pits and Brick & concrete pits and they were all created by communities*

### 3.7.3 Descriptive analyses of *An. funestus* positive habitats in areas with different land cover types

Tree covered areas were the most abundant land cover type within the surveyed area; and were also the land cover type with the highest number of aquatic habitats (Table 3.5). Other land covers such as grasslands, shrublands and agricultural fields also had significant numbers of aquatic habitats. In contrast, built-up areas had markedly lower presence of aquatic habitats.

**Table 3.5:** Area in square kilometres (km$^2$) and the percentage of each cover type; number of aquatic habitats found in each category, and number and percentage of habitats that were occupied by *An. funestus* larvae.

| **Land cover category** | **Covered area in km$^2$ (%)** | **Number of aquatic habitats found** | **Number of positive habitats for *An. funestus* (%)** |
|---|---|---|---|
| **Trees and forests** | 14 277 (63.0) | 486 | 194 (40) |
| **Shrubland** | 6 765 (30.3) | 399 | 108 (27) |
| **Grassland** | 59 (0.3) | 55 | 13 (24) |
| **Crops/ Agricultural fields** | 841 (3.8) | 380 | 75 (20) |
| **Built areas** | 84 (0.4) | 129 | 16 (12) |
| **Flooded vegetation** | 163 (0.7) | 11 | 1 (9) |
| **Bare land/ Open space** | 41 (0.2) | 0 | - |
| **Water bodies** | 116 (0.52) | NA | - |

The "Water bodies" category is marked as NA because these were large, fast-flowing rivers or lakes that were not surveyed due to their unsuitability for *An. funestus* larval development and the logistical challenges of conducting habitat assessments in these areas.

### 3.7.4 Environmental predictors of *An. funestus* presence in aquatic habitats

The $R^2$ of the final model of *An. funestus* presence in aquatic habitats was 0.28, indicating modest explanatory power but with high accuracy (AUC of the final model = 0.83) [261]. Among the 33 environmental and landscape variables investigated, 9 were retained based on statistical significance in the final model. These included habitat type, water movement, water clarity, water source, permanence of the habitat, shading over habitats, presence of algae, proportion of tree cover and built-up area within a 300m buffer zone.

Regarding habitat types, human-made (dug) pits and those classified as 'other' showed lower odds of hosting *An. funestus* larvae compared to natural river streams. Although ground pools showed a higher occurrence of larvae than river streams, this difference was not statistically significant (Table 3.6). *Anopheles funestus* larvae were less frequently found in stagnant (OR = 0.42, $P < 0.001$) and unclear water sources (OR = 0.67, P = 0.02) compared to clear, flowing waters. Notably, *An. funestus* preferred permanent as opposed to temporary habitats such as those formed from rainwater accumulation. Shaded habitats and algal absence were also positively associated with the occurrence of *An. funestus* larvae.

With respect to land cover types, *An. funestus* larvae were more likely to be found in aquatic habitats situated in areas with extensive tree cover and forest canopy (OR = 2.83, $P< 0.001$). In contrast, presence of *An. funestus* larvae was negatively associated with habitats within or near built-up areas (OR = 0.34, P = 0.025). Finally, no significant associations were observed between An. *funestus* positive habitats and either human population densities or the different landscape factors derived from the digital elevation model.

**Table 3.6:** Results of the generalized linear model of habitat suitability for *An. funestus* habitats.

| **Characteristics** | **Odds ratios (95% CI)** | **p-value** |
|---|---|---|
| **1. Habitat type** | | |
| **River streams** | 1 | |
| **Ground pools** | 1.32 (0.85, 2.04) | 0.20 |
| **Dug pits** | 0.25 (0.14, 0.43) | < 0.001*** |
| **Others** | 0.29 (0.16, 0.53) | < 0.001*** |
| **2. Water movement** | | |
| **Moving** | 1 | |
| **Stagnant** | 0.42 (0.29, 0.59) | < 0.001*** |
| **3. Water clarity** | | |
| **Clear** | 1 | |
| **Unclear** | 0.67 (0.48, 0.93) | 0.02* |
| **4. Water source** | | |
| **Non rainwater** | 1 | |
| **Rainwater** | 3.65 (2.57, 5.16) | < 0.001*** |
| **5. Water permanency** | | |
| **Permanent** | 1 | |
| **Semi-permanent** | 0.25 (0.14, 0.42) | < 0.001*** |
| **6. Shades over habitat** | | |
| **None** | 1 | |
| **Shaded** | 1.45 (1.08, 1.96) | 0.015* |
| **7. Algae status** | | |
| **None** | 1 | |
| **Present** | 0.64 (1.54, 4.18) | < 0.001*** |
| **8. Environment** | | |
| **Cattle grazing** | 1 | |
| **Cultivated field** | 1.83 (0.88, 3.79) | 0.07. |
| **Scrub** | 1.37 (0.65, 2.89) | 0.40 |
| **Mixed** | 2.55 (1.25, 5.18) | 0.008** |
| **Landcover significant parameters** | | |
| **9. Proportion of trees at 300m buffer** | 2.83 (1.73, 4.62) | < 0.001*** |
| **10. Proportion of built area at 300m buffer** | 0.34 (0.12, 0.98) | 0.025* |

*The table shows the odds ratios with 95% lower and upper confidence intervals (CI), and p-values of the variables retained in the best model. Significance levels are denoted as * p < 0.05, ** p < 0.01, and *** p < 0.001.*

### 3.7.5 Predicting suitability for *An. funestus* larvae presence

We used the final model to predict the expected suitability for *An. funestus* positive habitats throughout the entire study area, including villages from where no field surveys had been conducted (Fig. 3.5).

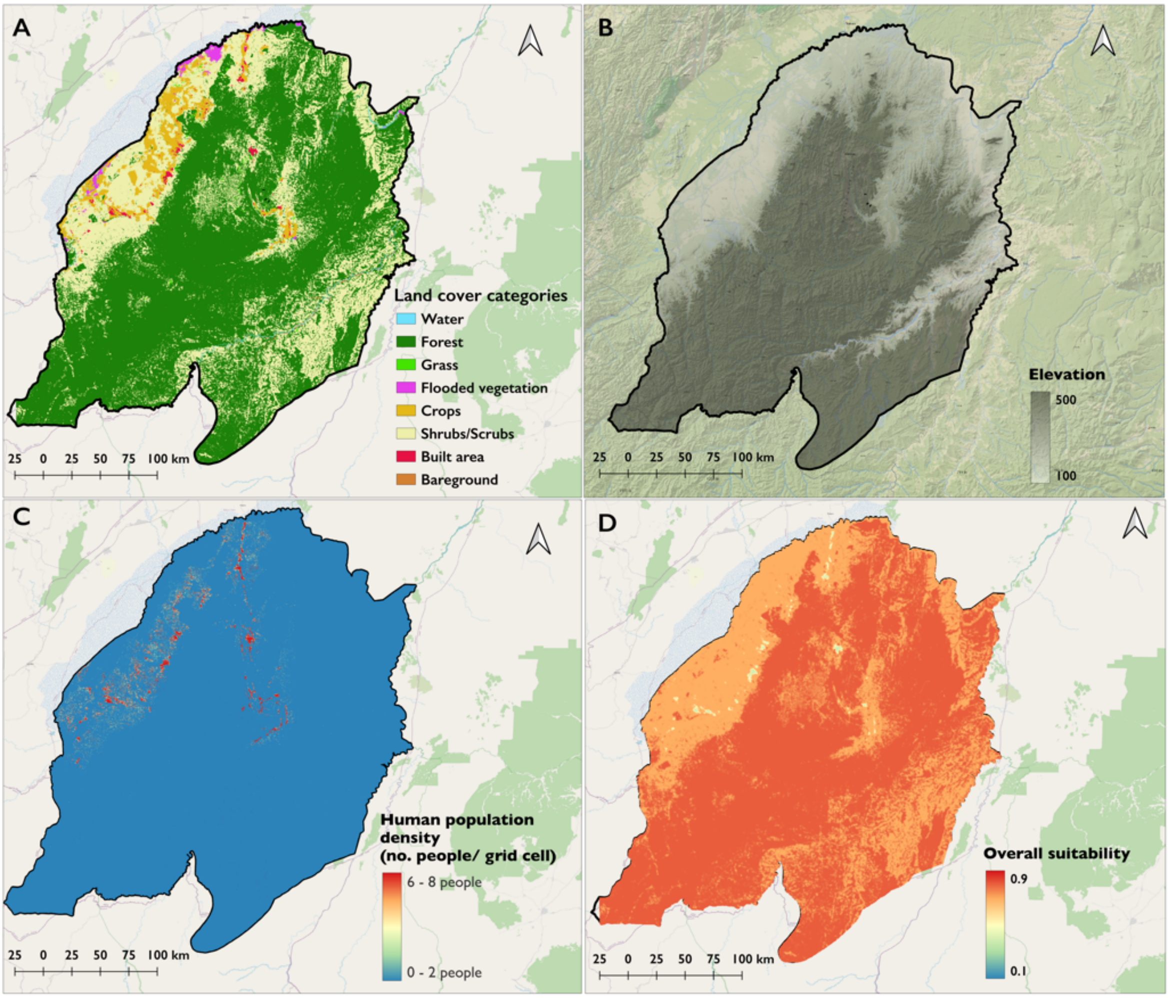

**Figure 3.5:** Showing the map of remotely sensed covariates across the study area and the predicted overall suitability for the presence of *An. funestus* in aquatic habitats: A) landcover, presents a classification of the area land cover B) Terrain elevation of the area, with grey shading indicating the gradient from lower to higher elevations. C) Density of human population per grid cell, with a colour gradient from blue to red, where blue represents lower density and red represents higher density areas. D) Overall Suitability: this synthesizes 8 significant remote sensing and habitat characteristics variables into an overall suitability map for *An. funestus* larval habitats.

Due to variability in environmental factors at different scales, we used interactive system, with different scenarios of how specific environmental conditions might influence the distribution and suitability of habitats for *An. funestus*, can be visualized and evaluated. Figure 3.6 shows examples of representative scenarios reflecting both the likelihood of an *An. funestus* habitat in that area being positive and the importance of specific condition of the individual habitats. This multifaceted approach allows us to explore how different combinations of environmental conditions can influence that habitat suitability for *An. funestus* larvae, under the assumption that aquatic habitats are present at these locations. Notably, the central region of the study zone consistently shows the highest suitability for *An. funestus* habitats, attributable to factors like dense tree cover, persistent water bodies, clear and flowing water, and shading conditions, all ideal for this vector species. In contrast, areas closer to built-up regions with temporary, unclear, and stagnant waters showed lower suitability.

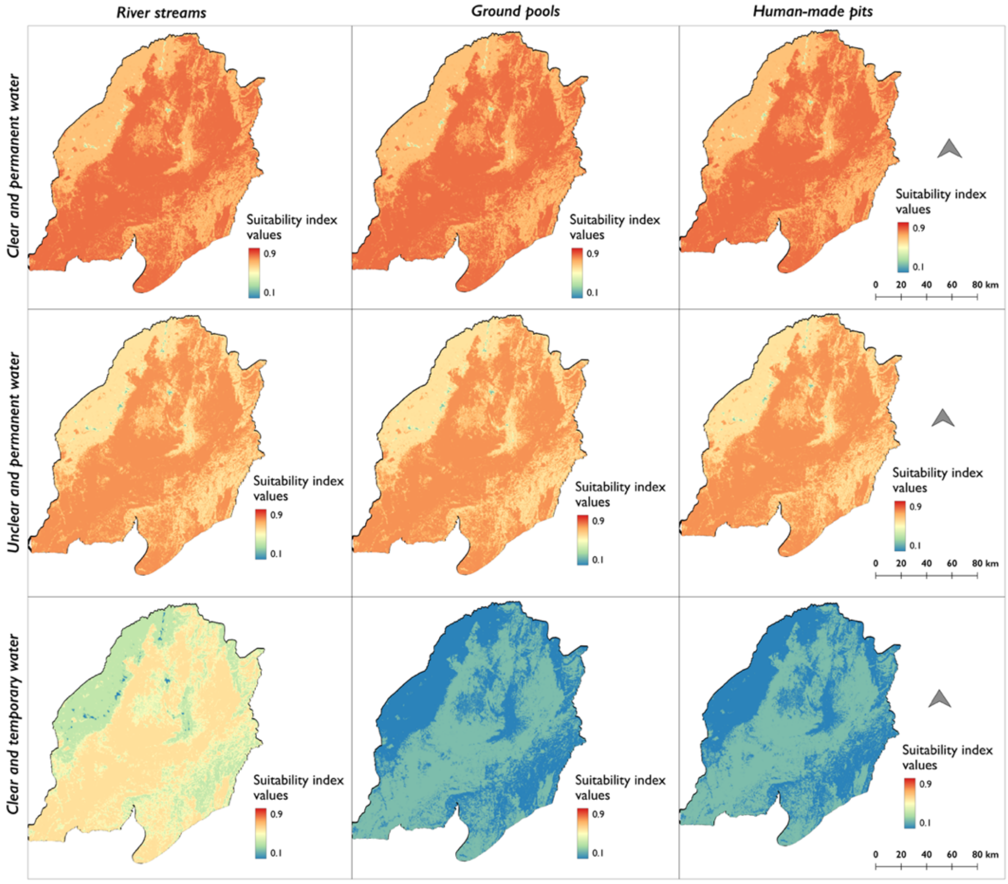

**Figure 3.6:** Examples of suitability maps for *Anopheles funestus* larval habitats under diverse environmental scenarios. This figure serves as an illustrative tool for understanding the varying suitability of larval habitats for *An. funestus* across

different environmental scenarios. These examples show how specific conditions such as water clarity, permanence, and habitat type (river streams, ground pools, and human-made pits) influence habitat suitability, while other environmental conditions remain constant (moving water, rainwater as water source, shaded habitats, absence of algae, areas with cattle grazing, cultivated fields and scrubs).

Ground pools and river streams, for instance, generally exhibit higher suitability compared to human-made pits. Further visualization is facilitated through an interactive web application, which can be accessed online for a comprehensive exploration of these environmental impacts on habitat suitability at http://boydorr.gla.ac.uk/lucanelli/kahamba_funestus/.

## 3.8 Discussion

Investigation of the aquatic ecology of *An. funestus* during the dry season can address a critical gap for planning larval source management (LSM) strategies. This study investigated the associations of habitat characteristics and land cover with the presence and distribution of *An. funestus* larvae in aquatic habitats in in two south eastern Tanzania districts. Our findings reveal that *An. funestus* larvae predominantly inhabit river streams and ground pools, with a marked association with areas characterized by extensive tree cover, grasslands, and shrublands. Conversely, larvae are less frequently found in built-up and semi-urban areas. Additionally, the study highlights the species' adaptability, thriving in both vegetated and non-vegetated habitats, and their preference for the more permanent water bodies over temporary ones.

*Anopheles funestus* larvae were found in various habitat types, including river streams, ground pools, and human-made pits such as spring-fed wells and dug pits. In the dry season, it was observed that some river streams undergo evaporation, resulting in the formation of isolated pools in the river stream bed. In some areas, local communities intentionally created artificial pools by blocking the natural flow of river streams for the purpose of agricultural irrigation, and these became important habitats for *An. funestus*. Other human-made pits, designed for various purposes such as construction, and domestic use, also served as larval habitats as previously observed [262]. We also found large ground pools characterized by stagnant water and emergent vegetation. It has been previously reported that river streams are an important feature of *An. funestus* ecology in east and west Africa [263,264]. Similarly, habitat stability and vegetation cover have also been reported as important for the vector species. For example, in one study in western Kenya near Lake Victoria, *An. funestus* larvae were found only during periods of high-water levels [265]; and in southern Mozambique, the species was most abundant in vegetated swamps where water accumulated throughout the year [266].

This study also identified a higher likelihood of finding *An. funestus* larvae in clear aquatic habitats compared to unclear habitats, which is consistent with previous research in the same settings [243], and in the western Kenya highlands, where *An. funestus* larvae had a high prevalence in clean water bodies [242,267,268]. Debrah et al and Nambunga et al, who worked in rural communities emphasized the importance of proximity to human dwellings as a significant factor for *An. funestus* habitation [242,243]. In our study, where this factor was analyzed from the perspective of landcover, we found that the likelihood of *An. funestus* habitation was lower in aquatic habitats in built up areas with concentrated human populations, which suggests that anthropogenic effects on the environments negatively impacts the ecology of *An. funestus*. Our expansive field surveys included inspection of even the most remote locations, allowing us to identify aquatic habitats far from residential areas. These remote habitats should not be ignored as they could serve as key refugia, supporting the persistence of mosquito populations.

Our study, conducted in the dry season, found that most aquatic habitats for *An. funestus* were permanent, corroborating earlier research by Mwangangi [263], Nambunga [243], and others [242,269]. However, a key limitation of these studies is not tracking these habitats across different seasons. To fully understand habitat permanence and its impacts on *An. funestus*, year-round monitoring across both wet and dry seasons is necessary. It therefore remains uncertain if these mosquitoes prefer permanent habitats all year-round or if these are simply the only option available in the dry season. To answer this question of temporal variation, a further investigation of the larval habitat use of An. funestus between the dry and wet season (Chapter 4) and across the year (Chapter 6) are presented in this thesis. Furthermore, contrary to previous studies reported a preference for dense vegetation in habitats [15,16], our observations indicate that this species is capable of laying eggs in both vegetated and non-vegetated habitats, with a particular affinity rich in algae. The occurrence of *An. funestus* larvae in habitats with algae suggests potential symbiotic relationship between larvae and algal blooms, possibly driven by the nutrients provided by the algae [28,270].

The study revealed localized differences in aquatic habitat distribution across several villages, underscoring *An. funestus* selective habitat use even within small geographical areas. *Anopheles funestus* positive river streams, for instance, were common in Chikuti, Lukande, and Mtimbira, but not in Kichangani and Ipera Asilia, indicating that habitat suitability is affected by the unique characteristics of each river and its ecosystem. Similar patterns observed in other studies highlight the complexity of *An. funestus* ecology [242,263], and emphasize that not all habitats, even within the same category, can support *An. funestus* larvae [271]. Habitats like rice fields, ditches, and hoofprints showed little or no presence of *An. funestus* larvae, aligning with research suggesting their selective breeding site preferences, often avoiding human-modified habitats [242]. This underscores the need for localized research and tailored interventions [93], as effective strategies in one area might not be suitable in another [272].

This study shows that *An. funestus* predominantly inhabits pristine environments like forests, grasslands, and shrublands, and is less common in human-modified areas such as urban or semi-urban settings. A significant association was found between *An. funestus* larvae and natural land cover types, with forested regions providing shaded, humid microclimates conducive to larval survival [273,274]. Factors such as shade from tree canopies and favorable microclimates enhance larval persistence as well. Moreover, river streams, which were the dominant habitat type in these tree-covered areas had the highest likelihood of larvae presence. In contrast, built-up areas and flooded areas limited the suitable breeding grounds, leading to reduced suitability for *An. funestus* larvae [275,276].

Although the larval ecology of *An. funestus* is not extensively studied [92], and its relationship with land cover needs more exploration, existing research, including studies in Kenya, supports our findings of an association between forested areas and larvae presence [267,277]. This study, along with other research identifies various land cover types such as forests, farmland, and pastures as potential habitats, underscoring the species' adaptability [268,276]. These variations underline the importance of understanding how different environmental and landcover factors contribute to the distribution of *An. funestus* larvae, which is vital for implementing effective larval source management (LSM) strategies and malaria control [272,278]. Targeting specific habitats that are hotspots for *An. funestus* larvae allows for a more efficient allocation of resources and implementation of interventions like larviciding or habitats modification [279]. However, the low $R^2$ in our model highlights a limitation in its predictive power, particularly when extrapolating results to regions not represented in the study area. Removing localized variables could make the model more widely applicable, but this would come at the cost of reduced precision in predicting larval habitats at a local level. This trade-off should be considered in public health applications of the model.

This study has some limitations. First, while our model accounted for a significant portion of the variability in *An. funestus* habitation, it may have omitted other influential factors. To enhance the understanding of the ecology of *An. funestus*, future research could include variables such as NDVI (Normalized Difference Vegetation Index), NDWI (Normalized Difference Water Index) and rainfall [280], as well as hydrological and geomorphology parameters [189,281,282]. Second, the accuracy of detecting mosquito larvae is influenced by sampling methods, including the number of samples, technician expertise, and spatial coverage. Our study may have been limited by the pre-specified nature of these parameters. Finally, integrating field environmental data with remote sensing land cover data presents multiple challenges. For example, the resolution of land cover data used here might have been insufficient to capture fine details like isolated residences and small water bodies, impacting habitat suitability mapping. To address these limitations, future studies may consider adopting higher-resolution remote sensing data sources, such as unmanned aerial vehicle (UAV) imagery [190,283,284], to capture finer details of habitats within a smaller geographical area and detect potential water sources for *An. funestus* [190].

## 3.9 Conclusion

This study comprehensively identified the main land use and environmental factors that influence larval habitat use by *An. funestus* in the dry season in southern Tanzania, where the species dominates malaria transmission. We found that river streams and ground pools were the primary larval habitats during the dry season, and that water bodies in forested areas, grasslands and shrublands are most likely to be positive for *An. funestus*. In contrast, larvae were least likely to be found in aquatic habitats in built-up and semi-urban areas. These insights are crucial for the strategic implementation of larval source management (LSM) strategies, particularly during the dry season when habitats are typically “few, fixed and findable”. The habitat suitability model developed here can be instrumental in pinpointing geographic areas where *An. funestus* larvae are most likely to be found, thereby facilitating targeted LSM deployment. Such targeted strategies, including larviciding and habitat modification, can be more effectively applied in high-risk zones identified through hour model, enhancing the efficacy of malaria control measures during the dry season. Building on these insights will further refine our understanding of mosquito dynamics, paving the way for enhanced strategies in malaria control and elimination.

# Chapter 4: Seasonal variation in availability and use of aquatic habitats by the malaria vector *Anopheles funestus*

*In preparation for submission to the Journal of Applied Ecology*

## Abstract

**Background:** Larval source management (LSM) can be highly effective for control of malaria vectors like *Anopheles funestus s.s.* that typically use large and permanent larval habitats. While *An. funestus* habitats can persist year-round, their diversity and quantity may shift between wet and dry seasons in response to rainfall-mediated availability. Understanding the seasonality in larval habitat availability and use is crucial for assessment of which aquatic habitats are most important for vector population persistence, including when and where LSM would be most effectively implemented. Here we investigated the availability and use of *An. funestus* larval habitats across the wet and dry season in Tanzania, and the environmental factors that influence these seasonal patterns.

**Methods:** Cross-sectional surveys were conducted in five villages, in south-eastern Tanzania during dry (September - November 2021) and rainy seasons (February - May 2022) to map and characterize potential aquatic habitats and identify those occupied by *An. funestus*. Complimentary data was extracted from remotely sensed satellite imagery and generalized linear mixed models were used to investigate seasonal and environmental predictors of *An. funestus* larval presence and abundance.

**Results:** A total of 2,844 aquatic habitats were identified in the wet and dry seasons. Both the number (2,485 versus 339) and types of habitats occupied by *An. funestus* were higher in wet season compared to the dry season, though a greater proportion of available aquatic habitats were inhabited by *An. funestus* in the dry season (dry = 44.5%, wet = 24.5%). Habitats used by *An. funestus* in both seasons included large and less-transient aquatic bodies such as river streams, ground pools (ponds) and ditches, with spring-fed wells being more significant in the wet season.

Rice fields and human-made habitats like dug pits played a modest role in the wet season but were absent or unused by the species during the dry season.

Mosquito species diversity and abundance were greater in the wet season, but the numerical dominance of *An. funestus* was more pronounced in the dry season. The presence and abundance of *An. funestus* were associated with the location (village), habitat type, physio-chemical properties of habitats, and the interaction between habitat type and season. This interaction suggests that *An. funestus* alters its habitat use based on seasonal changes, highlighting a significant shift in its ecological behaviour between wet and dry seasons.

**Conclusions:** This study revealed significant seasonal shifts in availability and types of habitats used by *An. funestus*. While the previous assertions that *An. funestus* prefers permanent and semi-permanent habitats remain generally true, our findings suggest that the species readily adapts to seasonal changes in availability of aquatic habitats and can occupy a diverse range of habitats during wet season. A major implication of this study is that in settings where *An. funestus* dominates malaria transmission, LSM strategies should be adaptable, targeting permanent habitats in dry months while accommodating diverse habitats in wet seasons.

## 4.1 Introduction

Understanding the relationship between the availability of aquatic habitats and their impact on mosquito vector populations is important for development of effective malaria control strategies [102]. The diversity and availability of aquatic habitats used by African malaria vectors undergo significant seasonal transformations, largely due to seasonal patterns of rainfall [285,286]. For instance, during the dry season, only larger and more permanent water bodies such as ground pools and river streams may be prevalent [22], whereas temporary habitats like puddles and flooded areas proliferate during and after the rains [244,287]. These changes in habitat distribution and availability impact the spatial and temporal distribution of adult mosquito populations, which are key to managing malaria transmission dynamics [288,289]. Understanding how these fluctuations in habitat availability translate into use by malaria vectors is crucial both for understanding the dynamics of their populations, and more effective targeting of vector control strategies [92].

Many African settings experience highly seasonal rainfall, which can lead to substantial variation in water availability and land cover [281,282,290]. Given the dependency of *Anopheles* mosquito vectors on water bodies for larval development, this seasonality is the major driver of their population dynamics [285]. While presence of suitable aquatic habitats is essential for vector population persistence, there can be substantial variation between the use (occupancy) and abundance of larvae in different aquatic habitats in relation to their physico-chemical properties such as temperature, nutrient levels, water movement, and presence of predators or competitors [22,242,265,291]. Additionally, there can be substantial variation in the use of aquatic habitats between mosquito species. For example, the major African malaria vector *An. gambiae s.l.,* can use diverse aquatics habitats including puddles, rice fields, and hoofprints [292,293]. In contrast *An. funestus s.l.,* another very important vector species, tends to occupy large more permanent water bodies with vegetation [22,23,154].

In most tropical settings, seasonal variation in rainfall is the most important driver of aquatic habitat availability, mosquito abundance and diversity [29]. While the role of seasonality on these attributes has been extensively studied [22,285], much less is known about how mosquitoes adapt their use of different habitat types to match seasonal changes in availability. Understanding how malaria vectors adapt their selection and use of aquatic habitats across the year is essential for guiding the design and implementation of effective Larval Source Management (LSM) control strategies that are optimally timed and targeted.

Despite the recognized importance of seasonality in driving malaria vector dynamics, there is still a considerable knowledge gap on how it influences the quantity, quality, and distribution of African *Anopheles* larval habitats. This is particularly true for *An. funestus,* the most important vector of malaria in southern Tanzania and several other settings in east and southern Africa [238]. On account of the unique tendency for this vector species to use larger, more permanent and easier to find aquatic habitats for larval development, it has been identified as good candidate for LSM on account of the World Health Organization’s criteria of having larval habitats that are “fixed, few, and findable” [61,70,102]. However, it is hypothesized that *An. funestus* might adapt its larval habitat use seasonally in response to availability, including possible expansion during the rainy season into temporary and or harder to reach habitats. Such seasonal adaptations in larval habitat use would increase the complexity of LSM and warrant careful consideration of the timing and selection of habitats for optimal impact.

Here, I investigated seasonal variation in the availability and environmental characteristics of aquatic habitats available to *An. funestus,* and how this influenced their pattern of habitat use. The study was conducted in south-eastern Tanzania where *An. funestus* is the predominant malaria vector and builds on our previous ecological research by providing the first analysis of seasonal shifts in habitat use. Specific objectives were: i) to estimate seasonal variation in the availability and type of aquatic habitats; ii) assess how the types of aquatic habitats used by *An. funestus,* and their abundance in them varies seasonally; and iii) examine the spatial distribution of aquatic habitats across season. Results will be of value for informing LSM strategies for improving malaria control initiatives, particularly in areas where *An. funestus* is the most dominant malaria vector.

## 4.2 Methods

### 4.2.1 Study area

The study was conducted in five villages in the districts of Ulanga and Malinyi in south-eastern Tanzania: Ikungua (-8.463384°, 36.687253°), Chikuti (-8.6028°, 36.7288°), Ebuyu (-8.9719°, 36.7608°), Itete (-8.720182°, 36.343460°), and Sofi Majiji (-8.927893°, 36.268187°) - approximately 100 km from Ifakara town (Fig. 4.1). Villages were selected based on known malaria prevalence [294], presence of *An. funestus* (Kahamba *et al.*, 2024) and to encompass both high (> 400 m) and low (=< 200 m) altitude sites. Monthly temperatures ranged between 20°C and 32.6°C [245] and average annual rainfall was approximately 1500 mm. There is marked seasonal variation in temperature and rainfall across the study area; with the dry season typically running from June to November and highest temperatures falling between September and November. There are two distinct wet seasons: the short rainy season (December - January) and longer rainy season (February - May).

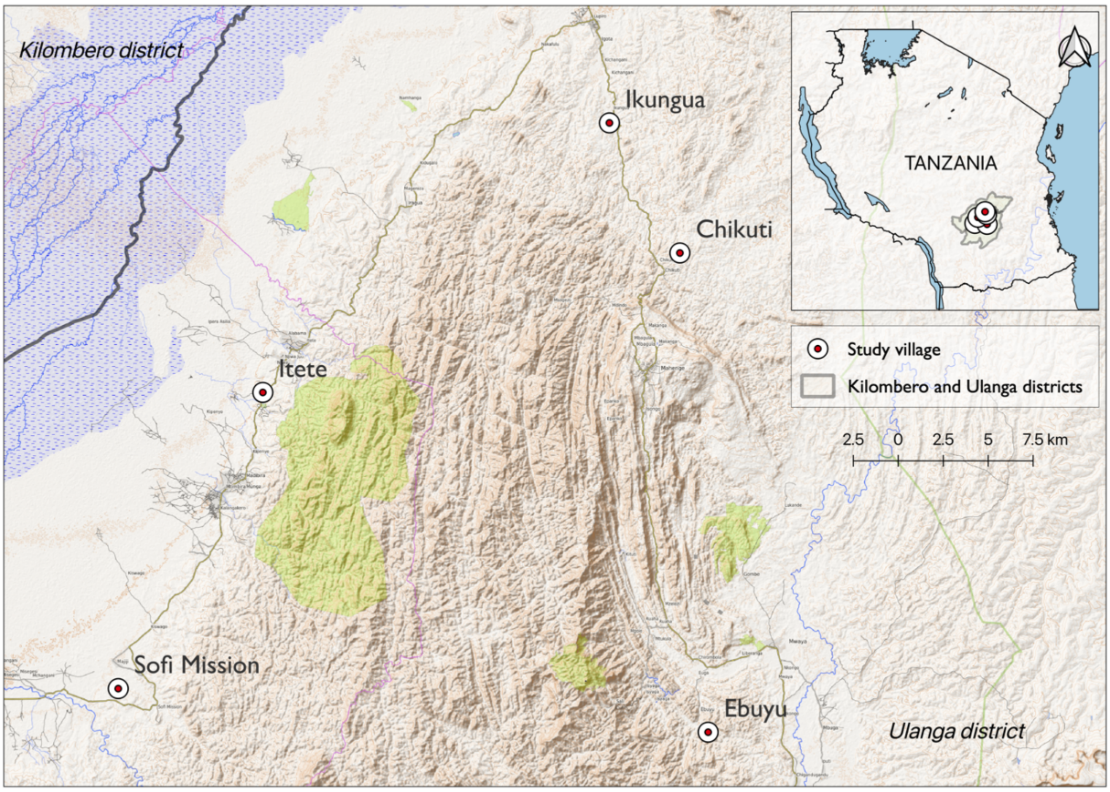

**Figure 4.1:** Showing the geographic locations of the five study villages (red circles) within Ulanga districts, in south-eastern Tanzania.

### 4.2.2 Study design

A cross-sectional survey was used to assess the distribution of aquatic habitats as well as the presence and abundance of *An. funestus* larvae during the dry (September - November 2021) and rainy season (February - May 2022). Comprehensive surveys of all aquatic habitats were conducted in each study village in each season. The mapping of habitats involved a structured approach as outlined in chapter 3.2.2, where transects were systematically walked across the area of each village to identify all potential aquatic habitats, ranging from large, permanent water bodies such as ponds and river streams to small temporary habitats like puddles, ditches, and hoofprints as described in chapter 3.

### 4.2.3 Characterization of aquatic habitats

The exact location of all identified aquatic habitats were recorded using a hand-held GPS receiver. Different physio-chemical characteristics of each habitat were then measured and recorded as described in chapter 3 (Table 3.1).

### 4.2.4 Larval sampling

Larval sampling was done in all potential habitats to determine if mosquito larvae were present. Here a water body, irrespective of the presence of larvae, was defined as an "aquatic habitat," whereas "larval habitat" refers to aquatic habitats where larvae were detected. Habitats with at least one *An. funestus* larva at any filial stage were classified as *An. funestus* habitats. Depending on the size and depth of aquatic habitats, larval surveys were performed using a standard 350 ml dipper or a 10-L bucket. Specifically, the 10-L bucket was employed for larger habitats, typically those with water depths exceeding 30 cm (as shown in chapter 3, Fig. 3.2). quantify the larvae, samples from each dip (standard dipper or 10-L buckets) were counted and recorded [22]. The contents of the dipper or bucket were carefully poured or filtered into a white tray for subsequent counting and sorting. For standardization, we defined a new variable named *'Total volume sampled (TVS)'*, calculated using the following formula:

$$TVS = \begin{cases} \text{Number of dips} \times 10 & if\ sampling\ method\ is\ 10l\ Bucket \\ \text{Number of dips} \times 0.35 & if\ sampling\ method\ is\ 350ml\ dipper \end{cases}$$

This formula calculated the total volume of water sampled based on the sampling method. This variable was used to control for variation in sampling effort when analyzing mosquito presence and abundance as described in analysis methods.

### 4.2.5 Taxonomic identification

All the collected mosquito larvae were morphologically identified in the field using established taxonomic keys by Gillies and De Meillon and Gillies and Coetzee (Gillies and de Meillon, 1968; Gillies and Coetzee, 1987). Larvae were identified into taxonomic groups of *An. funestus sensu lato* (*sl*), *An. gambiae sl*, and *Culex*, and others. Morphological identification was the primary method used for calculating diversity indices at taxonomic group level. Diversity in this context was quantified using two metrics: the Shannon diversity index and Simpson's diversity index, which consider both the number of different taxonomic groups species (richness) and their relative abundance (evenness).

The Shannon-Wiener Index quantifies diversity by considering both the abundance and evenness of mosquito species, with higher values signifying greater diversity. The Simpson's Diversity Index measures the probability that two randomly selected mosquitoes belong to the same species, with values near 1 suggesting lower mosquito diversity. In this study, members of the *An. gambiae* group were assumed to be *An. arabiensis*, while *An. funestus* group were assumed to be *An. funestus* s.s. as per previous observations [86,246].

### 4.2.6 Acquisition of remote sensing data

Satellite observation data were extracted from PlanetScope commercial satellites. These satellites are known for their high spatial (3 meters) and temporal (daily) resolution [295]). Images were extracted to align with the periods in the dry (September - November 2021) and rainy (February- May 2022) seasons when larval surveys were conducted. In both seasons, images with the best image quality and minimal cloud coverage within the specified duration were prioritized. The PlanetScope satellite data encompassed eight spectral bands: blue, green, red, near-infrared (NIR), coastal blue, green I, yellow, and red edge [295,296]. We used the green and NIR bands to generate Normalized Difference Water Index (NDWI). This index is appropriate for detecting water [297].

## 4.3 Data analysis

All analyses were done using R statistical software version 3.7.1 [258].

### 4.3.1 Seasonal variation availability of aquatic habitats

Seasonal variation in aquatic habitat availability was estimated in two ways. First the remote sensing data was used to assess seasonal shifts in the relative availability of aquatic habitats in terms of the proportion of land covered by water. In the *R* environment, training data for the classifier were generated through random sampling within water and non-water polygons, producing 1,000 points for each class. These points were then used to extract corresponding spectral band values from the multispectral images. A recursive partitioning decision tree model (*rpart* package) was employed to develop the classification algorithm [298]. This model utilized the extracted band values as predictors for the binary classification task.

The decision tree was visualized to ensure interpretability of the classification criteria. Model performance was evaluated using a confusion matrix, with accuracy and the Kappa statistic as key metrics. The confusion matrix was visualized using a customized *ggplot2* function, *ggplotConfusionMatrix*, which highlighted the proportion of correct and incorrect classifications within the dataset [299]. Classifications from this model were used to indirectly measure seasonal changes in water availability based on proportion of land classified as water. Additionally, seasonal variation in aquatic habitat availability was directly estimated from field observations by comparing the quantity and types of aquatic habitats recorded in each season.

### 4.3.2 Seasonal and environmental determinants of the presence and abundance of *An. funestus* larvae in the different aquatic habitats

Aquatic habitat used by *An. funestus* was defined in terms of positivity (% habitats in which *An. funestus* larvae were present) and productivity (mean number of *An. funestus* larvae per total number of dips taken). First, the number and types of aquatic habitats used by *An. funestus* larvae in different seasons was tabulated for descriptive analysis.  Second, statistical models were built to test for variation in *An. funestus* larval positivity and abundance as described below.

Generalized Linear Mixed Models (GLMM) were used to estimate the predicted mean larval positivity and abundance in habitats, and how they varied between seasons, habitat types and in association with physio-chemical characteristics (Bates *et al.*, 2015). In the GLMM used to analyse positivity, habitats were assigned a binary outcome of '0' if *An. funestus* larvae were absent, and ‘1’ if present. Given the primary emphasis on investigating seasonality, covariates were fit for season (rainy or dry), its interaction with habitat type, and environmental factors known to affect *An. funestus* larval ecology such as, vegetation quantity, water clarity, and water source (see Table 4.1 Model 2 for full details). A random effect for sampling date was included to account for potential dependencies in observations. The interaction between season and habitat type was specifically fit to allow testing of the hypothesis that the types of larval habitats used by *An. funestus* varied between seasons.

To analyze variations in *An. funestus* larval abundance across habitats, I employed a generalized linear mixed model (GLMM) with a negative binomial distribution. This approach was selected due to the overdispersion observed in the data, making a Poisson model unsuitable. A zero-inflated negative binomial (ZINB) model was fitted to account for the excess zeros and to provide a more accurate fit [300]. Before fitting the model, I tested the proportion of zeros and conducted a likelihood ratio test comparison between zero-inflated and non-zero-inflated models, which supported the inclusion of zero inflation.

Like the GLMM for analysis of positivity, this model focused on examining variation *An. funestus* larvae abundance between season, habitat type and their interaction, and in response to selected environmental habitat characteristics (Table 4.1, Model 3). Data in the ZINB model were divided into two categories: habitats with a larvae count greater than zero, and those with no larvae.

Both models incorporated a similar set of explanatory variables, with model selection conducted by likelihood ratio tests (LRTs) using stepwise backward selection. To account for the variability in sampling effort, we also included the standardized total volume of water sampled as an offset term.

### 4.3.3 Seasonal variation in the diversity of mosquito taxa

Descriptive analysis was conducted to summarize the composition of mosquito taxonomic groups (e.g., *An. funestus sl*, *An. gambiae sl*, *Culex*) between seasons and habitat types. The diversity and dominance of mosquito taxonomic groups in different aquatic habitats was then estimated in terms of species richness (S), Shannon-Wiener (H), and Simpson's (D) diversity indices. We did not have species-level taxonomic data as molecular identification was not performed to distinguish cryptic species. Thus, diversity indices were calculated with respect to species groups. Species richness was calculated as the total number of unique taxonomic groups, with the other indices calculated as defined in Table 4.2. Shannon-Wiener Index (H) was used to capture both species richness and species evenness, which refers to how evenly individuals are distributed among the species present. High evenness indicates that species are present in similar proportions, while low evenness suggests dominance by one or a few species. These indices were chosen to capture the variety of species (richness), the evenness of species distribution (Shannon–Wiener), and the dominance or concentration of abundance in a few taxonomic groups (Simpson’s). Finally, simple Kruskal–Wallis tests were used to determine statistical differences in species diversity between habitat types and season.

**Table 4.1:** Summary of Statistical Models used to include the primary response variable, explanatory variables, random effect variables, and statistical distribution used.

| Model | Response variables | Fixed Effect variables | Random effect variables | Statistical distribution |
|---|---|---|---|---|
| 1 | Availability of aquatic habitats (water and non-water classes) | Multispectral images with 8 bands: band 1 (coastal blue), band 2 (blue), band 3 (green i), band 4 (green), band 5 (yellow), band 6 (red), band 7 (red edge), band 8 (near-ir) | NA | Binomial distribution for binary classification |
| 2 | Presence or absence of *An. funestus* (positivity) | Season + Habitat type + Village + Habitat size + Water permanence + Water depth + Watercolour + Water source + Water movement + Algae type + Presence of shades + Vegetation + Distance from home + Habitat type: Season | Date | Binomial |
| 3 | Abundance of *An. funestus* larvae (productivity) | Season + Habitat type + Village + Habitat size + Water permanence + Water depth + Watercolour + Water source + Water movement + Algae type + Presence of shades + Vegetation + Distance from home + Habitat type: Season | Date | Negative binomial distribution |
| 4 | Species Diversity (Shannon Wiener) | Habitat type and Season | NA | Kruskal-Wallis |
| 5 | Species Diversity (Simpsons Index) | Habitat type and Season | NA | Kruskal-Wallis |

*Model 1 is a classification tree model. For model 2 and 3, an offset term based on the volume of the water sample was included to account for variations in sampling effort, "**:**" indicates an "interaction".*

**Table 4.2:** Description of indices used and their formular.

| **Indices** | **Formula** | **Definition of symbols** |
|---|---|---|
| Shannon-Wiener Index (H): combining species richness and abundance, provides a comprehensive measure of diversity | $H = -\sum_{n=i}^{n} \left(\frac{ni}{n}\right) ln \left(\frac{ni}{n}\right)$ | n = total number of individuals, *ni* = number of individuals for each species |
| Simpson's Index (D): This index measures the dominance or concentration of abundance in certain species | $D = \frac{1}{\sum_{n=i}^{n} \left(\frac{ni}{n}\right)^2}$ | n = total number of individuals, *ni* = number of individuals for each species |

### 4.3.4 Spatial distribution of habitats in wet and dry season

The spatial distribution of larval habitats was analyzed to examine patterns in the location and clustering of *An. funestus* larvae and how they varied seasonally. *Moran's* I statistic was calculated within the *spdep* package in R (Bivand, 2006) to summarize the degree of spatial autocorrelation in An. *funestus* positive habitats [301]. A *Moran's* I value close to +1 indicates strong clustering of habitats, while a value near -1 suggests dispersion. To complement this, we employed the method of Inverse Distance Weighting (IDW) interpolation using the *gstat* package in R. The IDW interpolation method was used to estimate the spatial distribution patterns of *An. funestus* habitats in areas that were not sampled, based on the proximity to sampled points. This method assigns more weight to nearer points, implying that habitats closer to sampled locations have a greater influence on the interpolation result. In our analysis, we used a 2-meter distance from the sampled points to estimate the weight of neighbouring points. A high IDW value would indicate a high likelihood of habitat occurrence in each area, which is important for understanding and predicting mosquito breeding sites' distribution.

## 4.4 Results

### 4.4.1 Seasonal variability in the availability and types of aquatic habitats

High-quality satellite imagery was obtained for only four of the five study sites (Ikungua, Sofi Majiji, Ebuyu, and Itete). The total area of land covered by water contracted by 40% between the wet (121.64 km$^2$) and dry season (72.92 km$^2$). The magnitude of change was consistent across villages; with Sofi Majiji and Ebuyu experiencing the greatest change between wet and dry season (approximately doubling, Table 4.3).

**Table 4.3:** Seasonal changes in water coverage by village

| | | **Rainy Season** | | **Dry Season** | |
|---|---|---|---|---|---|
| **Village** | Surface Area (km$^2$) | Area covered by water (km$^2$, %) | Accuracy %, (Kappa statistics) | Area covered by water (km$^2$, %) | Accuracy %, (Kappa statistics) |
| **Ikungua (Ulanga district)** | 60.40 | 36.4 (60.31%) | 87, (0.73) | 27.8 (46.14%) | 92, (0.84) |
| **Ebuyu (Ulanga district)** | 61.99 | 35.09 (56.59%) | 59, (0.13) | 16.7 (27.03%) | 93, (0.85) |
| **Sofi Majiji (Malinyi district)** | 72.12 | 26.56 (36.83%) | 85, (0.71) | 13.3 (18.31%) | 88, (0.77) |
| **Itete (Malinyi district)** | 50.64 | 23.59 (46.59%) | 94, (0.87) | 15.12 (29.86%) | 90, (0.81) |
| **Totals** | 245.15 | 121.64 | | 72.92 | |

*This table presents the data for dry and rainy seasons in the four villages, including total surface area, percentage water area, and accuracy along with Kappa statistics for the water classification.*

Mirroring trends predicted from remote sensing, the total number of observed aquatic habitat varied significantly across the seasons, from 2,485 in the wet season to 339 in the dry season (Table 4.4). Notably, observable stream segments decreased from 950 to 193, and there were substantial decreases in number of ditches (from 499 to 5) and ground pools (from 122 to 26). The diversity (types) of habitats also declined. In the wet season, 9 different aquatic habitats were present, with ~100 or more of each type being identified (including river streams, ground pools or ponds, dug pits & holes, spring-fed wells, brick & concrete pits, and rice fields). On the other hand, in the dry season, only 7 habitat types were detected and all in lower numbers than the wet season, with only the 'river and stream segment' category having more than 100 habitats. Habitats that were present during the wet season but completely disappeared during the wet season were rice fields and tyre tracks (Table 4.4).

### 4.4.2 Seasonal variability in proportion of habitats inhabited by *An. funestus* (larval positivity)

In the dry season 44.5% of aquatic habitats were positive for. *An. funestus;* with occupancy highest in river streams (69%). Positivity was intermediate in ground pools and ditches (42% and 60% respectively) with other habitats like dug pits, spring-fed wells, and brick and concrete pits having minimal to zero positivity (Table 4.4). The overall positivity rates fell to 24.5% in the rainy season; likely reflecting the huge upsurge in the total number of habitats available rather than a decrease in the *An. funestus* population. Stream segments, ground pools and ditches continued to have highest positivity in similarity to the dry season (Table 4.4). However, spring-fed wells, dug pits, and rice field habitats that had had low or no *An. funestus* in the dry season showed higher positivity in the wet season (Table 4.4). Notably the positivity of spring-fed wells for *An. funestus* increased from 5 to 34% between the dry and wet season.

**Table 4.4:** Total habitats, positivity, and abundance for *An. funestus* in dry rain and season

| | Rainy season | | | Dry season | | |
|---|---|---|---|---|---|---|
| **Habitat type** | Total habitats (N) | Habitats positive for *An. funestus* (%) | Mean ± SE | Total habitats (N) | Habitats positive for *An. funestus* (%) | Mean ± SE |
| **River/Stream segments** | 950 | 295 (31) | 0.38 ± 0.49 | 193 | 133 (69) | 0.57 ± 0.49 |
| **Ground pools** | 122 | 46 (38) | 0.05 ± 0.22 | 26 | 11 (42) | 0.08 ± 0.27 |
| **Ditches** | 499 | 140 (28) | 0.20 ± 0.40 | 5 | 3 (60) | 0.01 ± 0.12 |
| **Dug pits & holes** | 318 | 20 (6) | 0.13 ± 0.33 | 24 | 0 (0) | 0.07 ± 0.26 |
| **Spring fed wells** | 186 | 64 (34) | 0.07 ± 0.26 | 71 | 4 (5) | 0.21 ± 0.41 |
| **Brick & concrete pits** | 135 | 8 (6) | 0.05 ± 0.23 | 18 | 0 (0) | 0.05 ± 0.22 |
| **Rice fields** | 176 | 34 (19) | 0.07 ± 0.26 | 0 | 0 (0) | 0.00 ± 0.00 |
| **Tire track** | 95 | 3 (3) | 0.04 ± 0.19 | 0 | 0 (0) | 0.00 ± 0.00 |
| **Hoofprints** | 4 | 0 (0) | 0.00 ± 0.04 | 2 | 0 (0) | 0.01 ± 0.08 |
| **Totals** | **2485** | **610 (24.5)** | **-** | **339** | **151 (44.5)** | **-** |

*This table summarizes the overall number of habitats identified, the positive and proportion of An. funestus and their crude mean abundance across the season.*

Of the 15 explanatory variables considered in the GLMM, 11 were significantly associated with *An. funestus* positivity. *Anopheles funestus* positivity was generally higher in the dry season compared to the wet season across most habitat types, with a significant interaction between habitat type and season ($p < 0.001$). Specifically, positivity was predicted to be higher in the dry season in streams, ground pools, and ditches, while in dug pits, positivity was notably higher in the wet season (Table 4.5). Among the villages, positivity was significantly higher in Ebuyu (OR = 5.714, CI [4.190–7.792], $p < 0.001$) compared to Chikuti, which had the lowest positivity. Environmental factors played a key role, with positivity being higher in habitats with deep water (more than 50 cm; OR = 2.368, CI [1.452–3.861], $p = 0.001$), rainwater-fed habitats (OR = 2.874, CI [2.081–3.971], $p < 0.001$), and shaded habitats (OR = 1.536, CI [1.131–2.087], $p = 0.006$). Habitats containing filamentous algae (OR = 2.339, CI [1.249–4.382], $p = 0.008$) or mixed vegetation (OR = 2.422, CI [1.359–4.315], $p = 0.003$) were also associated with higher *An. funestus* positivity. Conversely, positivity was lower in polluted waters (OR = 0.278, CI [0.188–0.412], $p < 0.001$) and in stagnant waters (OR = 0.492, CI [0.321–0.755], $p = 0.001$) (Table 4.6).

**Table 4.5:** Summary of stepwise model reduction for predicting *An. funestus* presence. The table shows the chi-squared statistics, degrees of freedom (d.f.), and p-values for the likelihood ratio test of each variable.

| **Deleted Variable** | **Chi-Sq** | **d.f.** | **P** |
|---|---|---|---|
| **Habitat size** | 4.52 | 3 | 0.2108 |
| **Season * Village** | 8.91 | 4 | 0.0633 |
| **Distance from home** | 5.72 | 2 | 0.0573 |
| **Water type** | 3.42 | 1 | 0.0644 |
| **Included variables** | | | |
| **Season** | 116.98 | 4 | <0.001 |
| **Habitat type** | 58.34 | 6 | <0.001 |
| **Village** | 147 | 4 | <0.001 |
| **Water depth** | 12.09 | 2 | 0.0023 |
| **Water colour** | 66.01 | 1 | <0.001 |
| **Water source** | 38.39 | 1 | <0.001 |
| **Water movement** | 5.66 | 1 | 0.0173 |
| **Algae type** | 43.63 | 4 | <0.001 |
| **Shades** | 15.72 | 1 | <0.001 |
| **Vegetation type** | 57.49 | 3 | <0.001 |
| **Habitat type: Season** | 49.62 | 3 | <0.001 |

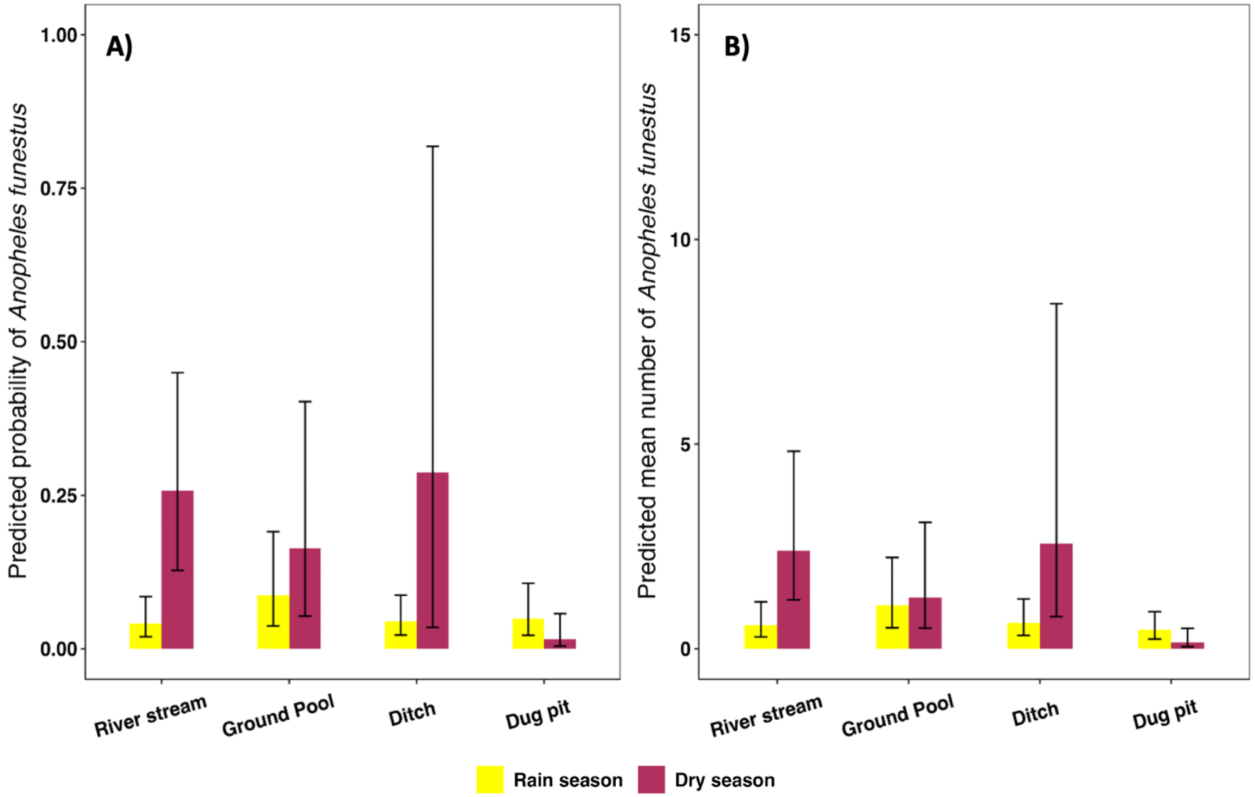

**Figure 4.2:** Seasonal comparison A) *An. funestus* positivity, B) *An. funestus* mean abundance per dip. Bar plots show the predicted probabilities and means for each habitat type during the rain and dry seasons, with error bars representing 95% confidence intervals. The statistical analysis was restricted only to 4 main habitat categories out of 9, where sample sizes were large enough to permit robust analysis in both seasons.

**Table 4.6:** Summary of the coefficient estimate (β), standard errors, z values and p-value for each explanatory variable included in the logistic model for predicting the presence of *An. funestus* aquatic habitats.

| Variable | OR | Lower CI | Upper CI | p-value |
|---|---|---|---|---|
| Intercept | 0.22 | 0.086 | 0.563 | 0.002 ** |
| Season: Habitat type | | | | |
| Dry season: River Stream | **1** | | | |
| Dry season: Ground Pool | 0.37 | 0.106 | 1.293 | 0.12 |
| Dry season: Dug pits | 0.054 | 0.016 | 0.179 | <0.001 *** |
| Dry season: Ditch | 1.115 | 0.101 | 12.275 | 0.929 |
| Village | | | | |
| Chikuti | 1 | | | |
| Ebuyu | 5.714 | 4.19 | 7.792 | <0.001 *** |
| Ikungua | 2.372 | 1.6 | 3.516 | <0.001 *** |
| Itete | 2.409 | 1.616 | 3.593 | <0.001 *** |
| Water depth | | | | |
| Less than 10 cm | 1 | | | |
| Between 10-50 cm | 1.128 | 0.773 | 1.646 | 0.532 |
| More than 50 cm | 2.368 | 1.452 | 3.861 | 0.001 ** |
| Water colour | | | | |
| Clear | 1 | | | |
| Polluted | 0.278 | 0.188 | 0.412 | <0.001 *** |
| Water source | | | | |
| Groundwater | 1 | | | |
| Rainwater | 2.874 | 2.081 | 3.971 | <0.001 *** |
| Water movement | | | | |
| Moving | 1 | | | |
| Stagnant | 0.492 | 0.321 | 0.755 | 0.001 ** |
| Algae type | | | | |
| No algae | 1 | | | |
| Filamentous | 2.339 | 1.249 | 4.382 | 0.008 ** |
| Green | 4.184 | 1.324 | 13.221 | 0.015 * |
| Mixed | 2.291 | 1.005 | 5.223 | 0.049 * |
| Shades | | | | |

|  |  |  |  |  |
|---|---|---|---|---|
| Unshaded | 1 |  |  |  |
| Shaded | 1.536 | 1.131 | 2.087 | 0.006 ** |
| Vegetation type |  |  |  |  |
| Submerged | 1 |  |  |  |
| Floating | 0.827 | 0.482 | 1.417 | 0.489 |
| Mixed | 2.422 | 1.359 | 4.315 | 0.003 ** |
| None | 0.682 | 0.522 | 0.89 | 0.005 ** |

### 4.4.3 Seasonal variability in abundance of *An. funestus* larvae

The predicted abundance of *An. funestus* larvae varied across different habitats, ranging from 0 to 2.3, with higher abundance generally observed in the dry season compared to the wet season, except in spring-fed wells (Table 4.5). Although the total number of larvae collected was greater in the wet season due to the increased number of available habitats, the abundance patterns remained distinct.

When testing for the proportion of zeros in the data, I found that approximately 70.4% of the values were zero, indicating a substantial amount of zero inflation, and the overdispersion ratio was 0.75, indicating slight underdispersion. To determine if a zero-inflated model was needed, a likelihood ratio test was performed between the zero-inflated (ZI) and non-zero-inflated (non-ZI) negative binomial models. The test results showed a significant improvement with the inclusion of the zero-inflated model ($X^2$ = 6.91, df = 1, p = 0.009), supporting the use of zero inflation for accurately modelling *An. funestus* abundance. Of the 14 explanatory variables tested (Table 4.1, Model 3), 11 were retained in the final model as statistically significant predictors of *An. funestus* abundance (Table 4.7).

Larval abundance was significantly associated with the interaction between season and habitat type (Table 5.8). *An. funestus* larval abundance was higher in Ebuyu (RR = 5.12, [CI: 3.38–7.76], p < 0.001) compared to the reference village, and also higher in Itete (RR = 2.20, [CI: 1.34–3.63], p = 0.002). Medium-sized habitats (10-100 $m^2$) showed greater larval abundance (RR = 1.54, [CI: 1.15-2.05], p = 0.004) compared to smaller habitats. Habitats with deeper water (more than 50 cm) were also associated with significantly higher larval abundance (RR = 2.48, [CI: 1.72-3.58], p < 0.001). Rainwater-fed habitats (RR = 1.82, [CI: 1.32-2.51], p < 0.001), shaded habitats (RR = 1.45, [CI: 1.15–1.81], p = 0.001), and those with filamentous algae (RR = 2.40, [CI: 1.40–4.11], p = 0.002) showed significantly higher larval abundance. In contrast, turbid habitats had significantly lower larval abundance compared to clear water habitats (RR = 0.45, [CI: 0.34–0.61], p < 0.001). Vegetation type was also influential, with mixed vegetation habitats showing higher larval abundance (RR = 1.61, [CI: 1.13-2.29], p = 0.008), which aligns with observations related to habitat positivity (Table 5.8).

**Table 4.7:** Summary of stepwise model reduction for predicting *An. funestus* abundance. The table shows the chi-squared statistics, degrees of freedom (d.f.), and p-values for the likelihood ratio test of each variable.

| **Variables** | **$X^2$** | **df** | **p-value** |
|---|---|---|---|
| Season | 106.69 | 32 | <0.001 |
| Habitat type | 67.76 | 26 | <0.001 |
| Village | 172.88 | 28 | <0.001 |
| Habitat size | 12.05 | 29 | 0.0072 |
| Water type | 6.71 | 31 | 0.0096 |
| Distance from home | 11.09 | 30 | 0.0039 |
| Shades | 11.18 | 31 | 0.0008 |
| Water depth | 23.96 | 30 | <0.001 |
| Water source | 44.12 | 31 | <0.001 |
| Algae type | 51.46 | 28 | <0.001 |
| Water colour | 51.78 | 31 | <0.001 |
| Vegetation type | 60.64 | 29 | <0.001 |
| Habitat type: Season | 46.19 | 29 | <0.001 |

**Table 4.8:** Regression Coefficients from GLMM Model predicting *An. funestus* abundance.

| **Variable** | **RR** | **Lower CI** | **Upper CI** | **p-value** |
|---|---|---|---|---|
| Intercept | 0.34 | 0.14 | 0.83 | 0.018 |
| Season: Habitat type | | | | |
| Wet season: River Stream | 1 | | | |
| Dry season: Ditch | 2.65 | 0.66 | 10.61 | 0.168 |
| Dry season: Ground Pool | 0.51 | 0.23 | 1.13 | 0.096 |
| Dry season: Dug pits | 0.08 | 0.03 | 0.25 | <0.001 *** |
| Village | | | | |
| Chikuti | 1 | | | |
| Ebuyu | 5.12 | 3.38 | 7.76 | <0.001 *** |
| Ikungua | 1.96 | 1.17 | 3.31 | 0.011 ** |
| Itete | 2.20 | 1.34 | 3.63 | <0.001 *** |
| Habitat size | | | | |
| Less than 10 m | 1 | | | |
| Between 10-100 m | 1.54 | 1.15 | 2.05 | 0.004 * |
| More than 100 m | 1.35 | 0.94 | 1.94 | 0.102 |
| Water type | | | | |
| Temporary | 1 | | | |
| Semi-permanent | 0.98 | 0.62 | 1.56 | 0.94 |
| Water depth | | | | |
| Less than 10 cm | 1 | | | |
| Between 10-50 cm | 1.29 | 0.95 | 1.75 | 0.09 |
| More than 50 cm | 2.48 | 1.72 | 3.58 | <0.001 *** |
| Water colour | | | | |
| Clear | 1 | | | |
| Turbid | 0.45 | 0.34 | 0.61 | <0.001 *** |
| Water source | | | | |
| Groundwater | 1 | | | |
| Rainwater | 1.82 | 1.32 | 2.51 | <0.001 *** |
| Algae type | | | | |
| No algae | 1 | | | |
| Filamentous | 2.4 | 1.4 | 4.11 | 0.002 ** |
| Green | 1.3 | 0.57 | 3 | 0.532 |

|  |  |  |  |  |
|---|---|---|---|---|
| Mixed | 2.22 | 1.19 | 4.14 | 0.0661 * |
| Shades |  |  |  |  |
| Unshaded | 1 |  |  |  |
| Shaded | 1.45 | 1.15 | 1.81 | <0.001 *** |
| Vegetation type |  |  |  |  |
| Emerged | 1 |  |  |  |
| Floating | 1.39 | 0.92 | 2.11 | 0.119 |
| Mixed | 1.61 | 1.13 | 2.29 | 0.008 ** |
| None | 0.69 | 0.56 | 0.86 | <0.001 *** |
| Distance from home |  |  |  |  |
| Less than 100 m | 1 |  |  |  |
| More than 100 m | 1.08 | 0.88 | 1.33 | 0.465 |

## 4.4 Seasonal variability in mosquito taxonomic diversity

A total of 69,241 immature mosquitoes, including larvae and pupae, were collected across different habitats in this study. Substantially more mosquito larvae were collected during the rainy (56,058) than dry season (13,062), reflecting the expansion of aquatic habitats (Table 4.9). The sampling effort involved 4,869 dips in the dry season and 33,285 dips in wet season. During the dry season, the predominant *Anopheline* species was *An. funestus s.l*, (37% of all collected mosquitoes, n= 4,840), followed by *An. gambiae s.l* (16%, n = 2,067) other *Anopheline* species (8%) and *Culicine* mosquitoes (39%). In the rainy season, *An. funestus* constituted only 20% of the mosquito larval community with *An. gambiae s.l* being the dominant Anopheline species (32%, Table 4.9).

All 4 taxonomic groups considered (*An. funestus sl, An. gambiae s.l,* other *Anophelines* and *Culex* mosquitoes) were present in both seasons; indicating constant taxonomic richness (Figure 4.3A). The Shannon-Wiener diversity index varied between habitats ($p < 0.001$) and across seasons ($p = 0.004$, Table 4.9, Figure 4.3B). Based on these indices, diversity was higher in the rainy season than dry season. During the dry season, river streams were identified as the most diverse habitats, with a relatively rich and balanced mosquito species distribution. In contrast, hoof prints exhibited the least diversity, with minimal mosquito presence. Ground pools and ditches presented moderate diversity levels. Similarly, the Simpson's diversity index showed a significant variation by habitat ($p < 0.001$) and season ($p < 0.001$); with values of this index being high in river streams and ground pools and peaking in hoof prints (Fig 4.3C) during the rainy season. This points to a substantial decrease in biodiversity within these temporary habitats, likely due to their high use by *An. gambiae,* which suggest dominance by this species in those areas.

**Table 4.9:** Mosquito larvae collected in dry and wet seasons.

| | **Rainy Season** | | | | | **Dry Season** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Habitat Type** | Count | *An. funestus* | *An. arabiensis* | Other *Anopheles* | *Culex* | Count | *An. funestus,* | *An. arabiensis,* | Other *Anopheles* | *Culex* |
| | (N) | n (%) | n (%) | n (%) | n (%) | (N) | n (%) | n (%) | n (%) | n (%) |
| **River streams** | 20,235 | 6,976 (34) | 4,604 (23) | 1,733 (9) | 6,922 (34) | 10,536 | 4,502 (43) | 1,150 (11) | 889 (8) | 3,995 (38) |
| **Ground pools** | 6,229 | 971 (16) | 1,987 (32) | 781 (13) | 2,490 (40) | 1,391 | 256 (18) | 544 (39) | 94 (7) | 497 (36) |
| **Ditch** | 8,749 | 1,719 (20) | 3,154 (36) | 1,357 (16) | 2,519 (29) | 134 | 52 (39) | 5 (4) | 27 (20) | 50 (37) |
| **Dug pits & holes** | 5343 | 336 (6) | 1492 (28) | 305 (6) | 3210 (60) | 382 | 0 (0) | 102 (26) | 94 (25) | 186 (49) |
| **Spring fed wells** | 4099 | 784 (19) | 498 (12) | 175 (4) | 2642 (64) | 519 | 30 (6) | 200 (39) | 27 (5) | 262 (50) |
| **Brick & concrete pits** | 5511 | 49 (1) | 3400 (62) | 29 (1) | 2033 (37) | 156 | 0 (0) | 66 (42) | 1 (1) | 89 (57) |
| **Rice fields** | 4337 | 199 (5) | 1956 (45) | 347 (8) | 1835 (42) | 53 | 0 (0) | 0 (0) | 53 (100) | 0 (0) |
| **Tire trucks** | 1533 | 24 (2) | 841 (55) | 33 (2) | 635 (41) | 12 | 0 (0) | 0 (0) | 12 (100) | 0 (0) |
| **Hoofprints** | 22 | 0 (0) | 10 (45) | 1 (5) | 11 (50) | 0 | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| **Totals** | 56,058 | 11,058 | 17,942 | 4,761 | 22,297 | 13,183 | 4,840 | 2,067 | 1,197 | 5,079 |

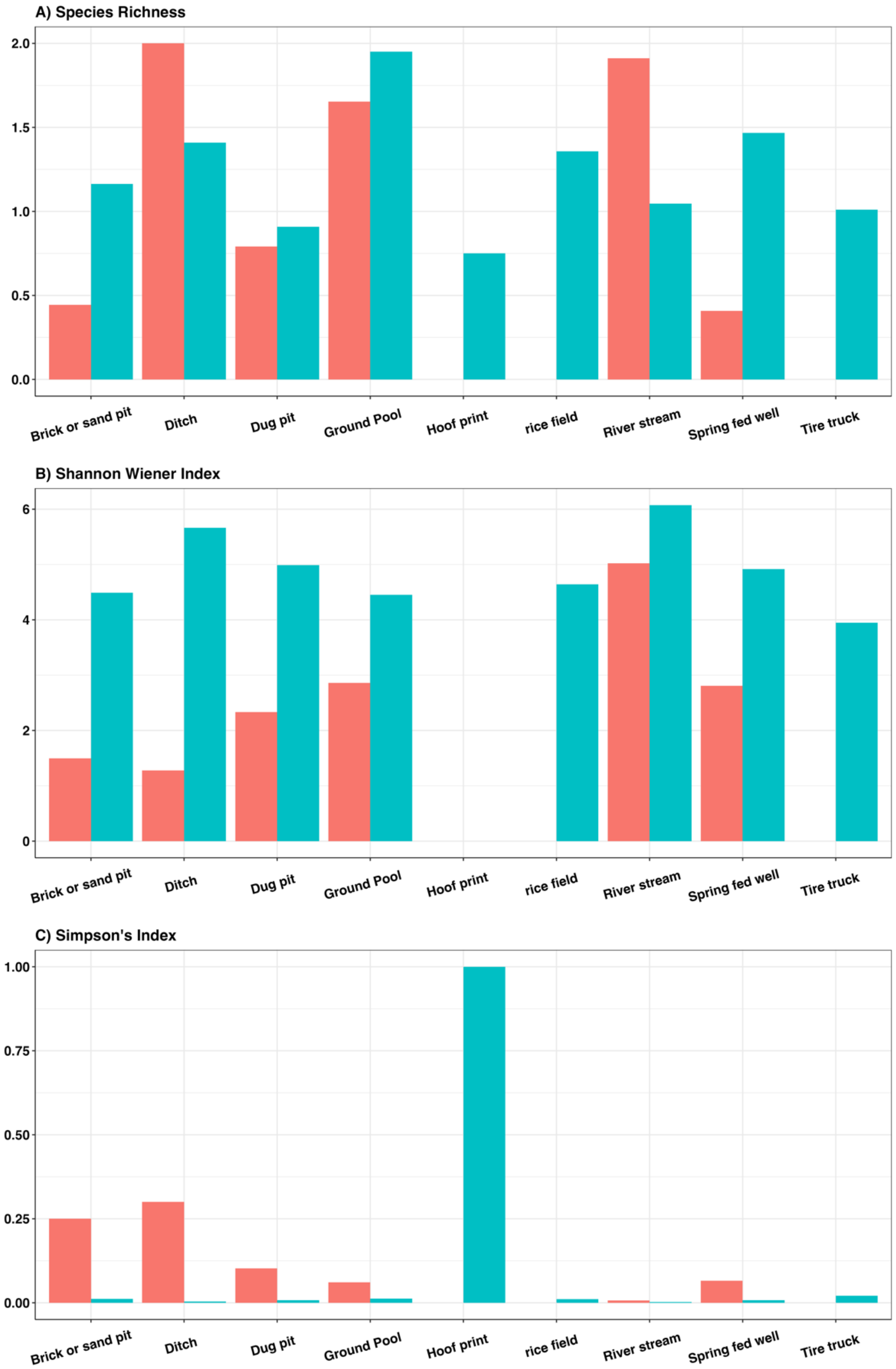

**Figure 4.3:** Showing summary of the diversity indices for mosquito taxonomic groups in each habitat type across the dry and rain seasons. A) Species Richness, B) the Shannon-Weiner Index and C) Simpson's diversity index.

### 4.4.5 Seasonal variability in the spatial distribution of *An. funestus* habitats.

During the wet season, the Moran's I statistic for *An. funestus* positive habitats had a value of 0.2891; z-score = 11.219, p-value < 0.001, indicating significant spatial autocorrelation. This reflects a clustering of habitats where *An. funestus* larvae were present, with the IDW map for the wet season (Fig. 4.4 A) showing a widespread yet concentrated presence of potential larval sites. Notably, a hotspot was identified in the central region of the study area during the wet season, with additional hotspots appearing around it.

In contrast, during the dry season, the distribution of *An. funestus* habitats became more heterogeneous as there was a reduction in the total number of habitats. However, the remaining habitats showed higher abundance within them. The Moran's I statistic for *An. funestus* habitats in the dry season was 0.4108; z-score = 5.951, p-value < 0.001, indicating greater spatial autocorrelation and a more clustered distribution of habitats across the study area (Fig. 4.4 B). This highlighted a large hotspot in the centre of the study area, which persisted from the wet season, alongside secondary foci to the west and north.

Moreover, the IDW maps for presence correspond closely with those for abundance (Fig. 4.4 C and D), indicating that the areas where *An. funestus* is present coincide with areas of higher larval counts.

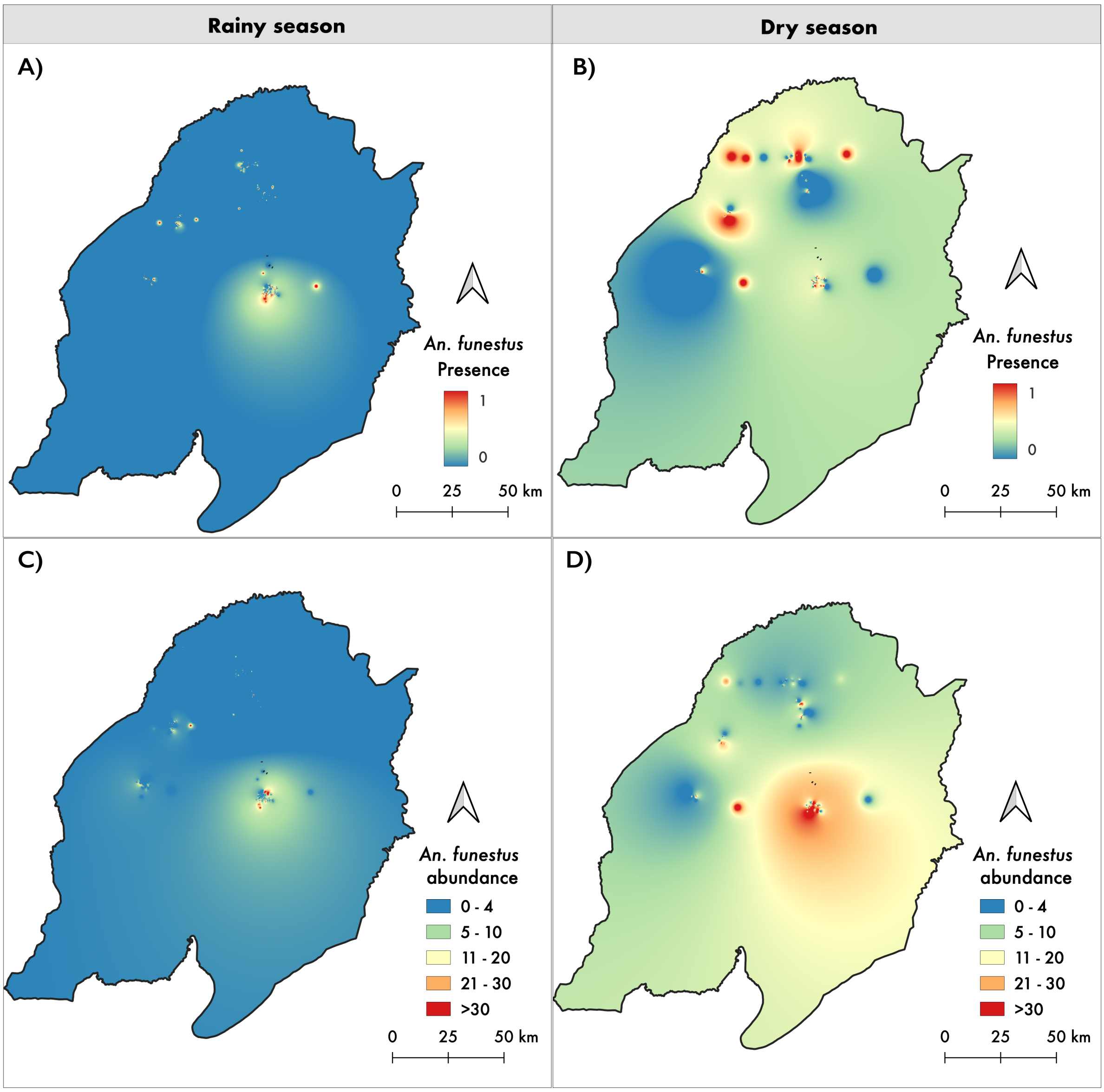

**Figure 4.4:** Inverse Distance Weighting (IDW) Maps of *An. funestus* Distribution across seasons. The top panels show the presence of *An. funestus* habitats during the rainy (A) and dry (B) seasons in the Ulanga and Malinyi district, with hotspots indicating a higher likelihood of habitat occurrence. The bottom panels illustrate the abundance of *An. funestus* larvae within identified habitats for the rainy (C) and dry (D) seasons, with warmer colours denoting higher larval counts.

## 4.5 Discussion

I tested if the larval habitat use by the major African malaria vector *An. funestus* shifts to adapt to seasonal variation in aquatic habitat availability and type. Our findings challenge the paradigm of this species being relatively fixed in its use of larger and more permanent larval habitats; indicating a much higher than previously described adaptability for exploitation of different larval habitat as they become more available during the year. This indicates resilience to environmental variability in this species, which may account for its unique ability to sustain year-round malaria transmission in highly seasonal settings in Africa. This adaptability also poses challenges for the development of effective Larval Source Management strategies for this species, highlighting the need to cover a wider range of habitats for population targeting. Moreover, mosquito immigration and emigration may play a crucial role in affecting the success of LSM. Movement between treated and untreated areas could undermine LSM effectiveness, as mosquito populations may be reintroduced into treated sites. Therefore, the LSM strategies must take these movement patterns into account.

While it is widely acknowledged that there is large seasonal variation in availability of larval habitats for African malaria vectors due to rainfall [282,302], this study is unique in quantifying its magnitude. In this study, remote sensing data indicated a 40% contraction in the area of land covered by water between the wet and dry season, with the total number of aquatic habitats identified falling by 7-fold (~2400 to ~300). These results align with broader trends observed in studies of seasonal changes in aquatic environments [282,302]. Additionally, we quantify changes in not only the number but diversity of aquatic habitat types across seasons. In correspondence with the greater number and diversity of aquatic habitats that were available, An. *funestus* larvae were observed to use a wide range of habitat types in the wet season. In general, the availability of aquatic habitats was considerably reduced in the dry season, resulting in a relatively high *An. funestus* positivity in the few remaining habitats [303]. The predominant use of river streams during this season aligns with their previously described affinity for selecting relatively permanent aquatic habitats [304]. However, the expansion of their habitat uses in the rainy season to include a wider range of aquatic habitats, including those typically considered rare or unsuitable for *An. funestus* [304] (e.g., rice fields, dug pits and even tire tracks) demonstrates ability to adapt to changing environmental conditions [305]. Despite this seasonal expansion of habitat use, most larvae were found in the same ‘top 3’ habitat types (river streams, ground pools, and ditches) in both seasons.

My finding of higher aquatic habitat uses (% positivity) during the dry season aligns with observations across sub-Saharan Africa [286,306–308] where *An. funestus* larvae are known to be more prevalent in drier periods [22]. This contrasts with the other major African malaria vector group *An. gambiae* sl, which is generally more prevalent and abundant during wetter months [244,292]. The decrease in *An. funestus* larvae (both presence and abundance) during the rainy season may be due to habitat flooding which disperses larvae of this species from habitats, and/or the proliferation of aquatic habitats during the wet season outpacing the rate at which *An. funestus* can use them [309].

In addition to the primary results of season and habitat type, several other environmental factors were associated with *An. funestus* larval habitat use and abundance of in this study. In general, environmental factors that had a significant impact on larval positivity were similarly associated with abundance (8 out of 10 variables associated with abundance were also associated with positivity). Both larval outcome variables were significantly associated with deeper, clear, and flowing aquatic habitats, habitats fed by rainwater, shaded, and surrounded by mixed vegetation, and that had green/filamentous algae (compared to no or brown algae). Many of these findings are in line with previous research on *An. funestus* larval ecology. For example, studies in western Kenya and south-eastern Tanzania found that the occurrence and abundance of *An. funestus* larvae were reduced in unclear waters [242,243,265]. However, *An. funestus* larvae have been observed in turbid water in temporary natural habitats in Ethiopia [310]. Studies also reported that *An. funestus* were more likely to be found permanent, medium to large habitats (ranging from 10 to 250 $m^2$ in surface area), and in habitats with emergent vegetation [22,243]; with similar effects for physio-chemical variables as detected here. The finding of a positive association between *An. funestus* larvae, filamentous and green algae, and mixed vegetation contrast with an earlier study by Kahamba et al. (2024) (chapter 3) conducted in dry season, where algae was negatively associated with the occurrence of *An. funestus* larvae [22]; suggesting seasonal variation in the importance and nature of environmental predictors of larval habitat use.

Additionally, our finding that *An. funestus* larvae abundance was slightly higher at habitats farthest away from houses (>500m) seems somewhat counterintuitive; and differs from previous findings in western Kenya and southeastern Tanzania where *An. funestus* larvae were more abundant at habitats close to houses (Nambunga et al., 2020; Debrah et al., 2021). However, another study in this area during the dry season also reported higher *An. funestus* larval abundance at habitats farther away from houses (Kahamba et al 2024, Chapter 3). The reason for the increase in *An. funestus* larvae as distance from houses is unclear but could be linked to variation in cryptic species composition within the *An. funestus* group, with larvae using habitats further away from houses being more zoophilic species such as *An. rivulorum*, which might prefer breeding sites that are not in immediate vicinity to human habitats [311]. These species could potentially exhibit different behaviors, including host preferences. Another possible explanation could be that the microclimatic conditions of habitats away from homes provide a more suitable environment for the larvae [25]. These locations might offer advantages like more consistent shading and the presence of undisturbed water, which are beneficial for larval development, while still being close enough for adult mosquitoes to seek out human hosts for blood meals [27,305]. Regardless of the mechanism, this finding indicates that more distant habitats which are farther away from houses should not be overlooked in larval source management strategies [303]. This finding highlights the need to extend our larval surveys beyond the immediate vicinity of human habitation to fully understand the diverse range of habitats utilized by this mosquito species.

In this study, we also observed cohabitation among *An. funestus, An. arabiensis*, other *Anopheline*, and *Culicine* larvae in various aquatic habitats. However, while *An. funestus* was found in a range of habitats alongside other mosquito taxa, some other mosquito groups were found only in a more limited range [244,312]. For example, hoofprints during the rainy season were primarily used by *An. arabiensis* and *Culex* mosquitoes. *Anopheles arabiensis* has been associated with these habitats in several previous studies [244,285,292,313]. It is also possible that habitats used by some mosquito genera in one season are used by others in another. For example, a ground pool that accommodates *An. funestus* during or after the rainy season might transform into a dry area with only wet animal footprints that attract *An. arabiensis* later in the year [189,281]. The dynamic nature of these habitats due to season highlights the importance of year-round monitoring to fully understand shifting characteristics and the implications for vector population dynamics [285].

Extending beyond our focus on *An. funestus*, we also qualitatively assessed how mosquito community structure varied between habitat types and seasons. The diversity of mosquito groups was generally highest in streams and ground pools, and in the wet than dry season. The number and evenness of mosquito groups was greater during the rainy season. Our findings differ from those of [308] in Kenya where mosquito species richness and evenness were greater in the dry than rainy season. This discrepancy highlights the variability in mosquito community dynamics across different geographical regions and environmental conditions, emphasizing the need of localized studies [314].

As well as variation in the availability and use of *An. funestus* larval habitats between seasons, we also detected differences in their predicted spatial distribution. There was spatial autocorrelation and clustering in the distribution of *An. funestus* habitats in both seasons, however this was more pronounced during the dry season. This can be explained by the contraction of habitats during the dry season, resulting in fewer and more spatially distinct clusters. This indicates LSM could be more effectively targeted and less expensive to implement during the dry season (with fewer habitats to target), assuming these persisting seasonal habitat hotspots are easy to find. A similar pattern of positive spatial autocorrelation in adult *An. funestus* has been documented in studies in Kenya [315] and Uganda [316]. Across both seasons, substantial hotspots of *An. funestus* larval habitats were detected the centre of the study, indicating a relatively fixed location of 'high' risk areas.

It is important to acknowledge some limitation of our study. First, habitats and larvae were monitored in only two cross-sectional seasonal surveys. While this provides a useful snapshot of seasonal differences, it may conceal more subtle and complex changes in habitat utilization and larval distribution that occur throughout the year. The possibility of these finer scale in *An. funestus larval* dynamics and habitat use across the year are investigated in a longitudinal study presented in Chapter 6. Furthermore, the potential impacts of interannual variability in weather patterns and mosquito behaviour could not be captured in a single cross section study design. Future work should consider a longitudinal approach, encompassing a broader range of seasonal transitions to deepen our understanding of these ecological dynamics. Another limitation of this study is that important environmental variables, such as land cover, which were identified as significant in the previous chapter, were not included in this analysis. Incorporating these factors could enhance the habitat predictions in both dry and rainy seasons. Additionally, we used Inverse Distance Weighting (IDW) for spatial interpolation, which has certain limitations. IDW does not produce uncertainty estimates, unlike Kriging or other geostatistical models, nor does it account for explanatory variables. Future studies could incorporate more robust models, such as Kriging or Generalized Linear Models (GLMs), to better capture uncertainty and the relationships between environmental variables and larval habitat dynamics. Such comprehensive assessments will be also important to redefine the larval source management strategies in order to improve their effectiveness when environmental conditions fluctuate.

## 4.6 Conclusions

This study underscores the importance of seasonal patterns of *An. funestus* habitats, and their implications for LSM strategies in malaria endemic regions, particularly in east and southern Africa where this vector species plays a major role in malaria transmission. Through extensive cross-sectional surveys in south-eastern Tanzania, coupled with satellite imagery and statistical modelling, we have shown that *An. funestus* not only uses permanent and semi-permanent aquatic habitats, but also demonstrates a high degree of adaptability by occupying a wider range of habitats during the wet season. This adaptability ensures their survival through the dry season when fewer habitats, typically the more permanent types such as river streams and ground pools (ponds) are available but are more intensively used for breeding. The findings reveal that LSM strategies need to be versatile, focusing on permanent habitats during dry periods while expanding to include diverse habitats in the wet season. Moreover, this study, by delving into the seasonal dynamics of *An. funestus* habitats, emphasizes the need for vector surveillance to include elements of aquatic ecology, so as to tailor intervention strategies effectively.

# Chapter 5: The influence of larval habitat type on the fitness, energetic reserves and insecticide resistance of an African malaria vector in the wild

*In preparation for submission to the Parasites and Vectors Journal*

## Abstract

**Introduction:** Malaria transmission dynamics are influenced by the fitness, ecology, and behaviour of mosquito vectors. In laboratory settings, many studies have shown that adult mosquito fitness is heavily determined by the conditions experienced during larval development. However, the importance of variation in larval habitats to the fitness and transmission potential of wild mosquito populations is much less understood. This study investigates whether the adult fitness and insecticide resistance of the African malaria vector *Anopheles funestus* are associated with the type of larval habitat from which they emerge. Here I compared the fitness and resistance traits of adult *An. funestus* emerging from different larval habitat types in southern Tanzania.

**Methods:** Fieldwork was conducted in two villages in southeastern Tanzania, targeting key larval habitats, including river streams, ground pools, and rice fields during the rainy and dry seasons of 2022 and 2023. These habitats were characterized based on their physical features. *Anopheles funestus* larvae and pupae were collected daily and reared to adulthood under standard insectary conditions. I measured the energy reserves (lipid, glycogen, and sugar contents), wing length, survival rates, and insecticide resistance profiles of the emerging adults. Generalized Linear Mixed Models (GLMM) and Cox proportional hazards models were used to evaluate the impact of habitat type on the productivity, energy reserves, survival, and resistance profiles of *An. funestus*.

**Results:** Significant variation in larval productivity, adult body size, energetic reserves, survival, and insecticide resistance was detected between different

natural larval habitat types. Ground pools were the most productive habitat, with a predicted mean larval density that was significantly higher than river streams and ditches. Productivity was approximately 5 times higher in ground pools compared to both river streams and ditches, which were not significantly different from each other. Mosquitoes from rice fields displayed elevated levels of energy reserves, with 2.25 times more lipids and 41% more sugar content than those from river streams. However, this did not translate into increased survival rates, as mosquitoes from rice fields had lower survival rates. Mosquitoes emerging from ground pools had significantly higher odds of survival, with a 14% lower risk of dying compared to those from river streams. Insecticide resistance varied by habitat, with mosquitoes from ground pools showing higher resistance to permethrin and deltamethrin compared to those from river streams.

**Conclusion:** This study supports the hypothesis that mosquitoes emerging from different larval habitats vary significantly in fitness and resistance phenotypes. Ground pools, identified as the most productive habitats, also produce mosquitoes with higher survival rates and resistance traits. Future studies should investigate how such habitat-dependent fitness characteristics may influence the overall malaria transmission, and the effectiveness of targeted LSM strategies for malaria control in specific areas.

## 5.1 Introduction

The transmission of vector-borne diseases (VBDs) such as malaria is linked with the fitness, ecology, and behaviour of mosquito vectors [27,239,305,317]. Most previous research has concentrated on the association between the environmental drivers of malaria transmission and the abundance of adult or larval mosquitoes [315,318,319]. While these studies provide valuable insights into vector population dynamics and transmission trends, they may overlook the full spectrum of transmission potential, which is influenced not just by the number of mosquitoes but also by their individual fitness traits [320].

Demographic, fitness, physiological and resistance traits of mosquitoes, including survival rates, body size, fecundity, feeding behaviour, and susceptibility to insecticides, play a critical role in the overall transmission capacity of their populations [321-323]. Despite their importance, the environmental determinants of these individual fitness traits and their variability across different habitats and seasons have received limited attention. This oversight may lead to a simplified understanding of mosquito populations by assuming individuals in the same population have a uniform 'quality’. There is a growing, albeit limited interest in understanding individual variation in mosquito fitness and resistance traits, and associated implications for their population dynamics and transmission capacity [324-326]. At least one study has shown that variation in mean *An. gambiae* body size is linked to population growth [326]. This is a relatively rare example of analysis of mosquito fitness traits and their link to demography in the wild, as most investigation of environmental determinants of mosquito fitness have been conducted under laboratory conditions, example impact of vegetation on larval survival [327].

Elucidating the role of natural habitat and environmental factors on mosquito fitness, resistance and transmission traits could benefit malaria control strategies [328,329]. This could be particularly useful in the case of larval source management (LSM strategies where aquatic mosquito habitats are targeted for removal, alteration or treatment with biocides) [72,303]. Typically, this approach is based on targeting aquatic habitats that are most frequently used by mosquito vectors, and that produce the largest number of individuals [61,102]. However, the aquatic habitats that produce the largest number of mosquitoes may produce individuals with suboptimal adult fitness. This could arise due to density dependence, with larvae developing in high density habitats having smaller body size and reduced survival and reproduction at the adult stage due to resource competition [293,328-331]. Density dependence has been demonstrated in numerous laboratory and field studies of African malaria vectors; with larvae emerging from high density habitats having reduced fitness [326,330-332].

Additionally, other environmental characteristics of aquatic habitats such as nutrient levels, or contamination with sub-lethal levels of insecticides or pollutants could influence insecticide resistance in adults emerging from them [293,333,334], it is clear that environmental conditions experienced during larval development shape mosquito fitness and resistance traits [234,335,336]; however, the magnitude and importance of these impacts are poorly understood in natural populations.

Here we conducted an extensive field-based investigation of the major African malaria vector *An. funestus* across multiple populations in southern Tanzania; with the aim of examining how the fitness and insecticide resistance of individuals varies seasonally, in response to larval habitat type and other environmental conditions in aquatic habitats. We focus on *An. funestus* because it is a key malaria vector species that dominates malaria transmission in many parts of southern and east Africa. [90] Additionally, this species is a particularly strong candidate for LSM because of its use of relatively large and stable aquatic habitats [61,102]; fulfilling the WHO's criteria for this control strategy of using habitats that are "few, fixed and findable". In planning LSM for *An. funestus*, there would be great value in understanding which aquatic habitats should be prioritized for targeting [72]. While the assumption is that habitats generating large numbers of adult mosquitoes are most important, this may not be the case if those emerging from other types of habitats have greater fitness, and potential to transmit malaria as reflected by higher adult survival and insecticide resistance. Identifying aquatic habitats and seasons that produce these "most epidemiologically relevant" could significantly enhance the impact of LSM [72].

I, therefore, assessed fitness traits and insecticide resistance in *An. funestus* emerging from different aquatic habitats in Southeastern Tanzania. The aims were to i) characterize the types of aquatic habitats used by *An. funestus* and their productivities, ii) quantify the teneral reserves of mosquitoes and their body size emerging from different habitat types, and iii) assess the adult survival of *An. funestus* emerging from different habitats, iv) test whether the insecticide resistance profile of *An. funestus* is associated with larval habitat type.

## 5.2 Material and methods

### 5.2.1 Study area

This study was conducted in two rural villages in south-eastern Tanzania: Itete in Malinyi District (-8.6630° S, 36.4170° E) and Ikungua in the Ulanga District (-8.490, 36.665; Figure 5.1). A detailed description of the study area is provided in Chapter 3.2.1. Resistance experiments took place during the rainy season (March and April

2022) and the dry season (September and October 2022). Additional experiments were conducted between May and December 2023, after the rainy season. These villages were selected due to the high abundance of *An. funestus*, as documented in Chapter 3.

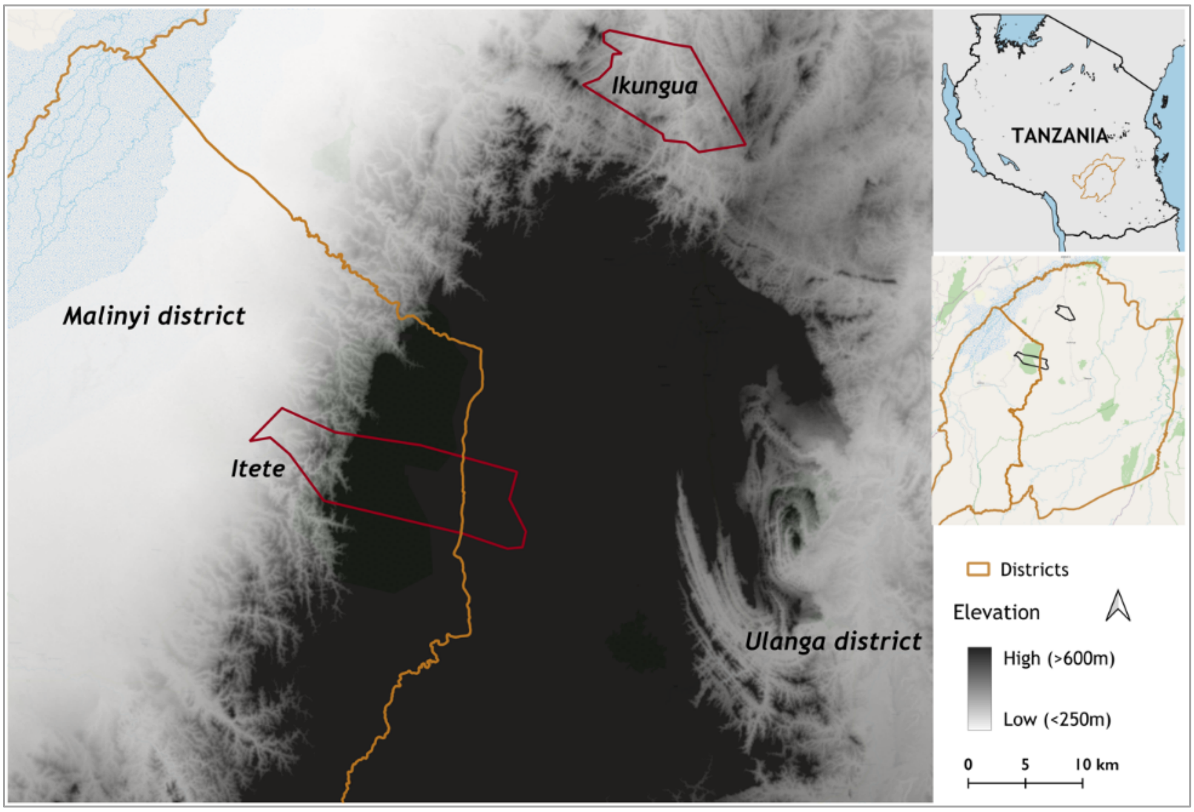

**Figure 5.1:** Study area, red polygons are the selected villages Ikungua and Itete in Ulanga and Malinyi respectively

## 5.2.2 Characterization and sampling of aquatic habitats

Our previous research had already shown that the most common aquatic habitat types by *An. funestus* in south-eastern Tanzania typically include river streams, ground pools, ditches and spring-fed wells, and with rice fields also playing a role in limited period of the year (Chapter 4) [22,243]. Given the prominence of these habitat types, this study focused on investigation of how *An. funestus* fitness and resistance traits varied between adults emerging from them.

Habitat were characterized as previously described in chapter 3 and 4, after which the fitness characteristics and insecticide-resistance profile of a representative sample of adult *An. funestus* emerging from these habitats were also assessed. Most of the traits were measured in these populations in only the dry season when the number of habitats was reduced, but the remaining ones were more concentrated and had higher mosquito abundance. Insecticide resistance was assessed in both the wet and dry season (Figure 2), on account evidence that resistance phenotypes can vary seasonally [337–341].

During the initial survey in (May – Oct /2022), all aquatic habitats in each study village were fully characterized based on their type, size, depth, clarity, presence of algae, permanency, vegetation around the habitats, and presence of shades. In habitats where *An. funestus* larvae were present, all mosquito larvae were then sampled, sorted, and counted according to a standardized protocol that adjusted the number of dips based on the habitat size, as detailed in Chapter 3.4.2. A total of 387 river stream segments, 94 ground pools, 100 habitats within rice fields, 164 spring-fed pools, and 240 ditches were initially characterized. Of these, only a few representative habitats as shown in Figure 2 were selected for subsequent analysis of adult fitness and resistance – based on having a sufficient number of larvae and pupae to provide appropriate sample sizes of emerged adults as detailed in table 5.1.

For the initial habitat characterization, a standardized number of dips per habitat was performed to allow for comparisons of productivity between habitats. The productivity here referred to mean number of *An. funestus* larvae per total number of dips. This protocol ensured that the number of dips was proportional to habitat size, as described in Chapter 3.3.3. However, the sampling strategy was adjusted when collecting larvae and pupae for fitness assays. In this case, dipping continued until no more larvae or pupae were obtained from the habitat. The larval collection process was repeated for 5-7 consecutive days to achieve the target sample sizes for subsequent tests of adult fitness and resistance.

Only *An. funestus* late instars larvae (3 & 4) and pupae were collected from these habitats, and they were immediately transported to the insectary for rearing. The total number of adult *An. funestus* used in each experiment varied due to the uneven emergence of adults from some habitats. Detailed information on the mosquitoes utilized in each experiment is provided in Figure 5.2.

### 5.2.3 Rearing of the mosquito larvae into adults

Two insectaries were used for rearing field-collected larvae and pupae to adult emergence: a field insectary set up in Ikungua village and another at the Ifakara Health Institute (IH) Vector Sphere insectary. The field insectary was created for convenience, as these villages were very far from the IHI insectary. Given the demand for these experiments, it was necessary to set up a field insectary and camp there until all experiments were finished (Figure 5.2a). This field insectary was established in a locally rented house. This setup included $30cm^3$ mosquito cages, a mini freezer which was used for killing mosquitoes and storing them before sent to the laboratory, tables for larval basins which were covered with netting, and a microscope. The temperature in the field insectary were ambient, reflecting the natural fluctuations in the study area (18-41°C, 53 and 92% relative humidity, Figure 5.2a). In contrast, the IHI Vector Sphere insectary provided a controlled environment. This facility maintained a consistent temperature range of 28 ± 2°C and a relative humidity of 82 ± 10% to ensure optimal rearing conditions. In each insectary type, late instars of *An. funestus* larvae and pupae were placed in medium basins (30 cm diameter, 10 cm depth) labelled with unique identification numbers corresponding to each habitat. Larvae were reared in the same water they were collected from their habitats and no additional food was provided in rearing basins. Each morning, pupae were collected, placed in 100 ml plastic cups, and then transferred to small cages (10 $cm^3$), each labelled with the date, habitat ID, and type.

Adult *An. funestus* emerging from field-collected juvenile stages were sequentially allocated to a series of experiments focused on different life history and resistance traits. Mosquitoes collected in the first months of the study period (March-April 2023, wet season) were allocated to resistance bioassays since these experiments required higher numbers compared to other experiments. After sufficient sample sizes were achieved for insecticide resistance bioassays, mosquitoes from further collections (May-late June 2023, Figure 5.3) were allocated for measurement of energetic reserves

Before analysing the energetic reserves in these adult mosquito specimens, one wing and one leg was removed and retained for estimating their body size (using wing size as a proxy) [342,343], and leg specimens were used to extract DNA to identify the sibling species of *An. funestus* s.l [344]. Wing length was measured using the micrometre ruler under a microscope (50mm micrometre scale in 0.1mm divisions, 70mm x 20mm x 3mm) The measurements were taken from apical notch to the to the auxiliary margin (Figure 5.2 b c).

Additionally, larvae and pupae emerging from selected habitats were used to measure habitat-specific adult survival (from collections between June- end of July 2023). The study aimed to achieve of target sample sizes for each trait of interest, within each habitat type combination. Target sample sizes were initially based on pragmatic consideration of measuring at least 200 mosquitoes per trait of interest per habitat type (Table 5.1). However, the total number acquired sometimes fell short of this target due to variability in in larval and pupal abundance. Procedures used to measure traits of interest are described below.

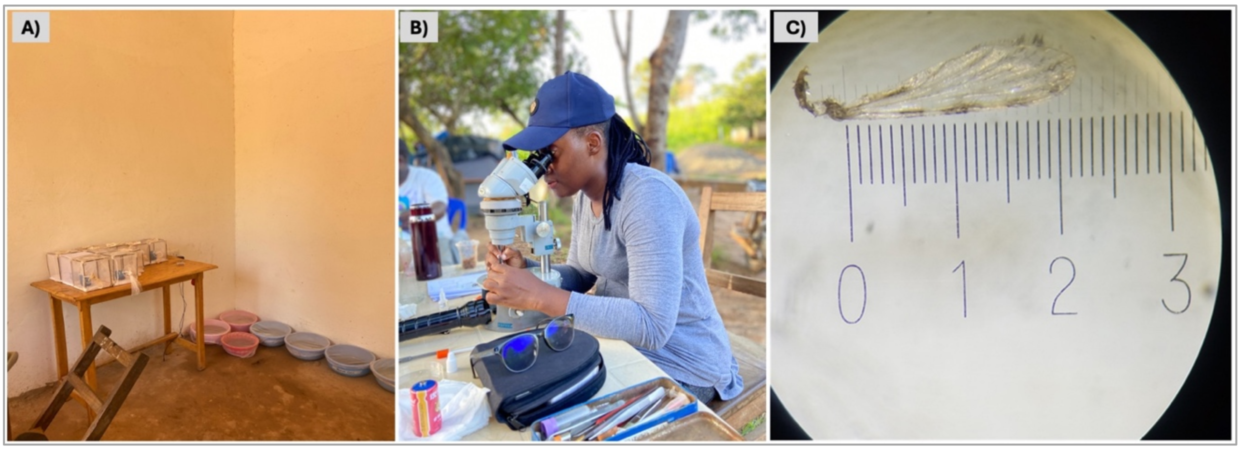

**Figure 5.2:** Field environment a) Field insectary, b) wing size measurement for *An. funestus* mosquitoes, c) wing measured from the notch to the end of the wing

### 5.2.4 Quantification of energy reserves

Adult mosquitoes emerging in cages in the field insectary were closely monitored and killed by freezing within 1–2 hours of emergence. This protocol was designed to ensure that the energy content from the larval stage was not utilized at adult stage. No additional food was provided to the emerging adults, ensuring their energetic reserves reflected only the resources accrued during larval development. The dead specimens were packed and transported to the IHI laboratory for quantification of their energy reserves.

Before the experiments, standard calibration was performed to ensure accuracy and precision. Serial dilutions were prepared to create standard curves. For glycogen and sugar, concentrations ranged from 0 to 100 µg/µL in 10 µg/µL increments. For lipids, concentrations ranged from 0 to 50 µg/µL in 5 µg/µL increments. Each concentration was tested in triplicate for reliability. The calibration curves were plotted using the *ggplot2* package in R, and linear models were fitted using the *lm* function. An R-squared ($R^2$) value of 0.85 or higher was considered indicative of successful calibration.

Energetic reserves were measured from individual adult (both males and females) according to established procedures [345]. Glycogen and sugar levels (µg per mosquito) were quantified using the Anthrone procedure, which involved heating the sample at 90-110°C for 17 minutes and measuring the Optical Density (OD) at 610 nm. Total lipids (µg/µL) were determined in a chloroform-methanol solvent solution via the vanillin-phosphoric acid reaction [346]. For each habitat category, 10 replicate groups of *An. funestus s.l.* were analyzed, with each group consisting of mosquitoes that emerged on different days. Twenty mosquitoes (both male and female) were analyzed from each replicate. In total, energetic reserves were measured from 800 mosquitoes (100 males and 100 females from 4 habitat types, as shown in Table 5.1).

### 5.2.5 Assessing survival of emergent adults

To quantify adult survival, individual mosquitoes were held in cages labeled with habitat ID and date of emergence. Each morning, the cages were checked, and the number of deaths were recorded until all the mosquitoes died. The dead mosquitoes were removed from the cage and the number of days they lived was recorded. One wing was removed from all mosquitoes on death and measured as a proxy of body size as described above.  Similarly, one leg was removed from all dead mosquitoes sent to the molecular laboratory for further identification to sibling species by PCR as described above. All survival experiments were conducted in the field insectary, sample size used are detailed in Figure 5.3 and Table 5.1.

### 5.2.6 Tests for insecticide resistance in emergent adults

Field-collected larvae and pupae were transported to Ifakara and maintained till emergence at the IHI's Vectorsphere laboratory. Adults emerging in the insectary were tested for resistance in according to WHO guidelines [347]. The insecticides tested included: organochloride (4% DDT), carbamate (0.1% bendiocarb), organophosphate (0.25% pirimiphos-methyl), and two types of pyrethroids - type I (0.75%, 3.75%, and 7.5% permethrin) and type II (0.05%, 0.25%, and 0.5% deltamethrin). For each bioassay, a total of 120 - 150 female *An. funestus* s.l mosquitoes aged 3 - 5 days were exposed to insecticide impregnated papers or papers impregnated only with oil (control group). For each insecticide class and concentration, six replicates were performed including four using the target insecticide and two controls. Each replicate tested 20 - 25 mosquitoes. The exposure time for mosquitoes was fixed at 1 hour, with the time to knockdown recorded every 10 minutes up to an hour. Post exposure, the mosquitoes were moved into insecticide-free tubes and provided with a 10% glucose solution. Mortality rates (proportion of dead mosquitoes) were then recorded after a 24-hour period. Whenever resistance to standard diagnostic doses of pyrethroids was observed, follow-up studies using 5× and 10× insecticide doses were done to assess the intensity of pyrethroid resistance.

**Table 5.1:** Target habitat replicates and adult mosquito sample sizes for different experiments in this study.

| Experiment type | Season targeted | Habitats types and habitat replicates targeted | | | | | |
|---|---|---|---|---|---|---|---|
| | | River streams | Ground pools | Spring fed wells | Rice fields | Target adult sample size* | Achieved adult sample size* |
| **Energetic reserves** | Dry | 200 (150$^{a}$, 50$^{b)}$ | 200 (130$^{a}$, 70$^{b)}$ | 200 (50$^{a}$, 150$^{b)}$ | 200 (120$^{a}$, 80$^{b)}$ | 1,000 | 800 |
| **Survival** | Dry | 70 (50$^{a}$, 20$^{b)}$ | 82 (60$^{a}$, 22$^{b)}$ | NA | 58 (40$^{a}$, 18$^{b)}$ | 500 | 210 |
| **Insecticide Resistance** | Wet & Dry | 2700$^{b}$ | 2700$^{b}$ | NA | NA | 13,500 | 5,400 |

In this table, the habitat types indicate the sources of individuals used in each experiment, with "NA" denoting habitat types that were not sampled. The numbers in brackets represent the number of habitat replicates (different habitats within a habitat type) that were sampled in each experiment, with "a" indicating the number in Ikungua and "b" the number in Itete. * Indicates the number of adult mosquitoes per individual habitat that were targeted or actually measured in the experiments.

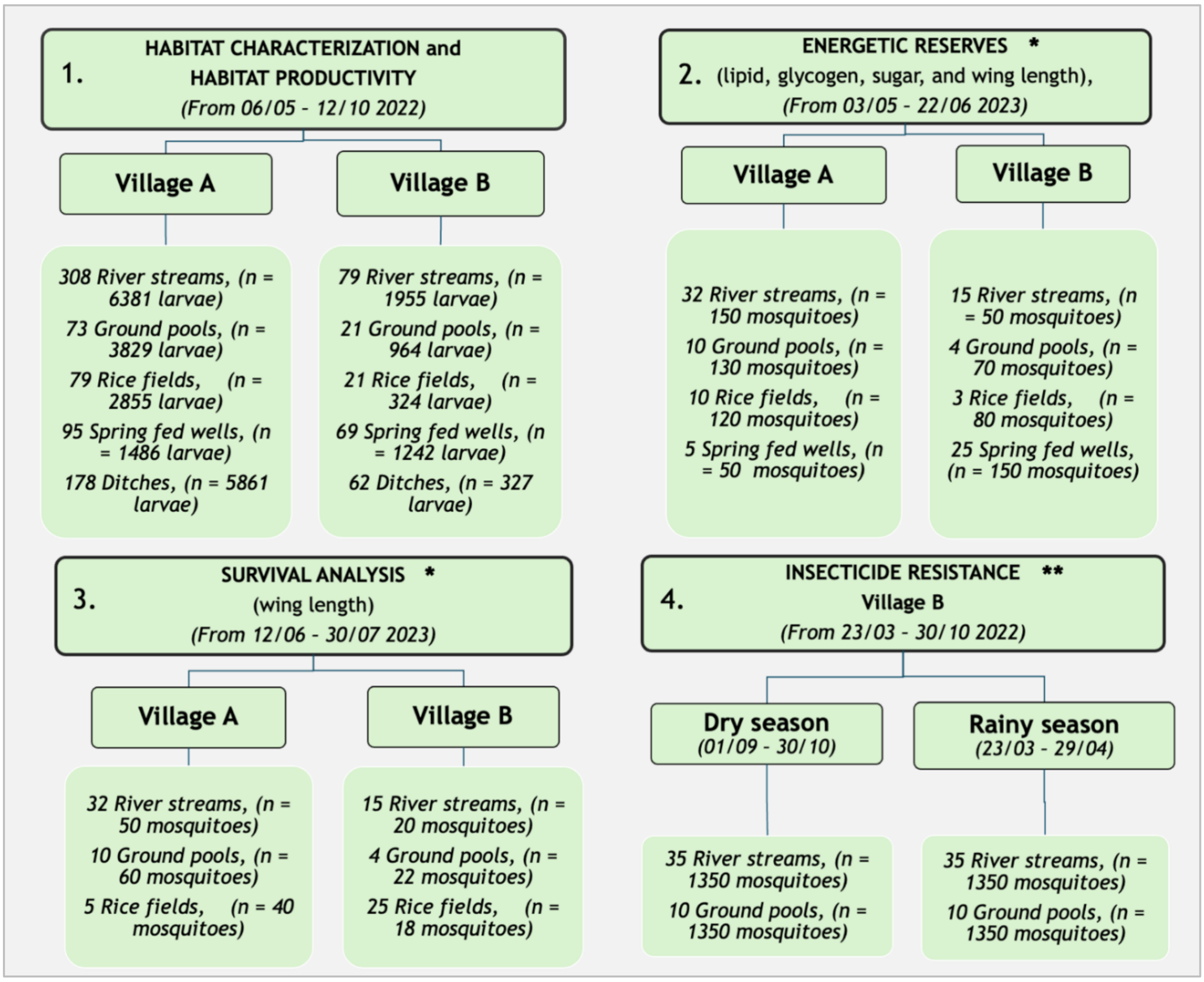

**Figure 5.3:** Summary of the different types of experiments and their timing in this study. For each experiment type (1-4), boxes indicate the villages where An. funestus (Village A = Itete, Village B = Ikungua), the number of different habitats sampled within each habitat type, and total number of specimens measured. For the first experiments those are the larvae collected. For experiment 2-3 those emerged *An. funestus* adults. The subscripts indicate which mosquitoes were identified to species level -* All mosquitoes were taken for molecular identification, ** only 10% were taken to the lab for molecular identification

## 5.3 Statistical analysis

All analysis was performed using R statistical software, version 4.0.3 [258]. Initially, a comprehensive descriptive analysis was conducted to summarize mosquito species and *An. funestus* s.l sibling species collected from various aquatic habitats. This preliminary step provided a broad overview of the data set, allowing a deeper understanding of the distribution and prevalence of different species across habitats. Following this, a focused examination was done to determine the proportions and predicted mean values for *An. funestus sl* in each habitat type. This initial analysis led to further examination using the Generalized Linear Mixed Models (GLMM) with a negative binomial distribution template model builder (*glmmTMB*) [348,349]. For each habitat type dataset, the selection of parameters to be included in the final model was achieved through stepwise backward selection, using the "*stepAIC*" function. Then, the "*ggeffects*" package was used to calculate the predicted mean of *An. funestus* across various habitat characteristics, with the total number of *An. funestus* per dip treated as the response variable and the habitat type and environmental characteristics as explanatory variables. The parameters incorporated into these models are detailed in Table 5.2. The models were adjusted to account for variations in sampling effort by including the volume of water sampled as an offset term, and date and village were included as a random effect to accommodate temporal and spatial variability.

To address the second aim of assessing the impact of habitat type on *An. funestus* energetic reserves and body size, we used Generalized Linear Mixed Models (GLMM). Here four models were built to examine impacts on the separate outcome variables of glycogen, lipid, sugar concentration and wing size. Each model incorporated habitat type, sex and their interaction as explanatory variables, and habitat id as a random effect (Table 2). Model selection was conducted by sequential backward elimination, using Likelihood Ratio Tests (LRTs) to assess the statistical significance of individual terms. Additionally, a post hoc analysis was conducted to test for differences in energetic reserves and wing sizes between individual habitats.

To address the third aim, mixed-effects Cox proportional hazards models were employed to investigate the impact of habitat type, sex, and wing length on adult mosquito survival. The comprehensive model included habitat type, sex, and wing length as fixed effects and habitat ID as a random effect. This model was implemented using the *coxme* package in R [350]. The statistical significance of the model was determined through likelihood ratio tests, comparing the full model with reduced models. These tests indicated that the inclusion of each predictor significantly improved model fit.

To complement the statistical analysis, survival probabilities across different habitat types and across sex were further explored through Kaplan-Meier survival curves. Using the Kaplan-Meier estimator, a survival object was constructed using the *survfit* function from the survival package. Visualization was subsequently achieved with the *ggsurvplot* function from the *survminer* R package [351], generating survival plots that highlighted median survival times, confidence intervals, and p-values.

Lastly, to address the fourth objective, a Generalized Linear Mixed Model (GLMM) was constructed to assess the impact of larval habitat type on insecticide resistance, and test whether it varies between seasons [352]. In this analysis, only two common aquatic habitat types were considered (river streams and ground pools, Figure 5.3 and Table 5.1) because insufficient numbers were achievable from other habitat types in both seasons. The primary outcome was the binary mortality 24 hours after exposure to insecticides, where ‘0’ indicated alive and ‘1’ indicated dead. The model included fixed effects for larval habitat type, insecticide treatment, season, and the interactions between habitat type and season, and insecticide treatment and season. A random effect for replicate was included to account for variability between replicates. The mean mortality rates and 95% CI were obtained from the best fit model were extracted and plotted using the *ggplot2* package. Data on insecticide susceptibility was interpreted based on the WHO-specified thresholds for resistance determination [347]; with susceptibility inferred when mortality was ≥98%, possible resistance indicated by mortality between 90-97% in which case the tests were repeated for confirmation, and resistance considered confirmed if mortality was <90% [353].

**Table 5.2:** Summary of Statistical Models used to include the primary response variable, explanatory variables, random effect variables, and statistical distribution used. “*” indicates an “interaction”.

| Aim | Model | Response variables | Fixed effect variables | Random effect variables | Statistical distribution |
|---|---|---|---|---|---|
| 1. Mean abundance of habitat-specific characteristics<br>i. river streams<br>ii. ground pools,<br>iii. rice fields,<br>iv. spring fed wells,<br>v. ditches | 1 - 5 | *An. funestus* total | Habitat size + Water permanence + Water depth + Watercolour + Water source + Water movement + Algae type + Presence of shades + Vegetation type + offset (log (volume sampled)) | Date + Village | Negative binomial distribution with zero inflation |
| 2. Energetic reserves and mean wing length | 6 | Mean Lipid (uL) contents | Habitat type + Sex + Habitat type: Sex | Date + Habitat ID + Village | Gaussian (Normal) distribution |
| | 7 | Mean Glycogen (uL) content | Habitat type + sex + Habitat type: Sex | Date + Habitat ID + Village | Gamma distribution |
| | 8 | Mean Sugar (uL) content | Habitat type + sex + Habitat type: Sex | Date + Habitat ID + Village | Gamma distribution |
| | 9 | Mean wing length | Habitat type + sex + Habitat type: Sex | Date + Habitat ID + Village | Gamma distribution |
| 3. Survival analysis | 10 | Death and failure | Habitat type + sex + wing length | Date + Habitat ID + Village | Semi-parametric model* |
| 4. Resistance profile | 11 | Mortality (dead, alive) | Habitat type + Insecticide + Season | Replicate + Date | Binomial distribution |

** This model focusing on the relation between the survival times and the covariates through the hazard function, without making assumptions about the overall shape of the survival distribution.*

## 5.4 Results

### 5.4.1 General results

A total of 31,412 mosquito larvae and pupae were collected from various habitats, including river streams, ground pools, rice fields, ditch, and spring-fed wells (Table 5.3), of which 4643 were *An. funestus sl*. Other common mosquito species and genera found in habitats were *Anopheles gambiae* s.l (hereafter referred as *An. gambiae*), and *Culex* species and other *Anopheles* species (Table 5.3).

Of all emerged *An. funestus* s.l., 1192 were analysed by PCR (Figure 5.2). *Anopheles funestus s.s.* (hereafter referred to as *An. funestus*) was the most prevalent within this species group across all habitat types, representing 52-85% of the samples. Overall, *An. funestus* was the most dominant species in the study, accounting for 69% of the total specimens analysed (Table 5.4).

Other species generally accounted for less than 5% within *An. funestus* s.l. However, *An. rivulorum* was notably the second most common species, particularly in ground pools where it occurred at a moderate frequency of 27%. *An. parensis* also occurred at higher frequencies in specific habitats, such as spring-fed wells (11%). Additionally, a relatively high proportion of *An. funestus* s.l. from all habitat types could not be identified to species level due to failure to amplify, ranging from 11-28%.

**Table 5.3:** Summary of all mosquito larvae and pupae collected from different aquatic habitats in this study.

| **Habitat, n** | **Mosquito Species** | **Number of larvae/pupae, (n)** | **%** |
|---|---|---|---|
| **1. River Streams (n= 387)** | *An. funestus* | 1531 | 18% |
| | *An. gambiae* | 3080 | 37% |
| | Other *Anopheles* | 1170 | 14% |
| | *Culex* species | 2555 | 31% |
| | Sub -total | 8336 | 100% |
| **2. Ground Pools (n= 94)** | *An. funestus* | 1284 | 27% |
| | *An. gambiae* | 1393 | 29% |
| | Other *Anopheles* | 546 | 11% |
| | *Culex* species | 1570 | 33% |
| | Sub -total | 4793 | 100% |
| **3. Rice Fields (n= 100)** | *An. funestus* | 840 | 9% |
| | *An. gambiae* | 4190 | 45% |
| | Other *Anopheles* | 1298 | 14% |
| | *Culex* species | 3039 | 32% |
| | Sub -total | 9367 | 100% |
| **4. Spring-fed wells (n= 164)** | *An. funestus* | 303 | 11% |
| | *An. gambiae* | 1174 | 43% |
| | Other *Anopheles* | 247 | 9% |
| | *Culex* species | 1004 | 37% |
| | Sub -total | 2728 | 100% |
| **5. Ditch (n= 240)** | *An. funestus* | 685 | 11% |
| | *An. gambiae* | 2815 | 45% |
| | Other *Anopheles* | 1010 | 16% |
| | *Culex* species | 1678 | 27% |
| | Sub -total | 6188 | 100% |
| **Grand total** | | 31412 | |

**Table 5.4:** Sibling species of *An. funestus* sensu lato collected from different habitat type.

| Habitat | Sibling species | Number, (n) | Percentage |
|---|---|---|---|
| **1. River Streams** | *An. funestus* | 468 | 85% |
| | *An. leesoni* | 6 | 1% |
| | *An. parensis* | 11 | 2% |
| | *An. rivolurum* | 8 | 1% |
| | Non ampl | 59 | 11% |
| | Sub -total | 552 | 100% |
| **2. Ground Pools** | *An. funestus* | 231 | 52% |
| | *An. leesoni* | 19 | 4% |
| | *An. parensis* | 17 | 4% |
| | *An. rivolurum* | 122 | 27% |
| | Non ampl | 57 | 13% |
| | Sub -total | 446 | 100% |
| **3. Rice Fields** | *An. funestus* | 53 | 54% |
| | *An. leesoni* | 10 | 10% |
| | *An. parensis* | 6 | 6% |
| | *An. rivolurum* | 2 | 2% |
| | Non ampl | 27 | 28% |
| | Sub -total | 98 | 100% |
| **4. Spring-fed Wells** | *An. funestus* | 68 | 71% |
| | *An. leesoni* | 4 | 4% |
| | *An. parensis* | 11 | 11% |
| | *An. rivolurum* | 0 | 0% |
| | Non ampl | 13 | 14% |
| | Sub -total | 96 | 100% |
| **Total** | | 1192 | |

**Non ampl indicates chances of other sibling species*

### 5.4.2 Productivity and environmental characteristics of different aquatic habitats

*i. Anopheles funestus productivity across aquatic habitats*

The productivity of aquatic habitats varied significantly, with ground pools being the most productive habitat, with a predicted mean of 20.06 *An. funestus* larvae

(95% CI: 15.78 - 24.34). This was significantly higher than the productivity in river streams (mean=3.96 larvae; 95% CI: 3.60 - 4.32), ditches (mean =2.85 larvae; 95% CI: 2.38 - 3.32) and spring fed wells (mean = 1.85 larvae ;95% CI: 1.49 - 2.21). Rice fields were the least productive, with a mean that significantly lower than all other habitat types (mean = 1.55 larvae;95% CI: 1.21 - 1.89; Figure 5.4).

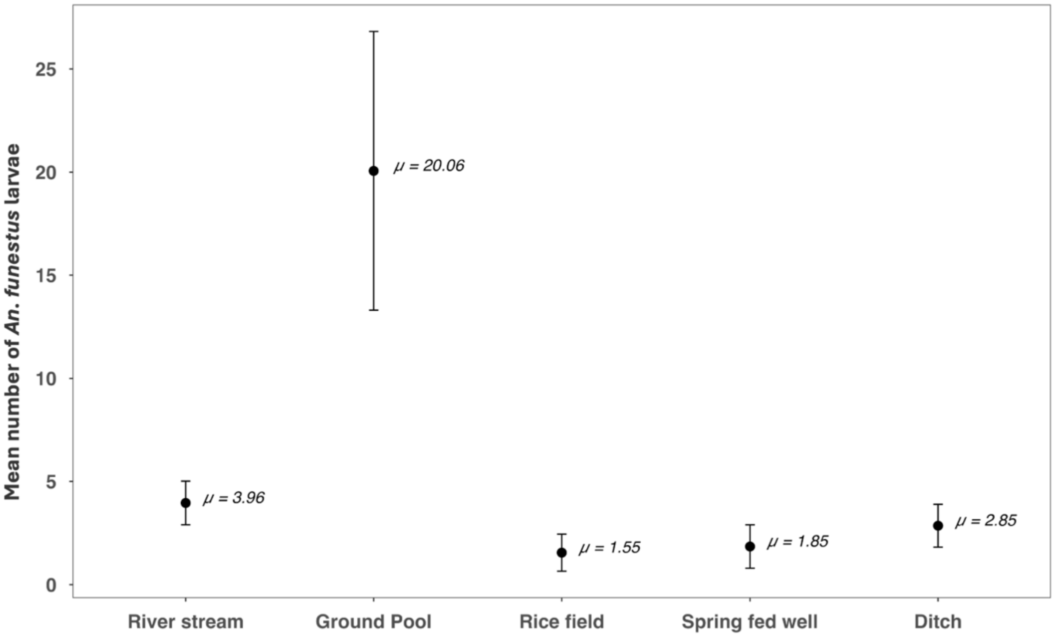

**Figure 5.4:** Mean number of *An. funestus* larvae by habitat type, μ shows the mean values for each habitat category.

*ii. Environmental characteristics of different aquatic habitats*

Environmental measurements taken at the time of first survey indicated notable differences between the 5 major aquatic habitats (Table 5.5). River stream habitats were generally large (surface area more than 250m and more than 50 cm depth), semi-permanent, coloured, fed by rainwater, had moving water and were shaded by vegetation. Within river stream habitats, *An. funestus* were more abundant in sites of larger surface areas (more than 250 m) and shallow depth (less than 10 cm), with floating vegetation and filamentous or mixed algae (Table 5.6). Similarly, ground pools were also relatively large (surface area more than 250m) and deep (>50 cm depth), semi-permanent, coloured, fed by rainwater, and shaded but with stagnant water. Within ground pools, *An. funestus* larvae were more abundant in larger and deeper habitats, and in permanent, coloured and stagnant water with brown algae and floating vegetation (Table 5.6).

While rice fields were also relatively large and semi-permanent, unlike river streams and ground pools these habitats were clear, and none were categorized as permanent. Rice field habitats were generally stagnant, shaded and had emergent vegetation (Table 5.5). Amongst rice fields, no *An. funestus* mosquitoes were collected from temporary habitats, and abundance was higher at medium water depths (between 10-50 cm), and sites with clear and stagnant water conditions, and that had no algae (Table 6).  In contrast to other habitat types, spring fed wells were generally small in surface area (10-100m) but deep (>50 cm, Table 5.6). Most wells were semi-permanent with clear, stagnant water without shade. Amongst spring-fed wells, *An. funestus* s.l larvae were significantly more abundant at habitats with clear water, shade and that were also fed by rainwater. Ditch habitats were also generally small (10-100m surface area) with moderate depth (10-50 cm), semi-permanent, clear stagnant, shaded and had emergent vegetation (Table 5.5). Within ditch habitats, *An. funestus* larval productivity was higher in smaller and shallow ditches, and that had shade and algae (Table 5.6).

**Table 5.5:** Environmental characteristics of the 5 main aquatic habitat types where *An. funestus* sl were sampled in this study.

| Variable | Categories | River streams, % | Ground pools, % | Rice fields, % | Spring-fed wells, % | Ditch, % |
|---|---|---|---|---|---|---|
| **Habitat size** | Less than 10m | 6 (2) | 2 (3) | 0 | 57 (35) | 2 (1) |
| | Between 10-100m | 101 (26) | 0 | 2 (2) | 94 (57) | 152 (63) |
| | Between 100-250m | 121 (31) | 13 (20) | 11 (11) | 4 (2) | 50 (21) |
| | More than 250m | 159 (41) | 49 (77) | 87 (87) | 9 (6) | 36 (15) |
| | | | | | | |
| **Water permanency** | Temporary | 2 (1) | 3 (5) | 8 (8) | 4 (3) | 3 (1) |
| | Semi-permanent | 284 (73) | 41 (64) | 92 (92) | 143 (87) | 235 (98) |
| | Permanent | 101 (26) | 20 (31) | 0 | 17 (10) | 2 (1) |
| | | | | | | |
| **Water depth** | Less Than 10 cm | 5 (1) | 0 | 6 (6) | 1 (1) | 8 (3) |
| | Between 10-50 cm | 189 (49) | 21 (33) | 72 (72) | 77 (47) | 210 (88) |
| | More Than 50 cm | 193 (50) | 43 (67) | 22 (22) | 86 (52) | 22 (9) |
| | | | | | | |
| **Watercolour** | Clear | 56 (14) | 31 (48) | 77 (77) | 118 (72) | 213 (89) |
| | Coloured | 331 (86) | 33 (52) | 23 (23) | 46 (28) | 27 (11) |
| | | | | | | |
| **Water source** | Rainwater | 347 (90) | 55 (86) | 99 (99) | 104 (63) | 231 (96) |
| | Non-rainwater | 40 (10) | 9 (14) | 1 (1) | 60 (37) | 9 (4) |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Water movement** | Moving | 348 (90) | 8 (12) | 33 (33) | 4 (2) | 227 (95) |
| | Stagnant | 39 (10) | 57 (88) | 67 (67) | 160 (98) | 13 (5) |
| | | | | | | |
| **Algae type** | Filamentous | 91 (24) | 23 (36) | 30 (30) | 28 (17) | 72 (30) |
| | Brown | 8 (2) | 4 (6) | 2 (2) | 12 (7) | 20 (8) |
| | Mixed | 4 (1) | 2 (3) | 7 (7) | 4 (2) | 6 (3) |
| | None | 283 (73) | 35 (55) | 61 (61) | 120 (73) | 142 (59) |
| | | | | | | |
| **Presence of shades** | Shaded | 285 (74) | 57 (89) | 90 (90) | 98 (60) | 182 (76) |
| | None | 102 (26) | 7 (11) | 10 (10) | 66 (40) | 58 (24) |
| | | | | | | |
| **Vegetation type** | Emergent | 271 (70) | 19 (30) | 91 (91) | 58 (35) | 197 (82) |
| | Floating | 2 (1) | 3 (5) | 0 | 11 (7) | 2 (1) |
| | Mixed | 13 (3) | 24 (38) | 7 (7) | 4 (2) | 0 |
| | None | 101 (26) | 18 (28) | 2 (2) | 91 (56) | 41 (17) |

**Table 5.6:** Mean abundance of *An. funestus* s.l and pupae in different habitat types in this study. Values represented mean predicted number in habitats with different environmental features; based on separate GLMMs fit to data from each habitat type.

| | | Mean number of *An. funestus* larvae and pupae [95% CI] | | | | |
|---|---|---|---|---|---|---|
| **Variable** | **Categories** | **River streams** | **Ground pools** | **Rice fields** | **Spring-fed wells** | **Ditch** |
| **Habitat size** | Less than 10m | 4.90 [0.93, 25.72] | 0 | - | - | 35.8 [2.42, 129.04] |
| | Between 10-100m | 4.25 [2.22, 8.16] | 0 | - | - | 2.30 [1.26, 4.21] |
| | Between 100-250m | 6.58 [3.81, 11.38] | 6.42 [1.71, 24.09] | - | - | 2.26 [1.35, 5.00] |
| | More than 250m | 15.33 [8.65, 27.15] | 11.07 [5.10, 24.03] | - | - | 6.3 [3.34, 11.85] |
| | | | | | | |
| **Water permanency** | Temporary | 0 | 1.42 [0.20, 10.00] | 0 | - | - |
| | Semi-permanent | 15.33 [8.65, 27.15] | 11.07 [5.10, 24.03] | 11.67 [6.63, 20.54] | - | - |
| | Permanent | 1.03 [0.34, 3.09] | 55.61 [38.39, 80.56] | 0 | - | - |
| | | | | | | |
| **Water depth** | Less Than 10 cm | 30.17 [11.82, 77.03] | 0 | 0 | - | 2.84 [0.62, 13.09] |
| | Between 10-50 cm | 6.87 [3.79, 12.47] | 12.38 [3.59, 42.73] | 11.67 [6.63, 20.54] | - | 2.3 [1.26, 4.21] |
| | More Than 50 cm | 15.33 [8.65, 27.15] | 11.07 [5.10, 24.03] | 0.61 [0.06, 5.9] | - | 0. 63 [0.20, 2.01] |
| | | | | | | |
| **Watercolour** | Clear | 15.33 [8.65, 27.15] | 6.22 [2.27, 17.01] | 11.67 [6.63, 20.54] | 8.48 [2.18, 32.97] | - |
| | Coloured | 4.83 [1.55, 15.04] | 11.07 [5.10, 24.03] | 0. 5 [0.04, 5.75] | 2.74 [0.81, 9.33] | - |
| | | | | | | |
| **Water source** | Rainwater | 15.33 [8.65, 27.15] | 11.07 [5.10, 24.03] | - | 8.48 [2.18, 32.97] | 2.3 [1.26, 4.21] |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Non-rainwater | 46.43 [14.67, 146.98] | 1.19 [0.22, 6.30] | - | 2.29 [0.66, 7.95] | 0.05 [0.00, 0.49] |
| | | | | | | |
| **Water movement** | Moving | 15.33 [8.65, 27.15] | 0.94 [0.07, 13.45] | 11.67 [6.63, 20.54] | - | - |
| | Stagnant | 46.41 [20.38, 105.72] | 11.07 [5.10, 24.03] | 30.41 [15.98, 57.89] | - | - |
| | | | | | | |
| **Algae type** | Filamentous | 41.11 [25.51, 66.25] | 19.03 [9.87, 36.67] | 3.12 [1.59, 6.11] | - | 8.64 [5.68, 13.14] |
| | Brown | 5.59 [0.56, 56.27] | 65.55 [8.76, 490.44] | 0 | - | 8.33 [4.48, 15.49] |
| | Mixed | 30.95 [7.07, 135.56] | 23.15 [9.67, 55.46] | 7.85 [2.89, 21.36] | - | 75.26 [41.54, 136.36] |
| | None | 15.33 [8.65, 27.15] | 11.07 [5.10, 24.03] | 11.67 [6.63, 20.54] | - | 2.3 [1.26, 4.21] |
| | | | | | | |
| **Presence of shades** | Shaded | 15.33 [8.65, 27.15] | 11.07 [5.10, 24.03] | - | 8.48 [2.18, 32.97] | 2.30 [1.26, 4.21] |
| | None | 7.29 [3.04, 17.47] | 4.91 [1.19, 20.19] | - | 3.8 [0.88, 16.38] | 1.02 [0.46, 2.27] |
| | | | | | | |
| **Vegetation type** | Emergent | 15.33 [8.65, 27.15] | 31.49 [17.41, 56.98] | - | - | 2.30 [1.26, 4.21] |
| | Floating | 227.54 [27.75, 1865.89] | 73.10 [24.89, 214.69] | - | - | 1.11 [0.13, 9.86] |
| | Mixed | 29.93 [11.35, 78.96] | 11.07 [5.10, 24.03] | - | - | 0 |
| | None | 22.71 [11.27, 45.76] | 186.18 [58.39, 593.64] | - | - | 9.93 [5.33, 18.49] |

For “-” refer that that specific parameter was removed from backward selection process.  Note that, this analysis was conducted separately in each habitat category

### 5.4.3 Nutritional reserves and body size of *An. funestus* emerging from different habitat types.

Descriptive analysis indicated lipid content in *An. funestus* adults was quite variable (0.01-0.09 µg) (Table 5.7). Mean glycogen and sugar levels were more uniform across samples. Statistical analysis indicated that both habitat type and sex had some impacts on *An. funestus* energetic reserves (lipid, glycogen, and sugar content) and body size. Specifically, *An. funestus* emerging from rice fields had 2 times more lipids (RR = 2.25, P<0.001), and 41% higher sugar content (RR = 1.41, P<0.001), than those from river streams (Table 5.8, Figure 5.5a). There was a trend of higher glycogen in *An. funestus* emerging from rice fields (Figure 5.5b), but there were no statistical differences between habitat types for this variable except for levels being lower in those from ground pools than river streams (Figure 5.6) There were no significant differences in energetic reserves between male and female *An. funestus* s.l (Figure 5.5a-c). In contrast, mosquito body size varied between sexes but in a habitat-specific way (habitat type*sex interaction, $\chi^2$ = 2.03, p = 0.0012). Females emerging from river streams and spring fed wells tended to be larger than males (Figure 5.5d), with males being of equivalent or slightly higher mean body size in ditches and ground pools than females.

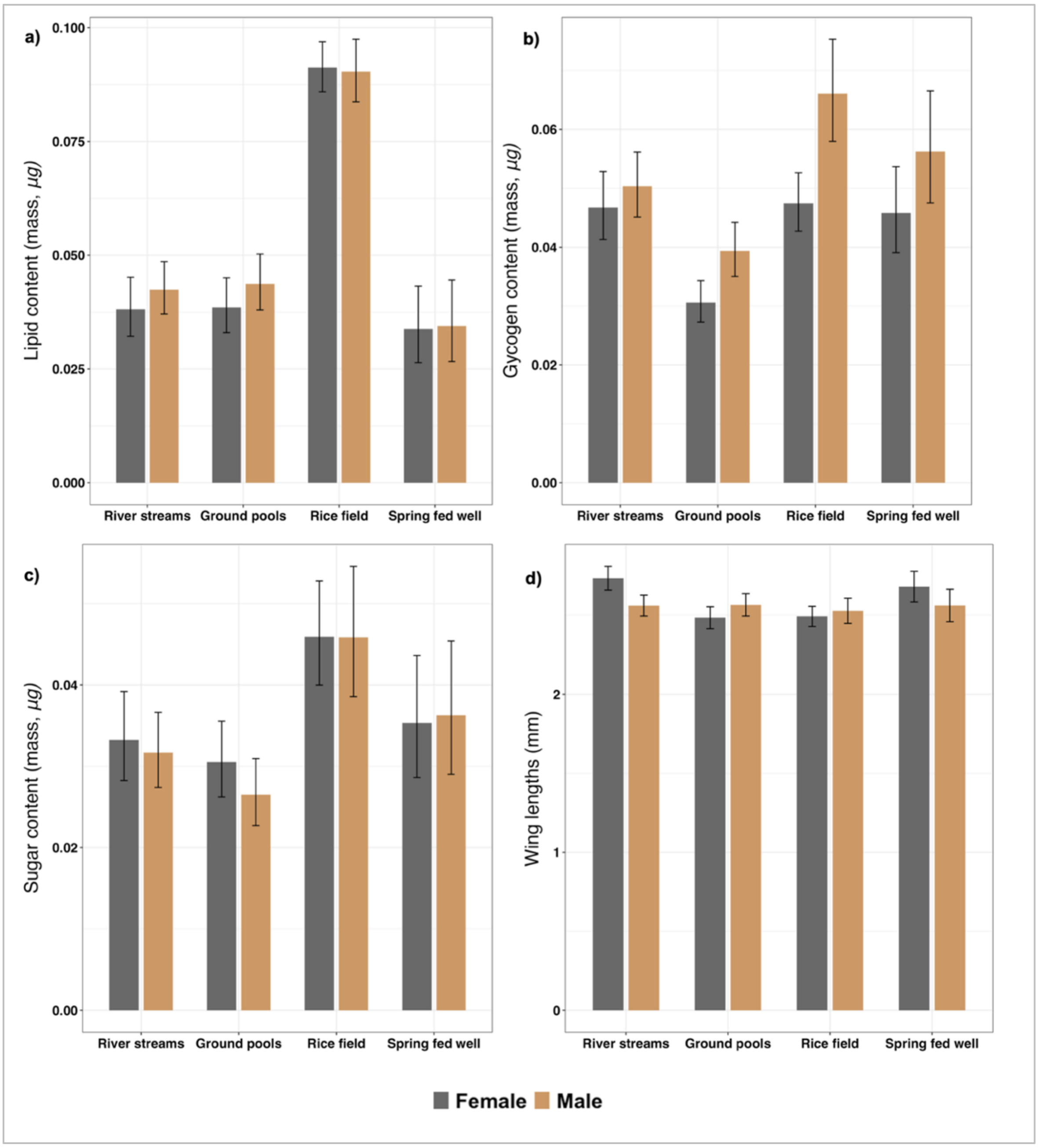

**Figure 5.5:** Means values for a) lipid content, b) glycogen content, c) sugar content, and d) wing length in *An. funestus*, separated by females (grey) and males (orange), from different larval habitat types (river streams, ground pools, rice fields, and spring-fed wells). These predictions show the impact of larval habitat type on the energetic reserves and body size of adult mosquitoes.

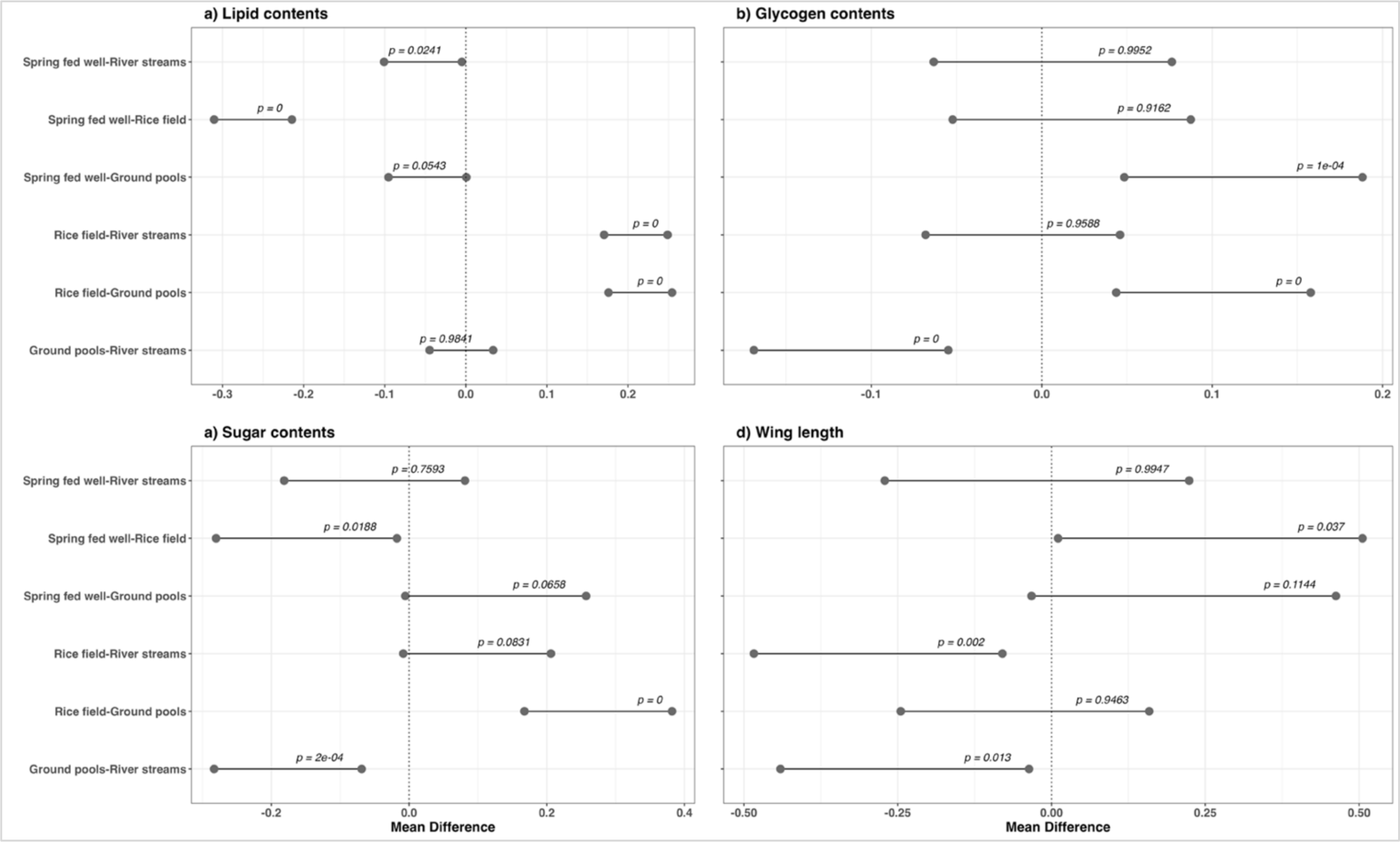

**Figure 5.6:** Post hoc analysis of *An. funestus* energetic reserves across different aquatic habitats. The mean differences (± 95% CI) between habitats are shown for various traits: (a) Lipid contents, (b) Glycogen contents, (c) Sugar contents, and (d) Wing length.

### 5.4.4 Survival of *An. funestus* emerging from different habitat types.

The final Cox proportional hazards model of adult *An. funestus* survival included habitat type, sex, wing length, and interaction between habitat type and sex (Table 5.7) Mosquitoes emerging from ground pool habitats had a significantly lower risk of dying compared to those from river streams. Odds of dying tended to be higher in rice fields than another habitat, but this was not statistically significant due to the large confidence interval around this estimate (Table 5.7). Survival was significant lower in females than males (Table 5.8). Additionally, adult survival was positively associated with wing length (Table 5.8). The Kaplan-Meier survival curves obtained to visualize differences between subgroups are shown in Figure 5.7. Median adult survival was relatively high, ranging between 26-40 days in different habitat type and sex groups. The median lifespan of mosquitoes emerging from ground pools (40 and 37 days for males and females respectively) was higher than those from river streams (32 and 30 days for females and males respectively) and rice fields (26 days for both males and females; Figure 5.7).

**Table 5.7:** Summary of stepwise forward selection for predicting survival probability of adult *An. funestus*.

| Variables | $X^2$ (Chi-squared) | p-value |
|---|---|---|
| **Habitat type** | 7.4 | 0.12 |
| **Sex** | 1.53 | 0.47 |
| **Wing length** | 78.5 | <0.001 |
| **Habitat type* Sex** | 1.529 | 0.46 |

*"$X^2$" is defined as the chi-squared statistic used to evaluate the relationship between the variables listed*

**Table 5.8:** Hazard ratios for factors influencing survival probability

| Predictor | Category | Hazard Ratio ± SE | p-value |
|---|---|---|---|
| **Habitat type** | River streams | 1 | |
| | Ground pools | 0.15 ± 0.82 | 0.016 |
| | Rice field | 2.01 ± 1.32 | 0.695 |
| | | | |
| **Sex** | Female | 1 | |
| | Male | 0.68 ± 0.28 | <0.001 |
| | | | |
| **Wing length** | - | 0.06 ± 0.30 | <0.001 |
| | | | |
| **Habitat type: Sex** | Female: River streams | 1 | |
| | Male: Ground pools | 0.95 ± 0.34 | 0.89 |
| | Male: Rice fields | 0.64 ± 0.40 | 0.27 |

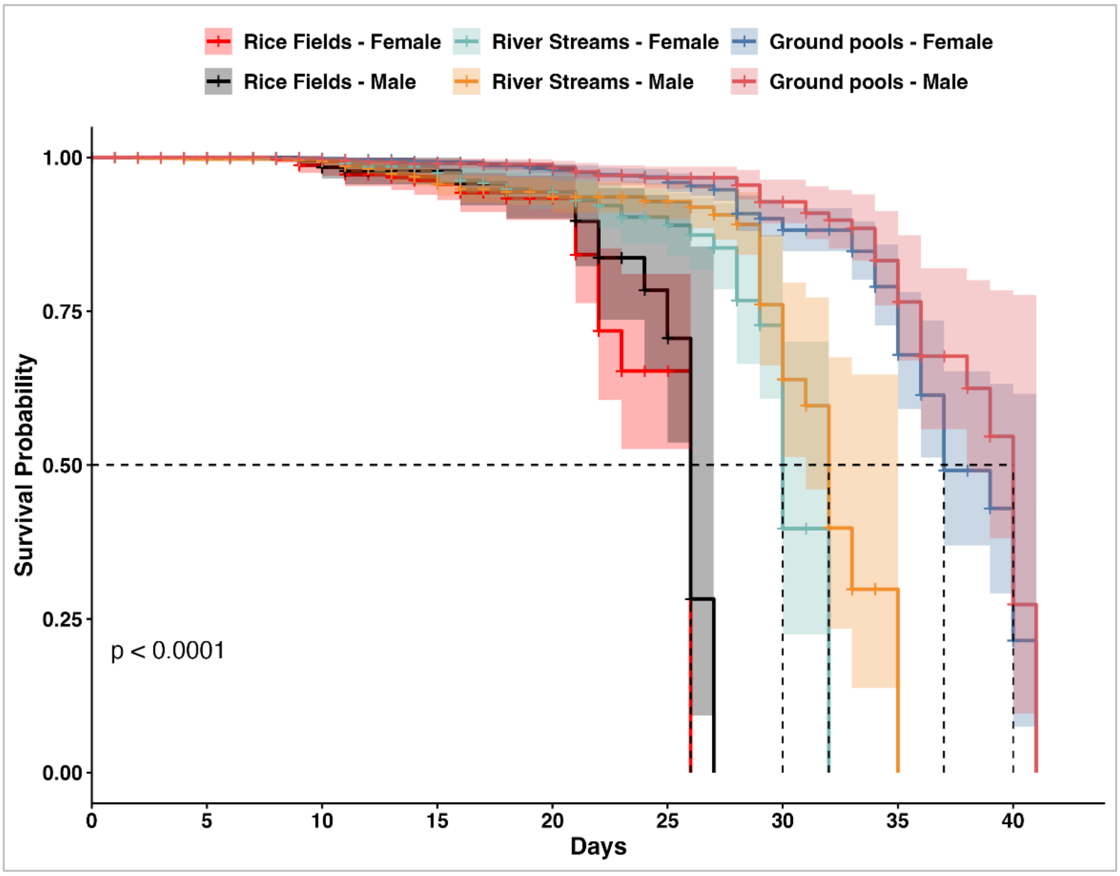

**Figure 5.7:** The survival curves, differentiated by colour and line type, represent the estimated probability of survival for mosquitoes from rice fields, river streams,

and ground pools, further categorized by female and male. The horizontal dashed line represents the median survival time, which is the day by which 50% of the population died. The survival probabilities are obtained from Kaplan-Meier estimates. The p-value is from the log-rank test. It indicates there is a statistically significant difference between the groups shown in the figure.

### 5.4.5 Insecticide resistance in *An. funestus* from different habitats and seasons

The final model for mosquito mortality following insecticide exposure included season, habitat type, and insecticide as main effects (Table 5.9). Neither the interaction between season and habitat type ($\chi^2$=0.05, p = 0.83) nor the interaction between insecticide type and season ($\chi^2$=11.88, p = 0.83) were significant, indicating that the impact of habitat and insecticide types does not vary between seasons. Season alone, however, shows a significant effect, with mortality decreasing in the rainy season compared to the dry season (OR = 0.76, p = 0.018). This suggests that seasonal factors may contribute to reduced insecticide efficacy during the rainy season.

While habitat type (ground pools and river streams) was included in the model, its effect was not statistically significant (OR = 1.19, p = 0.145), indicating only a marginal difference in mortality between habitats (Table 5.9). However, insecticide type played a crucial role, with Pirimiphos methyl showing high baseline efficacy, whereas Bendiocarb (OR = 0.3, p = 0.002) and other insecticides like DDT, Deltamethrin, and Permethrin exhibited significantly lower mortality rates at standard concentrations (e.g., Permethrin OR = 0.01, $p < 0.001$) (Figure 5.8), where mortality is generally higher in the dry season (Figure 5.8a), with increased dosages of Deltamethrin and Permethrin (5x and 10x) reaching nearly 100% mortality. In the rainy season (Figure 5.8b), mortality rates are notably lower for standard doses of these insecticides, highlighting the necessity of higher concentrations for effective control during the rainy season. Overall, this analysis shows the importance of adapting insecticide application strategies to seasonal conditions to improve control efforts against *An. funestus*.

**Table 5.9:** Odds Ratios (OR) for the Effect of Season, Habitat Type, and Insecticide on *An. funestus* Mortality after 24-hour Exposure

| **Variable** | **OR (95% CI)** | **p-value** |
|---|---|---|
| Intercept | 37.04 (18.71, 73.34) | <0.001 *** |
| **Season** | | |
| Dry season | 1 | |
| Rain season | 0.76 (0.6, 0.95) | 0.018 |
| **Habitat type** | | |
| Ground pools | 1 | |
| River stream | 1.19 (0.94, 1.49) | 0.145 |
| **Insecticide** | | |
| Pirimiphos methyl | 1 | |
| Bendiocarb | 0.3 (0.14, 0.65) | 0.002 |
| DDT | 0.1 (0.05, 0.2) | <0.001 *** |
| Deltamethrin | 0.06 (0.03, 0.11) | <0.001 *** |
| Permethrin | 0.01 (0.007, 0.03) | <0.001 *** |
| Deltamethrin 5x | 0.26 (0.12, 0.55) | <0.001 *** |
| Permethrin 5x | 0.23 (0.11, 0.5) | <0.001 *** |
| Deltamethrin 10x | 1.82 (0.6, 5.48) | 0.29 |
| Permethrin 10x | 2.28 (0.69, 7.48) | 0.175 |

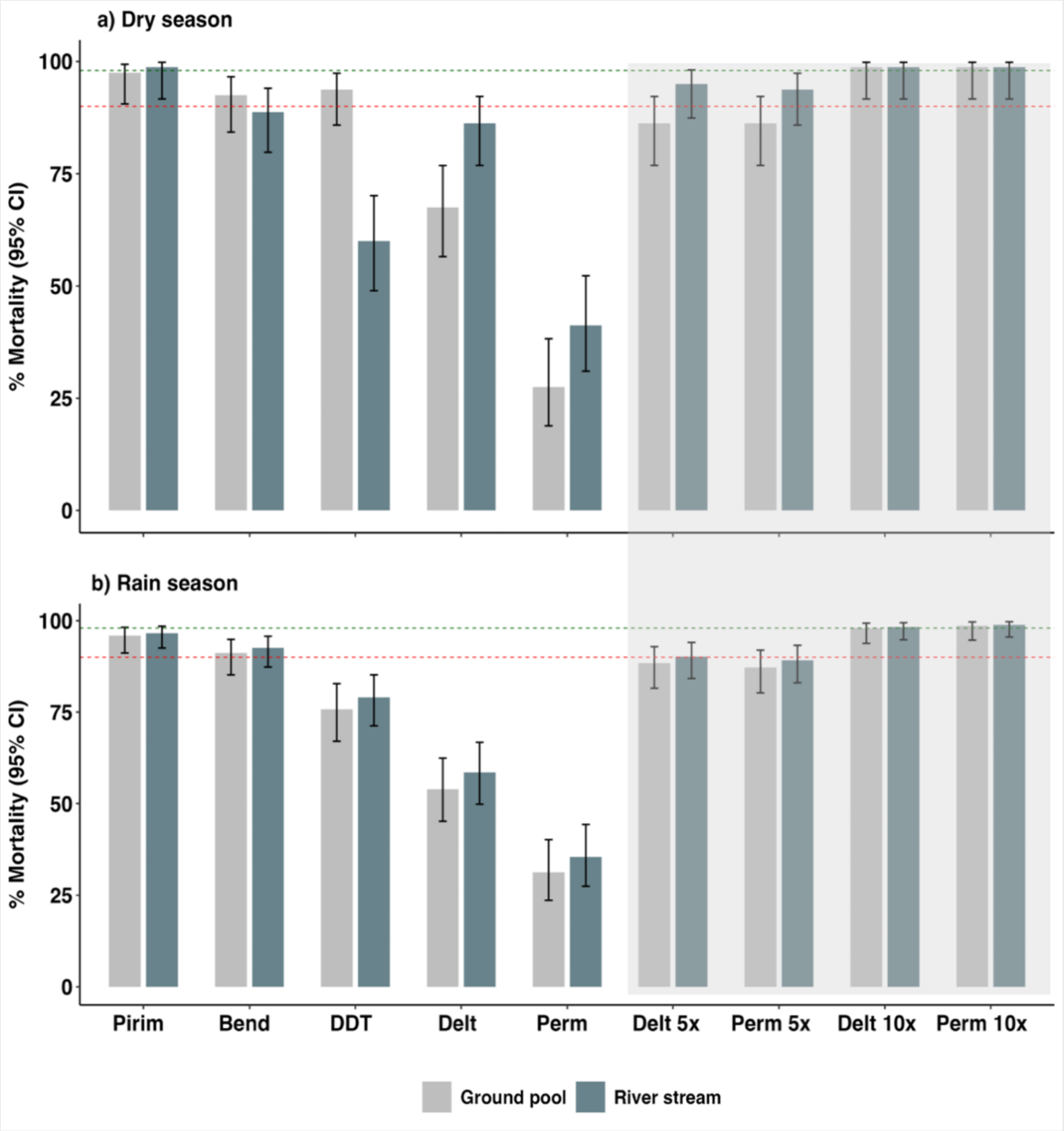

**Figure 5.8.** This figure shows the mean percentage mortality of *An. funestus* mosquitoes in common aquatic habitats across different seasons. In the figure, "Pirim" stands for Pirimiphos-methyl, "Bend" for Bendiocarb, "Delt" for Deltamethrin, and "Perm" for Permethrin. The labels "5x" and "10x" indicate concentrations increased by 5 and 10 times, respectively. Panel (a) represents experiments conducted during the dry season, while panel (b) depicts those carried out in the rainy season.

## 5.5 Discussion

In this study, we investigated how the type of larval habitat used by the malaria vector *An. funestus* impacted the quantity and "quality" of mosquitoes they produced, with 'quality' defined in terms of mosquito fitness and insecticide resistance traits. Our findings indicated significant differences in mean productivity between habitat types, with ground pools having 4-10 times more *An. funestus* larvae than other habitat types. Additionally, there were significant differences in fitness traits and nutritional reserves of adult mosquitoes emerging from different habitats. However, there was no one habitat type that consistently produced more or less fit mosquitoes than others. For instance, although adult *An. funestus* from rice fields had higher levels of key energetic reserves like lipids, glycogen, and sugar, mosquitoes from ground pools had the highest survival and insecticide resistance. At the level of habitat type (not individual habitat), ground pools were the most productive. They did not always have the lowest or highest fitness, but in some cases, mosquitoes emerging from ground pools were the most 'fit' in terms of pyrethroid resistance and the longevity. However, a formal analysis was not conducted to statistically confirm the relationship between productivity and fitness traits. Specifically, I did not assess potential interactions between productivity and fitness traits such as survival, lipid, and glycogen levels. While these patterns were observed, future studies should employ formal tests such as regression or correlation analyses to explore the potential relationships between productivity and mosquito fitness. Thus, while the habitat type with the highest productivity was not always the most or least fit, no individual analysis was done to test how productivity at the individual habitat level related to fitness.

In line with previous studies, our findings indicate a varied distribution of mosquito species across different habitats [267,354]. We observed that rice fields and ditches are prolific breeding grounds for *An. gambiae* s.l., while *An. funestus* is more commonly found in river streams and ground pools [242,244,277,355]. There was also substantial variation in larval productivity within specific habitat types in relation to physicochemical characteristics. For example, rice fields were unique in being clear and were stagnant water bodies. River streams had larger surface areas, deeper waters, and were most likely to be shaded compared to other habitat types. Ground pools and rice fields also tended to be semi-permanent, while spring-fed wells were mostly smaller in surface areas.

Larger surface areas and deeper waters were more productive for river streams, ground pools, and rice fields, whereas ditches and spring-fed wells showed higher productivity in smaller surface areas. This indicates that larval mosquito densities are influenced both by variation in habitat types and smaller physico-chemical differences within each habitat type. Additionally, environmental factors that have a strong link with larval density in some habitat types may not be as influential in others. Consistently, habitats with stagnant water and shade were associated with higher abundances of *An. funestus*. These findings align with the analyses presented in chapter 4, where similar environmental factors were identified as significant predictors of mosquito presence and productivity. The consistent association of stagnant water and shading with higher *An. funestus* productivity suggests these could be general environmental predictors useful for guiding LSM strategies. Prioritizing habitats that are shaded and have stagnant water, particularly ground pools which showed the highest productivity, could enhance the effectiveness of targeted mosquito control measures.

To our knowledge, this is the first study to measure nutritional reserves in wild *An. funestus* populations and demonstrate their strong association with larval habitat environments. Specifically, mosquitoes emerging from rice fields had higher levels of lipids and sugars, indicating higher fitness quality. This suggests a potential link between habitat-derived nutritional availability and physiological fitness [326,330]. Additionally, mosquitoes from river streams and spring-fed wells were generally larger than those from other habitats, which could contribute to higher survival rates. However, contrary to expectations, mosquitoes from rice fields, despite their higher energetic reserves, had the lowest survival. This finding challenges the assumption that greater energy reserves naturally lead to longer life [356,357].

Several factors could explain why habitats producing the longest-lived mosquitoes did not also generate those with the highest lipid reserves. First, body size differences could be a contributing factor, as larger mosquitoes tend to have longer survival rates [334]. Second, underlying variation in larval density may impact mosquito survival and energetic reserves in different ways. The higher productivity of ground pools indicates larvae developing in these habitats could face higher resource competition due to density dependence, potentially explaining their moderate levels of energetic reserves. However, it is unclear why larvae developing in these high-density habitats would have greater survival. One possibility is that the flow of water in these habitats led to higher nutrient flow through and reduced competition. Third, other environmental factors, such as pathogens or the microbiome, could vary between habitats and impact mosquito survival. Differences in microbial communities within habitats could affect mosquito health and longevity [358]. Finally, variations in survival could also be due to the presence of different *An. funestus* species across habitats [29]. All of these collectively highlight the complex interactions between habitat characteristics, mosquito physiology, and survival, suggesting that multiple factors beyond just energetic reserves influence mosquito longevity [334]. Further research is needed to fully understand these dynamics and their implications for malaria vector control strategies.

We also found notable variation in phenotypic resistance between *An. funestus* emerging from different larval habitat types. This is the first time such differences have been tested for *An. funestus* in these habitat types. However, these habitat differences were not consistent across insecticide classes. For example, mortality after exposure to DDT in the dry season was higher in larvae emerging from river stream than ground pools, whereas the reverse was true for permethrin and deltamethrin. The reasons for these differences in resistance between habitat types and insecticide classes are unclear. One possibility is that these specific insecticides or other related chemicals that generate cross-resistance accumulate at different rates in these habitats: exposing mosquitoes to variable selection pressures [359,360]. Alternatively, this variation could be a side effect of differential selection in adults using different larval habitats, with those using some habitats being more likely to feed on humans and be exposed to vector control chemicals [234]. Another factor could be the presence of different *An. funestus* species across habitats, which might exhibit varying resistance profiles. Regardless of the mechanism, the sustained high resistance to deltamethrin and permethrin in *An. funestus* across all habitats poses a significant challenge to the effectiveness of current pyrethroid-based vector control strategies in East Africa [106,361,362].

My results align with previous studies in Kilombero and other parts of Tanzania, showing highest resistance to deltamethrin and permethrin, followed by DDT, while susceptibility was highest for bendiocarb and pirimiphos-methyl [88,240]. These patterns of resistance are consistent with findings from other regions, indicating a widespread issue of pyrethroid resistance in *An. funestus*. Our study did find some differences in resistance levels between the dry and rainy seasons, although these were not consistent across all insecticides. For instance, there was a notable seasonal difference in mortality rates for DDT, with higher resistance observed during the dry season. However, for deltamethrin and permethrin, the resistance trends were relatively similar between seasons, consistent with findings by [359,363] in showing that seasonal variations in insecticide resistance can be chemical-specific.

This study has some limitations. First, we faced challenges in rearing *An. funestus* in the field insectary, where controlling the temperature and humidity was difficult. This led to low emergence rates for larvae from some habitats, limiting the range of different larval habitat types that could be compared in insecticide resistance bioassays. Variability in microclimatic conditions in the field insectary may have generated additional sources of error, making it harder to detect the effects of other variables. However, this heterogeneity in conditions is also a strength, as it ensured fitness differences were tested for under realistic conditions. Second, our investigation focused on a limited range of fitness and resistance traits (energetic reserves, body size, survival, and phenotypic resistance). Although informative, there may be other traits that are more indicative of fitness in the field. Future research could broaden this scope to include other fitness proxies such as larval development rates, fecundity, mating and blood-feeding success, and pre-gravidity rates [103,320,342]. Also, an in-depth analysis of chemical parameters (like dissolved oxygen levels, conductivity, nitrates, and phosphate levels) and biological factors (such as predator presence and microbial community) within these habitats would offer more comprehensive insights into the mechanisms through which habitat-specific fitness is generated [271,291,307,364]. Lastly, the extent of variation in fitness and insecticide resistance detected here may be underestimated. Sampling was biased towards relatively high-density habitats, because the availability of *An. funestus* larvae and their emergence into adults for conducting these experiments was low, potentially limiting the breadth of our findings.

## 5.6 Conclusions

Our study provided insights into the complex relationship between the use and fitness value of different aquatic habitats for African malaria vectors. Importantly, we demonstrated for the first-time notable differences in the energetic reserves, survival, and phenotypic resistance in *An. funestus* emerging from different habitats. In terms of mosquito “quality,” no single habitat was identified as optimal; habitats associated with higher values of some fitness traits had lower values of others. Ground pools, identified as the most productive habitats, also produced mosquitoes with higher survival and resistance traits. In contrast, rice fields, while less productive, played a crucial role by giving rise to mosquitoes with high energy reserves. This indicates that habitats producing the greatest quantity of mosquitoes may also produce high 'quality' mosquitoes in terms of longevity and resistance.

This distinction is crucial for the implications of our findings for larval source management (LSM). While our results suggest that targeting highly productive habitats, such as ground pools, which are also associated with high survival and resistance traits, may be an effective strategy, there are important aspect to consider. Due to the design limitations of our study, we were unable to fully control for larval density and other environmental factors, which restricts our ability to definitively conclude which habitats are the most epidemiologically important. Given these, future research should aim to incorporate more comprehensive environmental assessments and control for larval density to better understand the relationship between habitat productivity, and emerged mosquito fitness. It is recommended to conduct longitudinal studies across different seasons and include a broader range of fitness and resistance traits. This would provide a more robust basis for optimizing LSM strategies by identifying and prioritizing the habitats that are truly the most significant for malaria transmission.

# Chapter 6: Seasonal Variation in Aquatic and Adult Habitat Use by the Dominant Malaria Vector, *Anopheles funestus* in South-Eastern Tanzania

*To be submitted in Malaria Journal*

## Abstract

**Introduction**: The population dynamics of major African malaria vectors are closely tied to seasonal variations in climatic factors such as rainfall and temperature. While the seasonality in the demography of malaria vectors is general has well-documented, less is known about the *Anopheles funestus*, the dominant malaria vector in East and Southern Africa. This species is unique amongst the African malaria vectors in using larger more permanent aquatic habitats for larval development, giving rise to a distinct pattern of adult seasonality. Furthermore, it is possible that the seasonal dynamics of this species are habitat dependent due to shifts in larval and adult habitat use across the year in response to change availability and microclimatic suitability. Understanding such seasonal shifts in habitat use could significantly improve the timing and optimal deployment of vector control interventions. This study investigated the distribution and abundance of *An. funestus* in various aquatic habitats (larvae) and house types (adults) over 12 months to determine if their habitat use varies seasonally.

**Methods**: Longitudinal surveillance of larval and adult *An. funestus* was conducted from July 2022 to June 2023 in two villages in southeastern Tanzania, where this vector species is the major source of malaria transmission. Aquatic habitats were surveyed monthly for the presence and abundance of *An. funestus* larvae. Similarly, the abundance of adult *An. funestus* in different house types (with either mud, brick, or concrete walls, and thatch or metal roofs) was monitored bi-weekly, with nine replicates per house type in each village. Generalized Additive Mixed Models (GAMMs) were used to assess the seasonal trend in *An. funestus* population dynamics, and test whether the predicted patterns of seasonality varied between habitat types at the larval or adult stage.

**Results**: The dynamics of both aquatic and adult *An. funestus* were significantly influenced by habitat or house type and village, with peak densities observed beginning the end of the rainy season. While the presence and densities of mosquito larvae and adults followed rainfall patterns, *An. funestus* remained abundant long after the rains, especially in permanent aquatic habitats like ground pools, river streams, and spring-fed pools. Non-permanent habitats, such as rice fields, ditches, tire tracks, and puddles, were mainly occupied during the wet season, though there was a notable peak in irrigated rice fields in Ikungua at the start of the dry season. Thatched roof houses had higher mosquito densities compared to metal-roofed houses throughout the year, with slight differences between villages. There were statistically significant differences in the seasonal trends between house types, with thatched roof houses showing pronounced seasonal fluctuations, including multiple peaks, whereas metal-roofed houses exhibited less variability. Peak larval densities of this vector species occurred approximately one month after peak densities of adults, with larvae particularly concentrated in permanent habitats during dry months.

**Conclusion**: This study has demonstrated significant temporal and habitat-specific variations in the presence and abundance of *An. funestus* mosquitoes in the study area. The findings indicate that *An. funestus* exhibits significant seasonal shifts in habitat use at both the larval (different aquatic habitats) and adult (different house types) stages. We hypothesize that these seasonal shifts in habitat use are likely driven by changes in habitat availability and micro-climatic conditions inside houses, but more research is needed to identify the specific environmental cues involved. Notably, thatched roof houses exhibited higher mosquito densities and more pronounced seasonal fluctuations compared to metal-roofed houses, which showed lower densities and limited seasonal variability. Understanding these patterns is crucial for optimizing malaria control interventions, such as larval source management, insecticide-treated nets, and indoor residual spraying, to effectively target the vector during peak periods of habitat use.

## 6.1 Introduction

The ecology of *Anopheles funestus*, a key malaria vector, is shaped by several environmental factors such as temperature, humidity, and rainfall, which can cause fluctuations in mosquito populations throughout the year [311,365,366]. While populations of mosquito vectors such as *Anopheles gambiae* s.l are often closely linked to rainfall patterns [312,367], those of *An. funestus* have a less pronounced and more lagged relationship with rainfall, getting to peak several weeks after the rainy season and persisting at relatively constant numbers throughout the dry season. This persistence of *An. funestus* throughout the dry season is linked to their use of large, more permanent aquatic habitats for larval development that remain stable across the year (Chapter 3 and 4).

Whilst larvae are restricted to aquatic habitats, adult *An. funestus* spend a significant fraction of their life inside or near houses [27]. Previous work has shown that household characteristics such as wall and roof type have a significant impact on indoor microclimatic conditions [318]. Houses with poor structures, such as those with eave spaces or thatched roofs, may be cooler during hot months compared to those with metal roofs [99,108,368]. Such houses may provide more favourable microclimatic environments for mosquitoes to rest and have more entry points, leading to higher indoor densities of *An. funestus* [108,369]. Consequently, the suitability of different house types for mosquitoes may vary seasonally in response to shifting indoor microclimatic conditions.

An important yet unexplored question is whether seasonal changes in malaria vector abundance are accompanied by shifts in habitat use at either the larval or adult stages. Understanding the potential for such shifts is important as it may reveal adaptive strategies employed by the vector to cope with varying environmental conditions, which could significantly impact the effectiveness of control measures [325]. To effectively reduce malaria transmission in settings where *An. funestus* is the dominant vector, co-deployment of interventions targeting both the aquatic and adult stages may be necessary [92]. Strategies such as larval source management (LSM) and adult-stage interventions like insecticide-treated nets (ITNs) and indoor residual spraying (IRS) play crucial roles [92]. The timing and location of intervention deployment can be optimized by understanding these fine scale seasonal variations in mosquito habitat use [289,370].

While the seasonality in the demography of other malaria vectors, notably *An. gambiae*, is well-documented [312], there is still limited knowledge on the seasonality of *Anopheles funestus*, the dominant malaria vector in East and Southern Africa, and whether it adapts its habitat use across the year in response to the changing in availability and microclimatic conditions. Multiple studies on other mosquito species and insects have demonstrated that environmental variability can lead to changes in habitat preference and use [29,305,325]. For example, *Anopheles gambiae* has shown altered breeding site selection in response to seasonal changes in water availability [267,367]. Thus, *An. funestus* might similarly adjust its habitat use seasonally (see Chapter 5); showing flexibility to expand into temporary aquatic habitats during the rainy season when they proliferate. However, the fine-scale nature of these shifts remains unknown, representing a significant knowledge gap in *An. funestus* ecology.

This study aimed to investigate seasonal variation in the larval and adult ecology of *An. funestus*, focusing on potential seasonal shifts in habitat use. The distribution and abundance of *An. funestus* in various aquatic habitats (larvae) and house types (adults) were analysed and compared over a 12-month period to investigate (i) whether the seasonality of *An. funestus* larvae varies between aquatic habitat types, and (ii) whether adult *An. funestus* shifts the house types they use throughout the year. By addressing these gaps, I hope to enhance the understanding of *An. funestus* ecology and provide insights for the development of targeted and effective vector control strategies in southeastern Tanzania.

## 6.2 Methods

### 6.2.1 Study site

This study was conducted over one year, from June 2022 to July 2023, in two villages: Ikungua (-8.46338°, 36.68725°) and Chikuti (-8.6028°, 36.7288°), in rural south-eastern Tanzania (Fig. 6.1). Further details of these study sites are provided in Chapter 3.2.1. In summary, these sites vary in altitude, with Chikuti located in the highlands (550 m) and Ikungua in the lowlands (400 m, Fig. 6.1). Chikuti covers an area of 80 km$^2$ with a population of 31,787, and Ikungua cover an area of 60 km$^2$

with a population of 10,953. The sites were selected based on their characterisation in a previous study in which they had chosen for their distinct geographic features and prevalence of *An. funestus* (Chapter 3) [22]. Common aquatic habitats used by mosquito larvae in these areas include river streams, ground pools, ditches, spring-fed wells, and dug pits (Chapter 3 & 4) [22]. Houses in these communities were generally classifiable into four groups based on construction material as follows: 1) mud walls and thatched roofs, 2) brick walls and thatched roofs, 3) brick walls without plaster and iron roofs and 4) brick walls with plaster and iron roofs. The primary malaria vectors in these study sites are *An. funestus* and *An. arabiensis* [109,246,247].

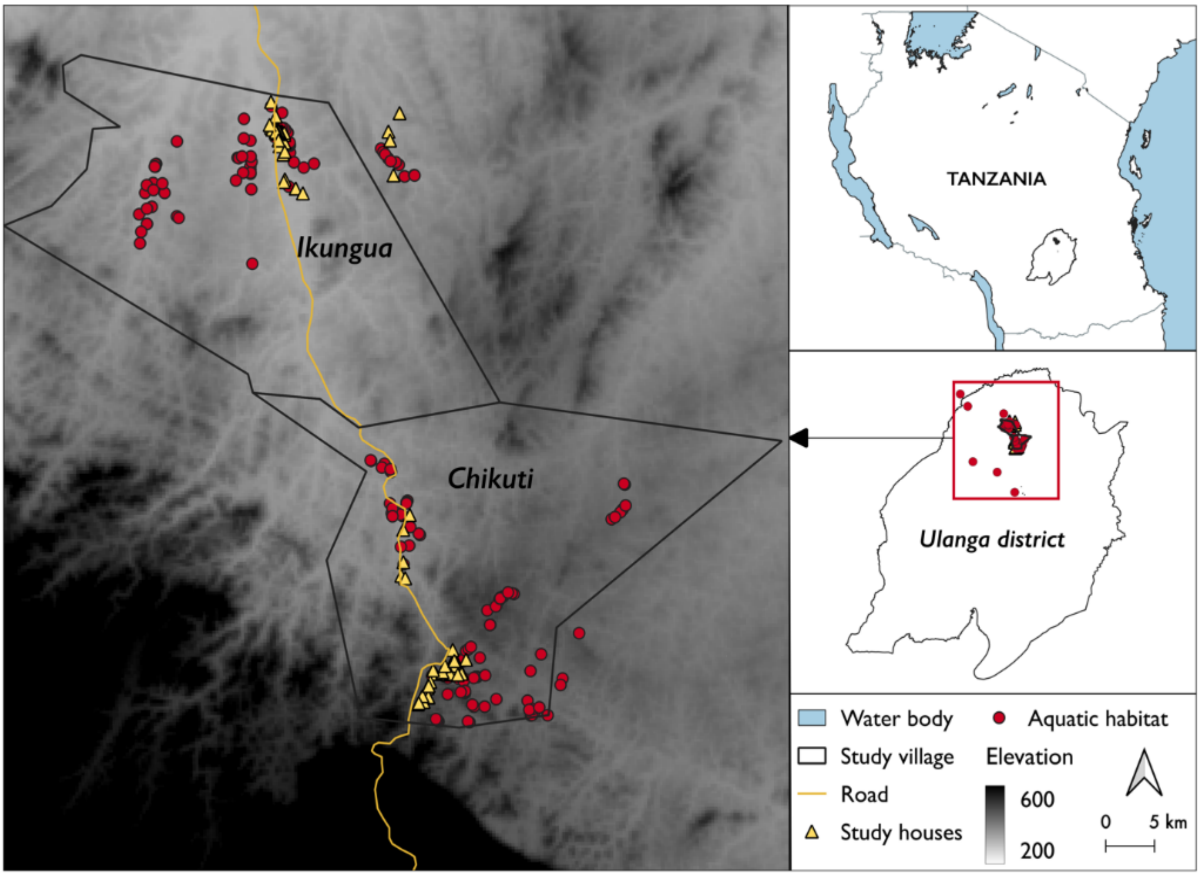

**Figure 6.1:** Map of study area, showing houses (yellow triangles) and aquatic habitats (red dots) that were monitored between July 2022 and June 2023.

### 6.2.2 Meteorological data

Seasonal variations in temperature and rainfall (Open-Meteo.Com Weather API, 2023) are shown in Figure 6.2a & b. The mean annual temperature was 24.9°C (min:

15.9°C, max: 36.9°C) in Ikungua and 23.8°C (min: 12.9°C, max: 35.5°C) in Chikuti. Precipitation levels were higher in Ikungua, (3.9 mm in October to 399.7 mm in December) than Chikuti (from 7 mm in October to 198.9 mm in December; (Open-Meteo.Com Weather API, 2023)

**Figure 6.2:** Annual temperatures and rainfall patterns in a) Ikungua and b) Chikuti (July 2022 - June 2023).

### 6.2.3 Identification, characterization and sampling in different aquatic habitats

As described in Chapter 4, a cross-sectional survey was conducted during the rainy season between February - May of 2022 to identify all aquatic habitats in these villages; and survey them for *An. funestus* larvae. A total of 67 aquatic habitats were examined in Ikungua and 114 in Chikuti. The survey identified different habitat types, such as river streams, ground pools, spring-fed well and dug pits, rice fields, ditches, puddles and tire trucks. Any aquatic habitats harbouring at least one *An. funestus* larvae classified as a positive and were included in the monthly follow-up surveys till June 2023 or when they dried up.

Sampling was conducted exhaustively once every month from July 2022 to June 2023. All aquatic habitats were sampled using a standard dipper (350mL) or a 10L bucket, depending on the size of the habitat as previously described in Chapter 3.4.2. During each visit, the number of dips increased incrementally with habitat size: one dip for habitats under 5 $m^2$, two for 6-10 $m^2$, three for 11-15 $m^2$, and up to 20 dips for habitats over 120 $m^2$. All mosquito larvae collected were identified by morphology/taxa, counted, and recorded as detailed in chapter four. Habitats wet for six to twelve months (50% of the year) were considered permanent. Those lasting three to six months (25% of the year) were labelled semi-permanent, and habitats wet for less than three months were classified as temporary (Table 6.5).

### 6.2.4 Identification, characterization and sampling in different house types

Different categories of house types were selected based on the common types observed in the study area [99,372]. These included houses with 1) brick walls and thatch roof, 2) mud walls and thatch roof, 3) plastered brick walls with iron roof, and 4) unplastered brick walls with iron roof. In each village, 36 houses were recruited, including nine of each of the 4 types, totalling 72 houses in the two villages (Figure 6.3). I selected nine houses per category because one house type (plastered brick walls with an iron roof) had only nine houses available. Convenience sampling was used to select houses that met the structural criteria, and whose occupants were willing to participate. These houses were also spread across the village.

In every recruited house, adult mosquitoes were sampled for one night after every two weeks for a period of one year (June 2022 to July 2023) using the CDC light traps, tent traps, and prokopack aspirators. Nine CDC light traps were installed in each house type in each village (9 traps x 4 house types x 2 villages). The collections were conducted between 6:00 p.m. and 6:00 a.m, each time setting the CDC light trap in the bedroom, at 1.5 m above the floor near a bed, on the foot side, next to a sleeping volunteer covered with a mosquito net. Household owners were trained on trap operation and instructed to ensure the trap remained operational throughout the night, closing the catch bag if the batteries ran out. The collection team retrieved mosquitoes from the traps each morning between 7:00 a.m. and

8:00 a.m. Additionally, Prokopack aspiration was performed in the same room where the CDC light trap was hung. This aspiration was conducted for 15 minutes per room, targeting all resting surfaces, including walls, ceiling, floor, and furniture, as detailed by Msugupakulya et al. (2022), to collect resting mosquitoes.

**Figure 6.3:** Examples of different house types recruited for this study

### 6.2.5 Ethical consideration

Ethical approvals for this project were obtained from Ifakara Health Institute's Institutional Review Board (Protocol ID: IHI/IRB/No: 26-2020) and the Medical Research Coordinating Committee (MRCC) at the National Institute for Medical Research (Protocol ID: NIMR/HQ/R.8a/Vol.IX/3495). Additional permission was obtained from District Medical Officers (DMO) and subsequently from each village executive officer (VEO). Permission was also obtained from the head of each family to carry out mosquito sampling. The aim of the study was explained to each family head, and they were asked for permission to collect mosquitoes on their premises. A written informed consent was obtained and recorded from each household head.

## 6.3 Data analysis

All the analysis were conducted in R statistical software version 3.6.0

### 6.3.1 Analysis of seasonality in larval habitat persistence

A descriptive analysis was performed to examine the seasonal dynamics of the aquatic habitats recruited for longitudinal surveillance, focusing on the proportion of habitats that remained wet and available for mosquito oviposition throughout the year. This was visualized by plotting the monthly proportion of wet habitats for each type in both villages.

### 6.3.2 Larval habitat use and its association with seasonality

Generalized Additive Mixed Models (GAMMs) were used to model seasonal variation in the presence and abundance of *An. funestus* larvae in different aquatic habitat types (Table 6.1). This was done by including the main effects for habitat type and a continuous temporal spline term defined by month of collection; whereby each collection month was assigned a numerical value ranging from 1 (January), to 12 (December). The GAMM structure accommodates non-linear variation in the temporal spline, allowing for multiple seasonal peaks and trough. An interaction between habitat type and the temporal spline term (Month) was fit to test whether seasonal trends in *An. funestus* larvae differed between habitat types. 'Village' was also included as a fixed effect to account for site differences, with a random effect also fit for habitat ID to capture unexplained within-habitat type variation. These models were fitted using the *gam* function from the *mgcv* package, which is particularly suited for data that does not exhibit a linear relationship with predictors [373]. This non-linear approach was necessary given the data covered a year-long survey period, marked by seasonal expansions, and declines in habitat availability. The smoothness of the splines in the GAMMs was optimized using the default generalized cross-validation criterion in the *mgcv* package. A binomial likelihood was used for modelling the presence of *An. funestus* larvae, while a *Poisson* distribution was applied for modelling their abundance. Forward stepwise selection added variables incrementally, comparing models with and without each variable using Likelihood Ratio Tests (LRT) to assess significance (Table 6.1).

### 6.3.3 House types used by adult mosquitoes and association with seasonality

A preliminary descriptive analysis was performed to visualize the distribution of adult mosquitoes across the 4 different house types in two villages. Here the proportion of *An. funestus* mosquitoes collected in different house types was visualized in *ggplots*.  Next, a GAMM model with a *Poisson* distribution was constructed to investigate seasonal trends in *An. funestus* adult abundance inside houses and whether it varied between different house types. This model included four explanatory variables: house type, a temporal spline defined by normalized month (nMonth), the interaction between nDate and house type, and village. The response variable was the total count of *An. funestus* females collected (Table 6.1). nMonth ranged from 1 (January) to 12 (December), assigning the same value to collections made on the same day in different years. Household ID and trap types were included as a random effect. The smoothness of the splines in the GAMM was optimized by the default generalized cross-validation method in the *mgcv* package. Model selection used backward selection, retaining variables based on their statistical significance and contribution to explaining variance in mosquito density, as indicated by changes in deviance. All the visualization in these models were done using *ggplots* [374].

**Table 6.1:** Summary of Statistical Models (Generalized Additive Mixed Models) used to analyse larval and adult mosquito densities.

| Aims | Model | Response variables | Fixed effect variables | Random effect variables | Statistical distribution |
|---|---|---|---|---|---|
| **1. Larval habitat uses and its association with seasonality** | 1.1 | Larval presence | Habitat type, month, village, month: habitat type | Habitat id | Binomial distribution |
| | 1.2 | Larval density | Habitat type, village, nMonth, nMonth: habitat type | Habitat id | Poisson distribution |
| **2. Adult habitat uses and its association with seasonality** | 2.1 | Adult density | House type, village, nMonth, and nMonth: house type | Household id + Trap type | Poisson distribution |

“**:**” indicates an “interaction, Smooth spline term used for aquatic model was nMonth, and the Smooth spline term used for adult was Day

## 6.4 Results

### 6.4.1 Densities of mosquito larvae

Over the study period, a total of 111,446 mosquito larvae and pupae were collected, with the majority being early instars (stage I and II, Table 6.2). More than half of all larvae and pupae were *Culex* species, followed by *An. gambiae* and *An. funestus* and *Anopheles* species (Table 6.2).

### 6.4.2 Densities of adult mosquitoes

A total of 35,590 adult mosquitoes were collected from 144 sampling events at 72 houses during this study. The majority of these were *Culex* species (~70%); with the malaria vectors *Anopheles gambiae* s.l and *An. funestus* s.l constituting 5.6% and 22.8% respectively (Table 6.3). Male mosquitoes comprised 15.2% of the total (Table 6.3). Of the 30,163 females collected, 2.9% were blood-fed, ~1% were gravid, and 0.6% were partly fed. Other mosquitoes detected included *Anopheles coustani*, *Coquillettidia* species, *Mansonia* species, and *Aedes* species, although they were rare (<1% of total in all cases).

CDC Traps caught the highest number, with 6,648 (65.5%) *An. funestus* s.l. and 2,658 (75.2%) *An. gambiae*, totalling 9,306 mosquitoes. Prokopack aspirators captured 823 (8.1%) *An. funestus* s.l. and 108 (3.1%) *An. gambiae* Tent Traps collected 994 (9.8%) *An. funestus* s.l. and 769 (21.7%) *An. gambiae*, amounting to 1,763 mosquitoes. In summary, the CDC Traps were the most effective, followed by Tent Traps, and then Prokopack Aspirators.

**Table 6.2:** Distribution of Mosquito Larvae and Pupae by Species Stage

| Species | Early instars (I and II), n (%) | Late instars (III and IV), n (%) | Pupae, n (%) | Total, n (%) |
|---|---|---|---|---|
| *Anopheles funestus* | 13,977 (18.8%) | 5,493 (17.0%) | 614 (13.0%) | 20,084 (18.0%) |
| *Anopheles gambiae s.l* | 19,056 (25.6%) | 4,708 (14.6%) | 275 (5.8%) | 24,039 (21.6%) |
| Other *Anopheles* | 2,648 (3.6%) | 6,578 (20.4%) | 2,934 (62.2%) | 12,160 (10.9%) |
| *Culex* Species | 38,824 (52.1%) | 15,444 (47.9%) | 895 (19.0%) | 55,163 (49.5%) |
| **Total** | 74,505 (100%) | 32,223 (100%) | 4,718 (100%) | 111,446 (100%) |

**Table 6.3:** Distribution of adult mosquitoes collected by Species and their physiological state

| Species | Blood Fed, n (%) | Gravid, n (%) | Unfed, n (%) | Partly Fed, n (%) | Male, n (%) | Totals, n (%) |
|---|---|---|---|---|---|---|
| *Anopheles funestus* | 340 | 139 | 7,299 | 36 | 285 | 8,099 (22.8) |
| *Anopheles gambiae s.l* | 107 | 34 | 1,818 | 13 | 29 | 1,962 (5.5) |
| *Anopheles coustani* | 2 | 3 | 61 | 0 | 38 | 104 (0.3) |
| *Coquillettidia* sp | 1 | 3 | 29 | 3 | 12 | 48 (0.1) |
| *Mansonia* sp | 12 | 10 | 332 | 8 | 38 | 400 (1.1) |
| *Culex* sp | 398 | 102 | 19,100 | 124 | 5,020 | 24,744 (69.6) |
| *Aedes* | 9 | 0 | 175 | 5 | 5 | 194 (0.5) |
| **Grand Total** | 869 (2.4) | 291 (0.8) | 28,814 (81) | 189 (0.5) | 5,427 (15.2) | 35,590 (100) |

### 6.4.3 Permanency of larval habitats

A total of 67 aquatic habitats in Ikungua and 114 in Chikuti were monitored monthly over the course of a year. Of these, 120 habitats remained wet for at least six months throughout the year, 38 were semi-permanent, staying wet for 3-6 months, and 23 were temporary, staying wet for less than three months (Table 6.4). These habitats included river streams (n = 64, 35%), ground pools (n = 14, 8%), ditches (n = 43, 24%), dug pits and spring-fed wells (n = 49, 27%), rice fields (n = 6, 3%), and puddles (n = 5, 3%). For Ikungua, the majority of ground pools were categorized as permanent, with a duration ranging from six to twelve months. River streams generally fell into the semi-permanent or permanent category. Other habitats, such as dug pits, ditches, rice fields, and puddles, were typically temporary, disappearing shortly after the rainy season ceased (Figure 6.4), Overall Chikuti village had higher ratio of permanent habitats (than Ikungua), particularly in the case of dug pits and spring-fed wells. However, ground pools in Chikuti were predominantly temporary.

Aquatic habitat availability varied seasonally and by habitat type across villages. In Ikungua and Chikuti, permanent habitats like river streams and ground pools remained wet throughout the year, while temporary habitats such as puddles and tire tracks exhibited significant seasonal fluctuations, becoming wet during the rainy season and drying out in the dry months (Figure 6.4).

**Table 6.4:** Number and percentage of aquatic habitats that were classified as permanent (wet > 6 month/year), semi-permanent (wet 3-6 months/year) or temporary (wet < 3 months/year) in the study area.

| | **Habitat types** | **Permanent, n (%)** | **Semi-permanent, n (%)** | **Temporary, n (%)** | **Total, n (%)** |
|---|---|---|---|---|---|
| **Ikungua village** | River streams | 30 (93.8%) | 2 (6.3%) | 0 (0%) | 32 (100%) |
| | Ground pools | 10 (100%) | 0 (0%) | 0 (0%) | 10 (100%) |
| | Dug pits & Spring fed wells | 3 (75%) | 1 (25%) | 0 (0%) | 4 (100%) |
| | Ditch | 13 (86.7%) | 2 (13.3%) | 0 (0%) | 15 (100%) |
| | Rice fields | 0 (0%) | 3 (75%) | 1 (25%) | 4 (100%) |
| | Puddle & Tire truck | 0 (0%) | 1 (50%) | 1 (50%) | 2 (100%) |
| | Total | 56 (83.6%) | 9 (13.4 %) | 2 (3 %) | 67 (100%) |
| | | | | | |
| **Chikuti village** | River streams | 28 (87.5%) | 2 (6.3%) | 2 (6.3%) | 32 (100%) |
| | Ground pools | 0 (0%) | 2 (50%) | 2 (50%) | 4 (100%) |
| | Dug pits & Spring fed wells | 24 (53.3%) | 14 (31.1%) | 7 (15.6%) | 45 (100%) |
| | Ditch | 12 (42.9%) | 10 (35.7%) | 6 (21.4%) | 28 (100%) |
| | Rice fields | 0 (0%) | 1 (50%) | 1 (50%) | 2 (100%) |
| | Puddle & Tire truck | 0 (0%) | 0 (0%) | 3 (100%) | 3 (100%) |
| | Total | 64 (56.1%) | 29 (25.4%) | 21 (18.4%) | 114 (100%) |
| | Grand total | 120 (66.3%) | 38 (20.9%) | 23 (12.7%) | 181 (100%) |

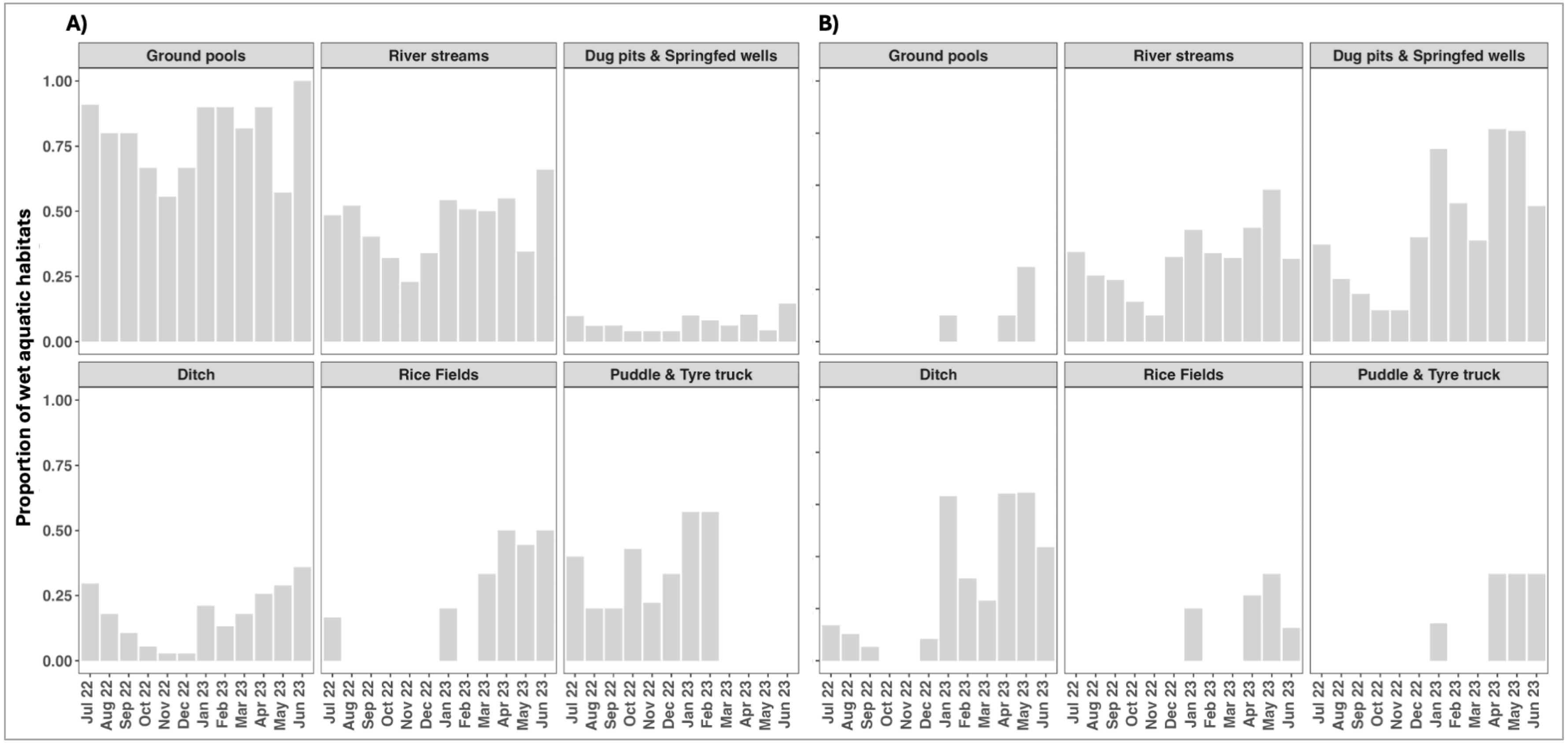

**Figure 6.4:** Seasonal dynamics of aquatic habitat availability in A) Ikungua and B) Chikuti villages. Each habitat type is represented by a bar showing the proportion of habitats that stay wet each month. NB: All habitats were wet when recruited into the study in Feb 2022, with values here showing their status when surveyed again at the start of the longitudinal surveillance in July 2022

### 6.4.4 Seasonal patterns in the use of aquatic habitats use by *An. funestus*

In Ikungua, *An. funestus* larvae were found in various habitats, with river streams and ground pools being consistently occupied year-round, while rice fields were utilized primarily from April to July, with peak in April (Fig. 6.5A). In Chikuti, *An. funestus* larvae showed a uniform pattern of habitat use throughout the year, with river streams being the most consistently used, and a notable increase in the use of ground pools and rice fields in May (Fig. 6.5B).

The presence and abundance of *An. funestus* larvae in aquatic habitats were significantly influenced by seasonality, habitat type, and village (Table 6.5). The model for larval presence explained approximately 35.2% of the variance, while the model for larval abundance explained 37.9% of the variance, both indicating moderate predictive power. Larval occurrence was relatively high throughout the year, with a small dip in February-March, a rise in April, and a decline until October-November, followed by an increase until January (Figure 6.6A). This contrasted with larval abundance, which peaked in June-July before declining towards the end of the year. The pattern of seasonality varied by habitat type, reflected by a significant interaction between habitat type and seasonality ($p = 0.003$, Table 6.6). Permanent habitats, such as river streams and ground pools, maintained stable and high predicted probabilities of larval presence year-round, whereas temporary habitats, like puddles, experienced significant seasonal dips due to lower stability (Figure 6.6B).

Mean larval abundance was also significantly associated with an interaction between habitat types and seasonality (Figure 6.6C). Dug pits and spring-fed wells had high larval abundance in June-July, while ditches peaked in July and August (Figure 6.6D). Rice fields peaked in July, whereas ground pools and river streams peaked in late August. Some habitats exhibited two smaller peaks around February and May; February peaks were noticeable in ground pools and puddles/tire tracks, while May peaks were evident in river streams and dug pits/spring-fed wells (Figure 6.6D).

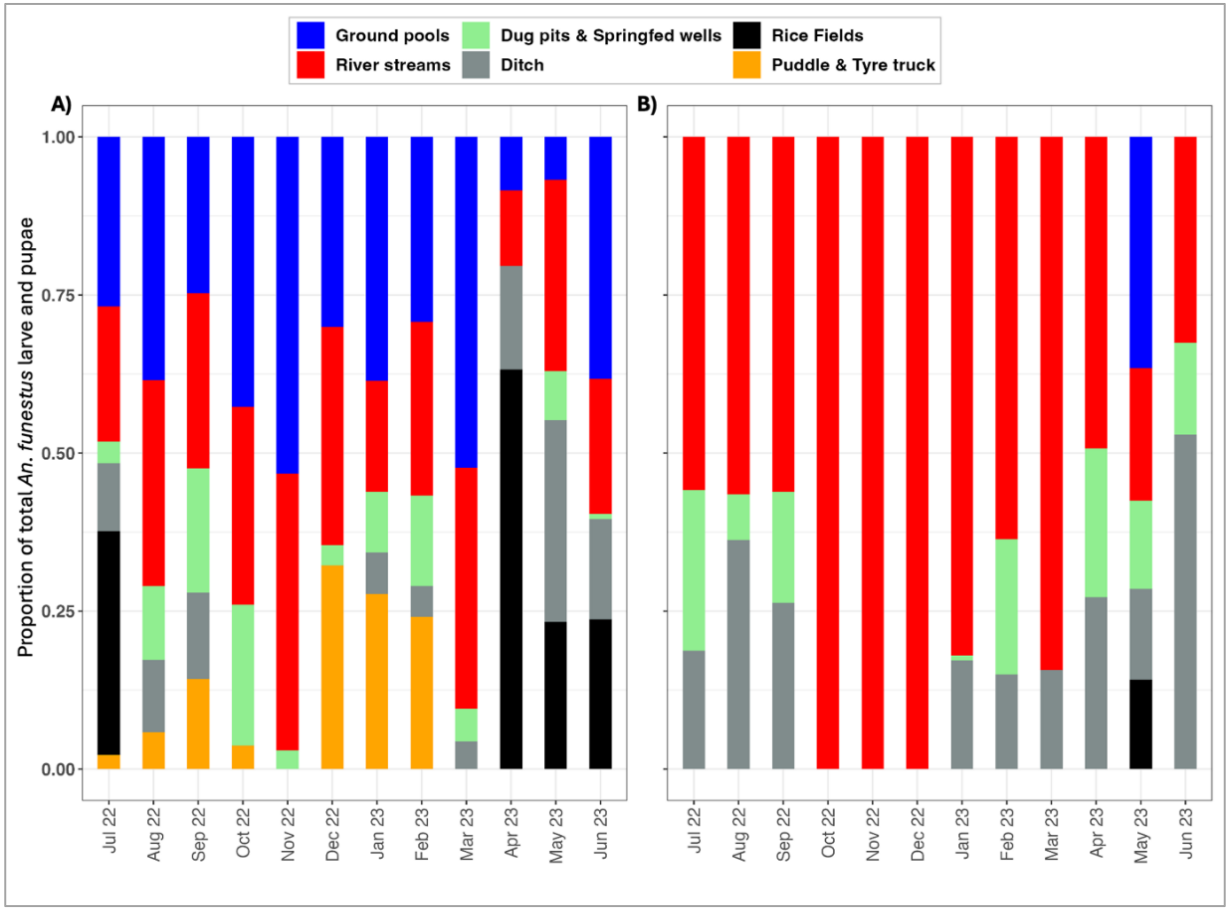

**Figure 6.5:** Distribution of *An. funestus* larvae in different aquatic habitat types across the year in in 2 villages of the Kilombero Valley A) Ikungua, and B) Chikuti

**Table 6.5:** Deviance change of the parameters included in the full GAMM models for assessing the association between the habitat type the larval presence and abundance.

| | Generalized Additive Mixed Model: response variable, *An. funestus* larval presence | | | Generalized Additive Mixed Model: response variable, *An. funestus* larval density | | |
|---|---|---|---|---|---|---|
| **Variables** | **Deviance** | **Degrees of Freedom, df** | **Pr(>Chi)** | **Deviance** | **Degrees of Freedom, df** | **Pr(>Chi)** |
| 1. Habitat type | - | - | - | - | - | - |
| 2. nMonths | 597.64 | 8.91 | <0.0001*** | 9880.2 | 8.99 | <0.0001*** |
| 3. Village | 160.66 | 1.02 | <0.0001*** | 379.5 | 0.99 | <0.0001*** |
| 4. Habitat type*Months | 32.97 | 10.14 | 0.0003** | 2444.4 | 21.98 | <0.0001*** |

**Table 6.6:** Factors affecting presence and abundance of *An. funestus* in aquatic habitats

| | | Larval presence | | Larval abundance | |
|---|---|---|---|---|---|
| **Factor** | **Category** | **Estimate (ß)** | **p-value** | **Estimate (ß)** | **p-value** |
| **Intercept** | | 0.68 | 0.04* | 2.64 | < 0.001 *** |
| **Habitat Type** | Ground pools | ref | | ref | |
| | River streams | 0.24 | 0.477 | -0.14 | < 0.001 *** |
| | Ditch | -1.51 | < 0.001 *** | -1.20 | < 0.001 *** |
| | Dug pits & Spring-fed wells | -0.75 | 0.03* | -1.48 | < 0.001 *** |
| | Rice Fields | -3.55 | < 0.001 *** | -568.27 | 0.24 |
| | Puddle & Tire truck | -1.98 | < 0.001 *** | -1.12 | < 0.001 *** |
| **Village** | Chikuti | ref | | ref | < 0.001 *** |
| | Ikungua | 1.70 | < 0.001 *** | 0.31 | < 0.001 *** |
| **Smooth spline term (nMonth)** | | **edf** | **p-value** | **edf** | **p-value** |
| **nMonth** | | 8.46 | < 0.001 *** | 8.83 | < 0.001 *** |
| **Habitat Type*nMonth** | Ground pools | 0.01 | 0.50 | 3.81 | < 0.001 *** |
| | River streams | 1.00 | 0.55 | 3.83 | < 0.001 *** |
| | Ditch | 1.03 | 0.006 ** | 3.84 | < 0.001 *** |
| | Dug pits & Spring fed wells | 2.22 | 0.02 * | 3.86 | < 0.001 *** |

| | | | | | |
|---|---|---|---|---|---|
| | Rice Fields | 1.85 | 0.46 | 3.84 | < 0.0001 *** |
| | Puddle & Tire truck | 2.67 | 0.29 | 2.83 | < 0.0001 *** |

*Summary of the estimate (β) and p-value of explanatory variables in the final Generalized additive mixed model using the presence and mean number of An. funestus collected (as response) at each collection date over six habitat types. nMonth is a discrete variable representing the longer-term starting from January to December. The term "edf" stands for "effective degrees of freedom." This is a measure of the complexity of the smooth term in the model. Ground pools were selected as the reference category for the fixed effects, as they were the most productive and stable habitat across the study area, serving as the primary breeding site for An. funestus.*

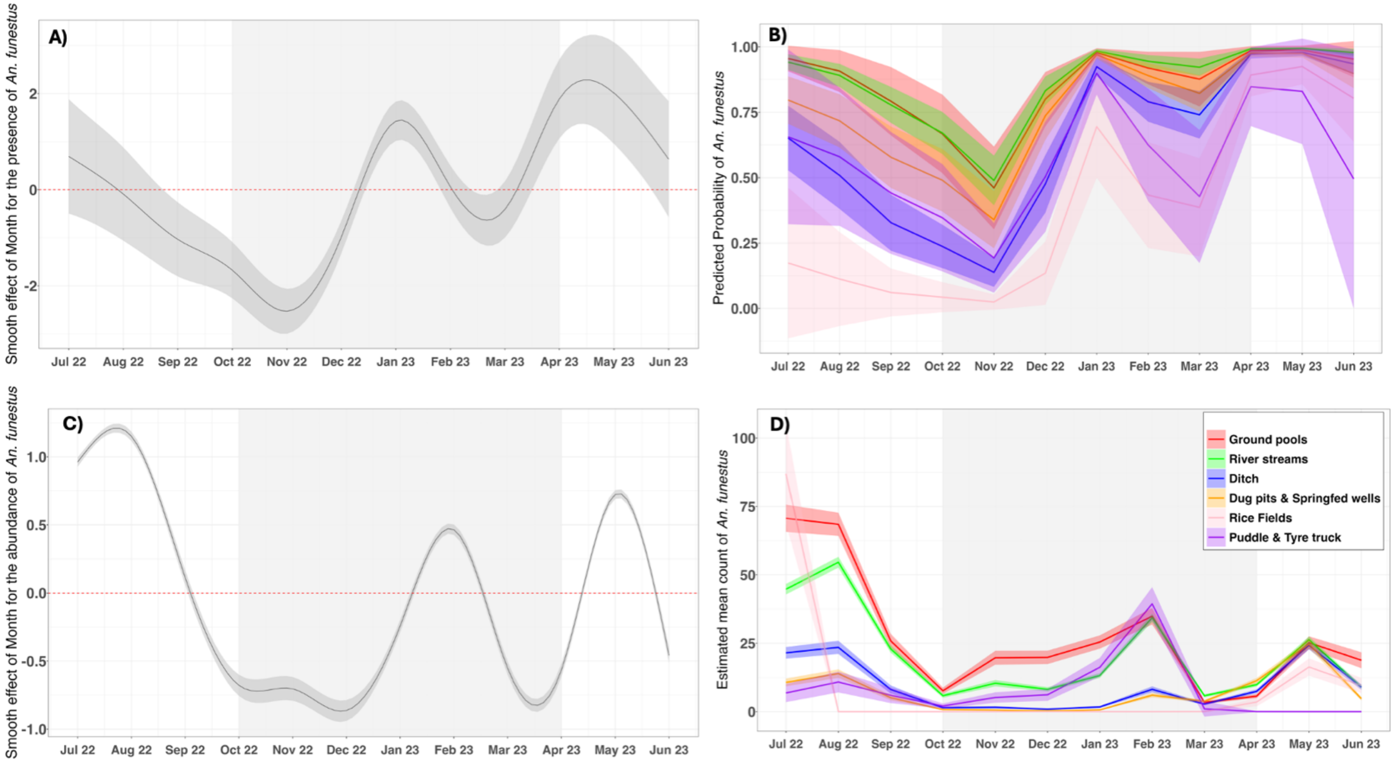

**Figure 6.6:** Panels A and C illustrate seasonal variation in the presence and abundance of *An. funestus* using cyclic cubic splines, predicted by a Generalized Additive Model (GAMM). Panels B and D show predictions of *An. funestus* presence and abundance across different habitat types, with colour codes indicating various aquatic habitats as detailed in the legend. The month numbers correspond to months of the year, with the wet season shaded grey from November (11) to April (4).

### 6.4.5 Mosquito abundance in different house types

A total of 7,132 female *An. funestus* mosquitoes collected, with 5,740 collected in Ikungua and 1,392 in Chikuti. In Ikungua, just over half of *An. funestus* females came from one house type (thatched roofs and mud walls). In contrast, *An. funestus* were split more evenly between the four house categories considered in Chikuti, with the greatest number collected in brick walled, and thatched roofed houses (34.6%. Table 6.7). However, in both villages, the majority of *An. funestus* were from thatched roofed houses (mud or brick-walled) rather than iron-roofed houses (plastered or unplastered).

**Table 6.7:** Female *An. funestus* collected from various house types.

| Village | House types | n | (%) |
|---|---|---|---|
| **Ikungua** | Thatched roofs and mud-walls | 2,932 | 51.1 |
| | Thatched roofs and brick-walls | 923 | 16.1 |
| | Metal roofs and unplastered mud or brick walls | 1,119 | 19.5 |
| | Metal roofs and plastered mud or brick walls | 766 | 13.3 |
| | Total | 5,740 | 100 |
| **Chikuti** | Thatched roofs and mud-walls | 430 | 30.9 |
| | Thatched roofs and brick-walls | 481 | 34.6 |
| | Metal roofs and unplastered mud or brick walls | 189 | 13.6 |
| | Metal roofs and plastered mud or brick walls | 292 | 21.0 |
| | Total | 1,392 | 100 |

### 6.4.6 Seasonal distribution of adult *An. funestus* across house type

Monthly analyses from January to December revealed distinct seasonal fluctuations in adult *An. funestus* populations inside houses. In Ikungua, the distribution of *An. funestus* adults between house types was relatively consistent across the year, with the highest proportion being found in mud thatched houses (35-65% per month), and a reasonably even split (with some month-to-month) variation between the remaining 3 house types (Figure 6.7). In contrast, unplastered thatched houses accounted had the highest proportion of *An. funestus* in most months in Chikuti, with the exception of February and April. Particularly high proportions of *An. funestus* were observed in March for mud thatch house types in both villages.

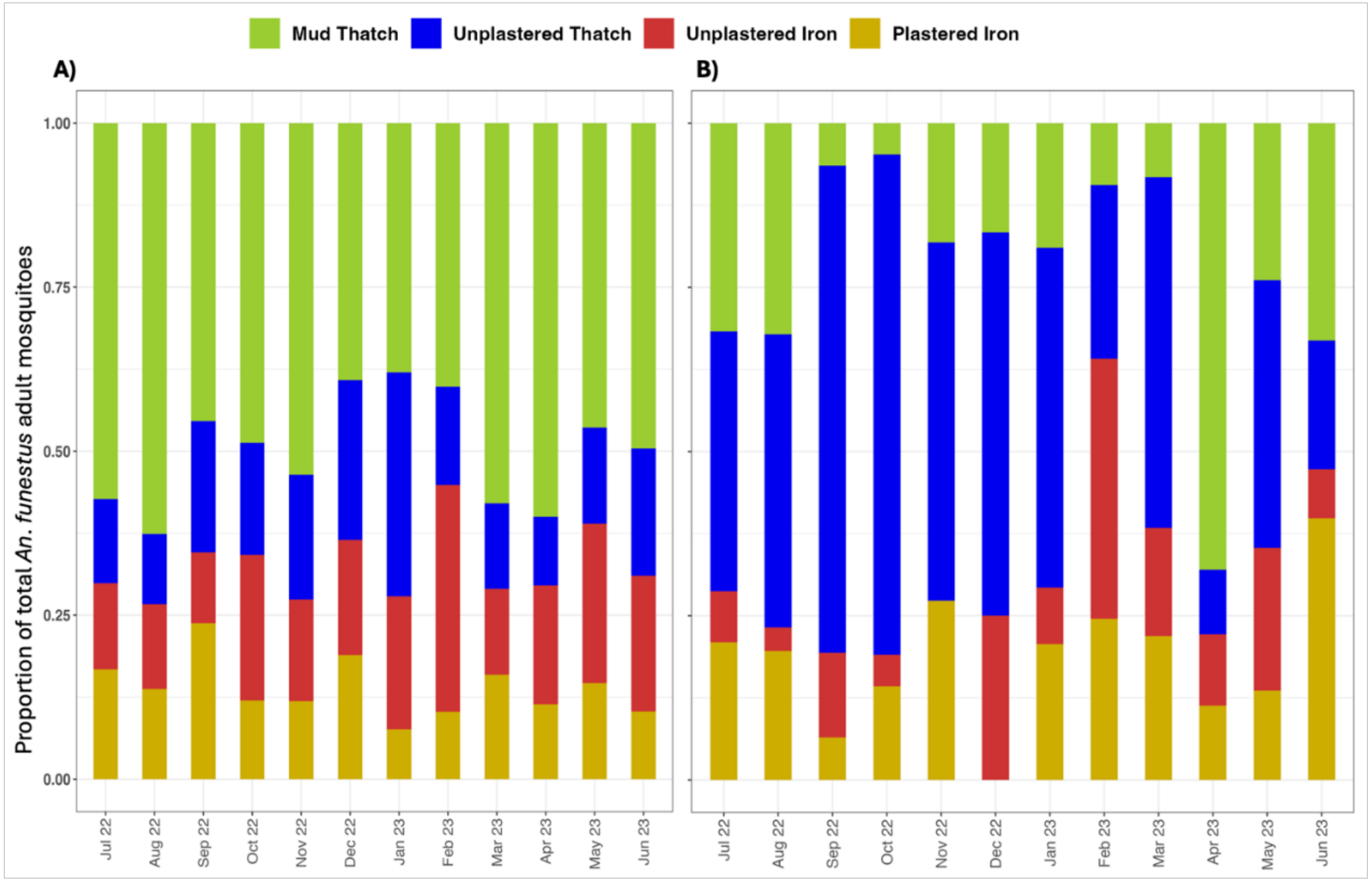

**Figure 6.7:** Seasonal variation of *An. funestus* density in different house type a) Ikungua village, B) Chikuti village.

### 6.4.7 Seasonality in adult densities in different house types

All initially tested variables were retained as statistically significant in the final generalized additive mixed model (GAMM) of *An. funestus* abundance in houses, including the seasonal spline term (nMonth), house type, the interaction of the seasonal spline term and house type, and village (Figure 6.8 A, Table 6.8). The model explained approximately 38.5% of the variance, as indicated by the R-squared value, reflecting a moderate level of predictive power.

In all house types, *An. funestus* abundance varied significantly throughout the year, with peaks generally occurring in the dry season, approximately 1 month after the end of the rainy season (Figure 6.8A). There were statistically significant differences in seasonal trends between house types (Figure 6.8B). Thatched roofed houses with mud walls had the most pronounced seasonal variation, with significant fluctuations in mosquito densities throughout the year (edf = 3.98, $p < 0.001$), showing multiple peaks, particularly following the rainy season. Unplastered iron-roofed houses also demonstrated considerable seasonal variation (edf = 2.26, $p = 0.002$), with peaks and troughs aligning with seasonal changes, but less pronounced than in mud thatch houses. In contrast, brick thatch houses (edf = 2.05, $p = 0.096$) and plastered iron houses (edf = 1.46, $p = 0.06$) exhibited less pronounced seasonal trends, with fewer and less distinct peaks. The timing of these peaks and troughs varied slightly between house types, indicating an interaction between house types and seasonal changes (Table 6.9).

**Table 6.8:** Showing parameters included in the full models

| | Generalized Additive Model: response variable, adult density *An. funestus* | | |
|---|---|---|---|
| Variables | Deviance | Degrees of Freedom, df | Pr(>Chi) |
| 1. House type | 261.7 | 1.76 | <0.0001 *** |
| 2. Village | 2755.1 | 1.41 | <0.0001 *** |
| 3. nDate | 1660.7 | 3.75 | <0.0001 *** |
| 4. nDate: House type | 137.41 | 10.75 | <0.0001 *** |

* *indicates significant term retained in the final model with* $p < 0.05$ *and deviance here measures the goodness-of-fit. When comparing two models, the deviance is computed as the deviance of the simpler model minus the deviance of the more complex model.*

**Table 6.9:** Statistical summary of final GAMM for adult density of *An. funestus* as the response, using month as smoothed term

| Factor | Category | Estimate (β) | p-value |
|---|---|---|---|
| **Intercept** | | 0.68 | < 0.0001 *** |
| **House Type** | Thatched roofs and brick-walls | ref | |
| | Thatched roofs and mud-walls | 0.36 | < 0.0001 *** |
| | Metal roofs and plastered mud or brick walls | -0.22 | < 0.0001 *** |
| | Metal roofs and unplastered mud or brick walls | -0.20 | < 0.0001 *** |
| **Village** | Chikuti | ref | |
| | Ikungua | 1.53 | < 0.0001 *** |
| **Smooth spline term (nMonth)** | | **edf** | **p-value** |
| **nMonth** | | 8.97 | < 0.0001 *** |
| **House Type*nMonth** | Thatched roofs and brick-walls | 2.05 | 0.09 |
| | Thatched roofs and mud-walls | 3.98 | < 0.0001 *** |
| | Metal roofs and plastered mud or brick walls | 1.46 | 0.06 |
| | Metal roofs and unplastered mud or brick walls | 2.25 | 0.002 ** |

*Edf is an effective degree of freedom which measure the complexity of the smooth term in the model. A higher edf indicates a more flexible model fit to the data, allowing for more complex shapes to adapt to variations in the data.*

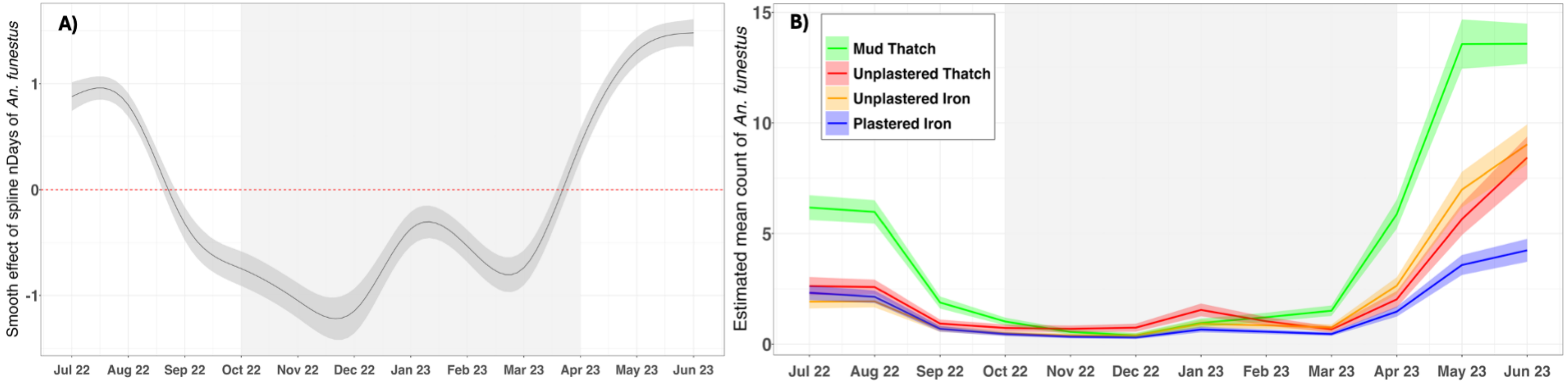

**Figure 6.8:** Seasonal trends in *Anopheles funestus* abundance from July 2022 to June 2023. (A) Smooth effect of time (months) on *An. funestus* abundance, represented by a spline curve with 95% confidence intervals (shaded area). The y-axis represents the smooth effect size, with positive values indicating higher abundance relative to the baseline. (B) Estimated mean count of *An. funestus* by house types. The shaded regions indicate 95% confidence intervals around each line. Shaded gray regions in both plots represent the rainy season, highlighting seasonal influence on *An. funestus* population dynamics.

## 6.5 Discussion

This study investigated the seasonal variation in the habitat use of larval and adult *An. funestus*. As expected, both larval and adult dynamics exhibited strong seasonality, with larval prevalence in aquatic habitats increasing during the end of wet season (January - February) and mean abundance of larvae peaking in dry season (June - July), while adults peaked in mid-May to June following the peak rainfall. While this study focused on specific habitat and house types to provide actionable insights, it is important to note that rainfall and temperature, although not included in the analysis, are known to significantly influence mosquito dynamics [27,29]. Rainfall can create breeding habitats, while temperature affects mosquito development and survival. These factors likely contribute to the seasonal patterns observed and should be considered in future studies for a more comprehensive understanding [375]. The study highlighted significant interactions between seasonality and habitat type and house type, suggesting that habitat-specific seasonality plays a crucial role in the population dynamics of *An. funestus*. Understanding these interactions is important for optimizing mosquito surveillance and control strategies, as it can inform the National Malaria Control Programs (NMCP) the timing and targeting of interventions such as larval source management (LSM), to correspond with periods of high larval density in specific habitats and targeting indoor interventions like insecticide-treated nets (ITNs) or indoor residual spraying (IRS) in houses that exhibit higher mosquito densities.

The results showed that in Ikungua, populations of mosquito larvae in permanent habitats, such as river streams and ground pools, were relatively stable across the year. In contrast, Chikuti exhibited more pronounced seasonal fluctuations in mosquito larvae populations, particularly in temporary habitats. Additionally, higher adult mosquito densities were found in mud thatch houses, which exhibited a strong pattern of seasonality with two distinct peaks, while other house types showed less variability and generally lower mosquito densities. These insights are critical for designing targeted interventions, such as improving house structures to reduce mosquito entry and survival.

Seasonal variation in *An. funestus* larval habitat use can be largely explained by differences in aquatic habitat availability and permanency across the year and between villages. In Chikuti, *An. funestus* larvae were consistently found in river streams, followed by ditches. Conversely, larval habitat use was more variable in Ikungua village, where temporary habitats like dug pits and ditches exhibited more pronounced seasonal variability. These findings align with previous research that highlighted the significant influence of habitat stability on mosquito population dynamics [22,243,312,367]. I hypothesize that local variations in habitat stability across the year are driving these differences. For example, in Chikuti village, the predominance of temporary habitats like ground pools and rice fields means that habitat availability fluctuates more throughout the year compared to Ikungua, where ground pools provide a more stable environment. This suggests that general predictions about habitat stability cannot be generalized; rather they need to consider local environmental conditions.

The peak larval abundance observed in mid-year aligned with the start of the dry season, indicating that decreased water availability during this time concentrates the available aquatic habitats, making them more favourable for *An. funestus*. In the study area, the dry season typically spans from May to October. During this period, I identified two distinct peaks in larval abundance: a smaller peak in May and a larger peak from July to August. These peaks varied between habitat types; for instance, dug pits exhibited an earlier peak in larval abundance shortly after the rainfall compared to other habitats. The smaller peak in May can be attributed to the initial accumulation of water pools as the rains cease. The larger peak from July to August corresponds to the persistence of concentrated habitats, particularly permanent and semi-permanent ones like ground pools and river streams, which continue to provide suitable breeding conditions throughout the dry season. In contrast, river streams and ground pools maintain their suitability for a longer duration, supporting higher larval densities later into the dry season.

The sudden increase in mosquito abundance in rice fields in July corresponds to periods of rice cultivation when they are flooded, allowing for mosquito oviposition. These rice fields were available for a few months during and shortly after the rainy season, specifically in May and June, but were completely absent in the dry season.

Rice cultivation in this area relies exclusively on rainfall, and these fields are typically located in low-lying areas that flood during the rainy season. During the harvest period (typically dry season), which lasts from August to November, these fields are dry. Additionally, a small peak in larval abundance during the rainy season was attributed to the proliferation of temporary habitats like puddles and tire tracks. These findings highlight the importance of habitat stability and the timing of water availability in influencing larval abundance, emphasizing the need for targeted vector control strategies that consider these seasonal and habitat-specific dynamics.

Several factors may account for the observed variations in larval populations between habitats and villages. Environmental conditions, particularly rainfall and temperature, play a crucial role in determining the availability and suitability of aquatic habitats [93,376]. More permanent habitats were present in Ikungua due to the topography of the area which is in the lowlands where the soil allows the accumulation of rainwater, while at the higher altitude of Chikuti village, the natural flow of water in this area led to more drainage and temporary habitats [282,290]. These geographical differences likely drive the distinct seasonal patterns observed in the aquatic habitats' permanency, larval presence and abundance. Previous research by [272,325] also emphasized the importance of climatic factors in influencing mosquito populations.

Similar to larvae, there was notable seasonality in adult *An. funestus* densities across the year, with peaks observed in May. This timing aligns with the transition from the rainy to the dry season, suggesting that adult mosquito populations respond to the increased availability of aquatic habitats during the preceding wet months, while populations were significantly lower during the rainy season (October - May). The variation in adult densities across different house types shows the impact of household characteristics on mosquito ecology [377]. Mud thatch houses consistently showed higher mosquito densities, likely due to their structural features that provide favourable resting conditions and more entry points for mosquitoes. Notably, the pattern of seasonality varied between house types, with mud thatch houses exhibiting high density and pronounced seasonal fluctuations, including two clear seasonal peaks, while other house types experienced lower densities and limited fluctuations.

Many studies support these findings, indicating that poorly structured houses with thatch roofs and eave spaces offer conducive microclimatic conditions for mosquitoes, leading to higher indoor densities of *An. funestus* [369,377,378]. For example, houses with thatch roofs and mud walls create microclimates that are more favourable for mosquito survival compared to metal-roofed houses, which tend to be hotter and less hospitable [377]. This suggests that improving housing structures, such as sealing eaves and using materials that reduce indoor humidity and temperature, could significantly reduce indoor mosquito densities [378,379]. This aligns with findings from other studies that have shown lower mosquito densities in houses with improved structures, emphasizing the need for integrated vector management strategies that include housing improvements [377,378].

This study has significant implications for malaria vector control strategies, specifically in the timing and targeting of interventions such as LSM, ITN, and IRS. Our findings indicate that LSM efforts would be most effective during the dry season when larval habitats are fewer and more concentrated, particularly in permanent water bodies such as ground pools and river streams. This period offers a strategic opportunity to target these productive habitats and reduce larval populations before the onset of the rainy season [22,92]. In contrast, temporary habitats like puddles and tire tracks, which become prominent during the wet season, should also be monitored and managed during this time to prevent an increase in mosquito populations (Chapter 4).

For indoor interventions like IRS and ITNs, this study suggests prioritizing mud thatch houses, which consistently showed higher adult mosquito densities. Enhancing coverage of these interventions in these houses, especially during the peak adult density periods following the rainy season, can significantly reduce indoor mosquito populations [377]. Additionally, improving the structural of mud thatch houses to limit mosquito entry and provide less favourable resting conditions can complement these efforts (Kahamba 2022 et al). By aligning these interventions with the observed seasonal and habitat-specific dynamics, vector control programs can be more precisely targeted.

While this study has provided valuable insights into the use of aquatic and adult habitats of *An. funestus*, it is important to acknowledge several limitations that could influence the interpretation and application of the findings: Firstly, the reliance on a one-year observational period may not capture the full extent of inter-annual variations in environmental conditions and mosquito population dynamics. Long-term longitudinal studies are needed to better understand these patterns over multiple years. Secondly, the study did not collect data on socioeconomic activities that could influence mosquito densities and malaria transmission [325]. The observations indicated that some improved houses were near local bars and social gathering spots attracted adult mosquitoes, suggesting that human behaviour and community structure significantly influence mosquito populations and malaria risk [247]. In addition, the analysis of this study did not include rainfall and temperature variables, which are critical factors known to influence mosquito life cycles, breeding patterns, and malaria transmission. Finally, during larval surveillance, only the habitats identified during the initial visit were monitored throughout the study period duration. This means that any new habitats that emerged after the first survey were excluded from ongoing observations, even if they later harboured *An. funestus* larvae. Consequently, our follow-up efforts were confined to those positive *An. funestus* aquatic habitats that were initially recruited. This approach might restrict capturing the full dynamics of habitat utilization and population changes.

These limitations highlight the need for integrating other data such as socioeconomic factors, climatic variables like rainfall and temperature, by linking human behaviour, environmental conditions, and mosquito density to provide a more comprehensive understanding of malaria transmission dynamics. Future studies should also employ more comprehensive sampling methods. By expanding the scope of research to include all potential habitats whether they are positive or not throughout the study period, a more complete understanding of mosquito breeding dynamics can be obtained.

## 6.6 Conclusion

This study provides significant insights into the seasonal dynamics and habitats used by *Anopheles funestus* in southeastern Tanzania. The distinct seasonal peaks in larval abundance during the dry season (July and August) and in adult densities in May underscore the influence of rainfall and temperature on breeding activities and survival conditions. Permanent water bodies in Ikungua supported more stable mosquito populations, while temporary habitats in Chikuti showed more pronounced seasonal fluctuations. Higher adult mosquito densities in mud thatch houses highlight the importance of household structure in creating favourable resting environments. Importantly, this study shows that the pattern of seasonality in *An. funestus* populations varies between aquatic habitat and house types, likely due to differences in habitat permanency and microclimatic conditions. These findings highlight the need for targeted malaria control strategies that consider these seasonal and habitat-specific dynamics. Interventions such as LSM should be intensified during the dry season for permanent habitats, while IRS and ITNs should focus on mud thatch houses during peak adult density periods.

# Chapter 7: Societal Uses of the Main Water Bodies Inhabited by Malaria Vectors and Implications for Larval Source Management

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## Abstract

**Introduction**: Larval source management (LSM) can effectively suppress mosquito populations at source and provides an opportunity to address major challenges such as insecticide resistance that undermine primary interventions like insecticide-treated nets (ITNs). While mostly implemented in urban and arid settings, emerging research indicates its potential in some rural settings in east and southern Africa, where the main malaria vector, *Anopheles funestus*, prefers permanent and semi-permanent water bodies that support year-round transmission. Targeting these unique habitats could amplify the effectiveness of LSM but requires careful consideration of local societal practices and expectations - particularly since mosquito breeding sites often also serve as community water resources. The aim of this study was to explore how the societal uses of aquatic habitats by local communities in rural south-eastern Tanzania might influence LSM strategies, focusing on habitats used by *An. funestus*.

**Methods**: This study was conducted in three villages in the Ulanga and Malinyi districts of southeastern Tanzania using a mixed-methods approach. Quantitative data were collected through a cross-sectional surveillance of all aquatic habitats, while qualitative data were gathered via a combination of individual unstructured interviews, focus group discussions with various community group and field observations of community practices and activities. Data analysis employed weaving and inferencing techniques to integrate findings from both quantitative and qualitative components, thereby developing a comprehensive understanding from the respondents' perspectives.

**Results:** A survey of 931 aquatic habitats revealed that 73% contained mosquito larvae, with late instar *An. funestus* identified in 23%. River streams segments were the most common habitat type, accounting for 41% followed by ground pools (4%); other types included pits, rice fields, ditches, and puddles. Ninety percent of aquatic habitats were used by communities, including 95% of those with *An. funestus* larvae, for activities such as cooking, washing utensils, washing clothes and bathing, agriculture, livestock rearing, brickmaking, and fishing. Focus group discussions indicated community readiness to implement LSM, favoring larviciding and habitat manipulation over habitat removal. Community concerns regarding LSM centred on the safety of larvicides for animal and human health and their environmental impact. The discussions proved the need for LSM interventions to integrate seamlessly with daily activities; and for community education on LSM safety and efficacy.

**Conclusion**: This study offers valuable insights into community perspectives on LSM for malaria control in rural settings, emphasizing the dual role of aquatic habitats as both mosquito breeding sites and community water sources. This presents a set of unique challenges and opportunities - suggesting that LSM strategies must address both the biological aspects of mosquito control and the socio-economic realities of local communities. Notably, there was a marked preference for larviciding and habitat manipulation over habitat removal, with a strong emphasis on health and environmental safety. Overall, the study highlights the critical importance of educating communities, adopting culturally sensitive approaches to LSM, and aligning LSM strategies with the needs, perspectives, and daily lives of local communities.

## 7.1 Introduction

Over the past two decades, significant progress has been made in the fight against malaria, primarily due to large-scale deployment of preventative and therapeutic measures [235,237,380]. Vector control strategies, notably insecticide-treated nets (ITNs) and indoor residual spraying (IRS), have been at the forefront; accounting for over 70% of the progress achieved [380]. Despite these advancements, malaria remains a public health concern in sub-Saharan Africa, with some areas seeing unchanged or increasing case numbers [235]. Among other challenges, malaria control efforts are being complicated by the rise of drug-resistant parasites, the spread of insecticide resistance in mosquitoes, and mosquito behaviour adaptations that reduce the effectiveness of existing controls [341,381,382]. In response to these ongoing challenges, the World Health Organization (WHO) recommends larval source management (LSM) as a supplementary intervention in malaria-endemic countries across Africa [383]. This approach is increasingly recognized for its potential against malaria, though there are still multiple uncertainties and conflicting statements about its viability [72].

*Anopheles funestus*, one of the most efficient malaria vectors, has contributed significantly to the persistence of malaria due to its adaptability and widespread presence [109,238,304]. Understanding the mosquito life cycle is crucial for appreciating the relevance of LSM. The mosquito life cycle includes four stages: egg, larva, pupa, and adult, with the first three stages being aquatic [27,29]. LSM disrupts the mosquito lifecycle through three primary approaches: i) habitat modification, which involves the complete removal of breeding sites, for example, filling the breeding habitat with sand or constructing structures to eliminate it entirely; ii) habitat manipulation, involving routine activities to make environments less conducive to mosquito breeding, for example flushing streams, removing vegetation and debris, and exposing habitats to the sun; and iii) larviciding, the application of biological or chemical insecticides to water to halt larval development [279].

By targeting the mosquito populations at its source, LSM can be particularly relevant for overcoming challenges such as insecticide resistance that diminish the efficacy of conventional vector control measures like insecticide-treated nets (ITNs). Additionally, the strategic use of LSM offers a way to manage mosquito populations effectively, without solely relying on chemical interventions [72]. Indeed, microbial larvicides, like *Bacillus thuringiensis israelensis (bti)* and *Bacillus sphaericus (bs),* have been effective and can overcome problems like insecticide resistance and environmental damage often associated with other chemical treatments [102,384]. At its core, the approach reduces mosquito populations and, as a result, can effectively suppress malaria transmission [72,102,385], and reduced incidence of malaria [102,386].

While LSM holds significant promise in the fight against malaria, its adoption by global funding bodies has encountered several obstacles. For example, the World Health Organization (WHO) recommends LSM for areas where suitable mosquito breeding sites are few, fixed and findable (FFF) [235,237]. Because of these guidelines, LSM is currently mostly implemented in urban and arid settings. However, in many malaria-endemic regions, these larval habitats are abundant, widespread, and often located in areas that are difficult to access, making the implementation of LSM strategies difficult. Additionally, larviciding, one of the key components of LSM, is often costly and labour-intensive [186,387]. Another challenge facing larviciding is the diversity nature of malaria vectors and their unique aquatic habitat usage, making it difficult to address all vectors simultaneously and effectively with this approach [22,243].

In southeastern Tanzania and other regions of the country, *An. funestus* has emerged as a major vector in malaria transmission, accounting for about 90% of the overall entomological inoculation rate (EIR) [86,88]. This trend is also seen across other parts of east and southern Africa, where the species contributes to the majority of ongoing transmission [238]. Given the unique traits of *An. funestus*, such as its breeding in fixed permanent and semi-water bodies, which persist into dry months and can help sustain year-round malaria transmission [22], LSM is argued to be a potential strategy against this vector. On account of this unique ecological suitability, and the fact that the adults, despite being highly resistant to insecticides, remain mostly endophilic and endophagic, a combined approach of LSM and adulticides such as dual-active ITNs or non-pyrethroid IRS, has been suggested as particularly valuable for not only reducing An. *funestus*-mediated malaria transmission but potentially even crushing the local populations this this species [92].

Targeting these unique habitats could be significantly magnify the impact of LSM in these rural settings, but it requires an in depth understanding of the interactions between communities in malaria-endemic areas and the aquatic habitats of malaria vectors. Insights into how communities use these habitats, and their overall opinions can shape the way larval source management (LSM) strategies are designed and implemented [269]. For example, if communities regularly use the same habitats for drinking, bathing, or other daily activities, they may be strongly against habitat removal but supporting larviciding, especially if they report a biting nuisance from these habitats and have information on the safety of the approach.

Many studies from different locations have demonstrated the correlation between community engagement and LSM success [74-76,269,387,388], indicating the need for strategies that are adapted to meet community experiences and needs. Efforts should therefore be made to ensure that LSM practices adequately account for local societal experiences, needs and expectations, especially since the same water bodies where mosquitoes breed tend to be the same as those used by communities for other purposes.

The aim of this study was therefore to explore how the use of aquatic habitats by local communities in rural south-eastern Tanzania might influences LSM strategies, focusing on habitats frequented by *An. funestus*. To achieve this, we first identified and quantified the main aquatic habitats used by local malaria vector species, and assessed if and how these habitats were being used by local communities. Lastly, we assessed community perspectives and recommendations on LSM approaches for malaria vector control.

## 7.2 Methods

### 7.2.1 Study area

This study was conducted in three malaria endemic communities in south-eastern Tanzania, namely Ikungua and Chikuti villages in Ulanga district, and Sofi Majiji village in Malinyi district (Fig. 7.1). Detailed description of these communities is provided by Kahamba *et al* 2024 [22]. The residents primarily engage in subsistence farming and pastoralism, with small groups involved in artisanal mining, brick making, fishing, and small-scale business-like food vendors, general stores, and market.  Rice cultivation occurs year-round, depending on natural rainfall during the rainy season and irrigation during the dry season [389]. Other food crops include maize, beans, sesame seeds, and cassava. These villages are situated at an altitude of approximately 300-450 m above sea level, with major rivers like the Ruli river providing essential water sources for irrigation and daily use. Access to electricity and clean water is limited, so most residents depend on shallow wells for domestic water needs. Cooking is mostly done using wood or charcoal. The environmental features, including the presence of major rivers and low-altitude settings, contribute to mosquito transmission by providing abundant breeding sites.

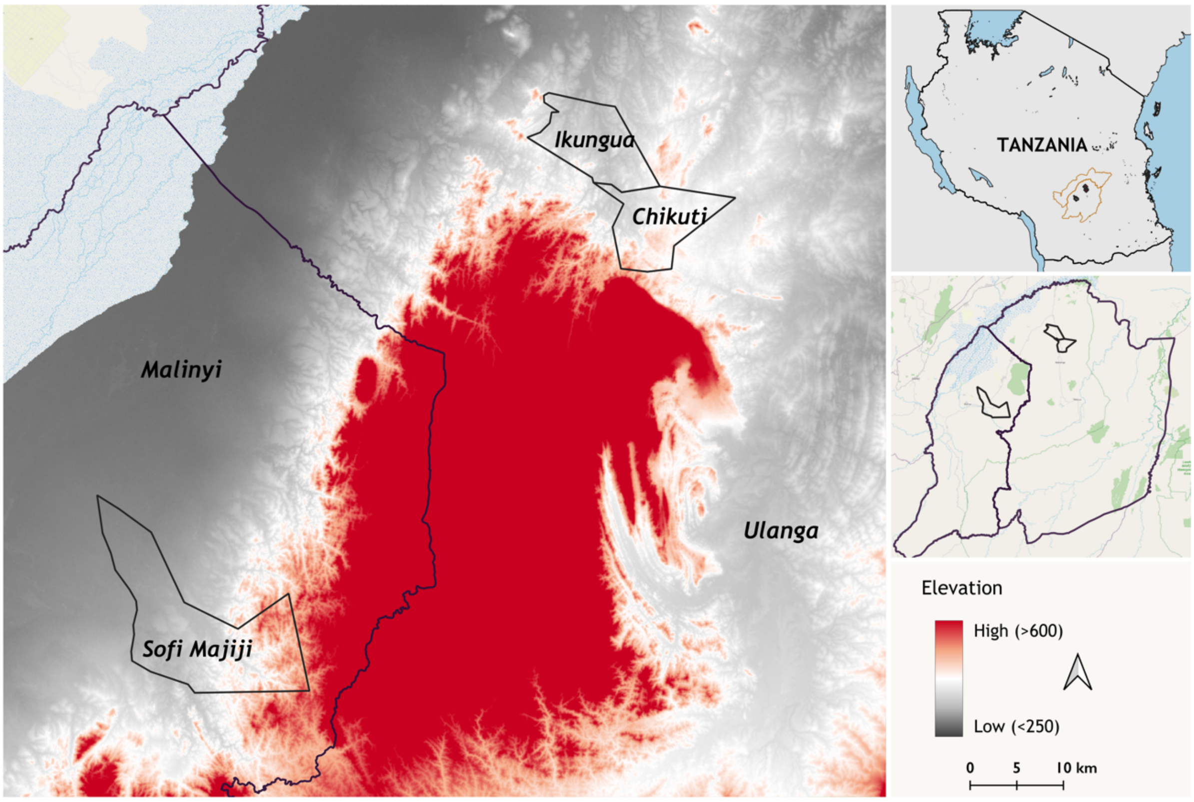

**Figure 7.1:** A map showing study villages in Ulanga and Malinyi districts.

### 7.2.2 Study design

This study used a sequential mixed-method research design for the main study objectives. Quantitative data was collected first during the dry season (July - November 2022), which included assessment of aquatic habitats, respective densities of *An. funestus* larvae or pupae, and domestic uses for the aquatic habitats. Subsequently, the qualitative component included a series of focus group discussions (FGDs) to explore communities' perspectives and recommendations on how LSM approaches can be integrated into their daily practices was conducted in November 2022 (Figure 7.2). Additionally, field visits and direct observations were made to assess the actual community practices and uses of these habitats.

### 7.2.3 Habitat characterization and entomological surveys

Quantitative data collection followed the procedure detailed in Kahamba *et al* 2024 [22]. A cross-sectional larval survey was done to identify and characterize aquatic habitats containing *An. funestus*. As previously detailed in chapter 3 (Table 3.1), this process involved recording various environmental characteristics such as habitat type, size, watercolour, permanence of water, water movement, water source, presence of shades, presence of vegetation, and the presence of algae (Fig. 7.3). Immature mosquitoes were collected using either 350ml dippers in small habitats or 10L buckets in larger habitats as described in (Chapter 3), and the mosquitoes were identified based on their morphological characteristics using established taxonomic keys by Gillies and De Meillon and Gillies and Coetzee (Gillies and de Meillon, 1968; Gillies and Coetzee, 1987). Larvae were identified into taxonomic groups of *Anopheles funestus sensu lato* (*sl*), *Anopheles gambiae sl*, and *Culex*, and others. Pictorial data was also collected for all the habitats.

### 7.2.4 Assessment of how communities use water bodies occupied by *Anopheles* mosquitoes.

Once the main habitats occupied by the dominant malaria vector *An. funestus* were identified, follow-up observations were conducted to identify and estimate the proportion of those habitats that were being used for domestic activities. This was done by directly observing and recording environmental indicators such as footprints, hoof prints, and signs of human and livestock waste within 10m around the habitats, using a prepared checklist. Additionally, we conducted several unstructured interviews with consenting community members living near the habitats to understand how they use them. During these interviews, we gathered information on both the frequency of use, and type of use activities conducted.

Following these initial assessments, we conducted focus group discussions (FGDs) with community members to gain deeper insights into their perceptions of malaria transmission risks within their homes and communities, the connection between local water sources and malaria, and their methods for mitigating transmission risks.

Additionally, participants' attitudes towards the potential and practicality of three LSM approaches were evaluated.

Discussions were structured into three main sections. Initially, participants shared their understanding of malaria transmission, factors contributing to its persistence, and their efforts to mitigate these risks. The second part focused on identifying different types of mosquito larval habitats and strategies for their control. Finally, participants evaluated the feasibility and effectiveness of the three LSM strategies; larviciding, habitat manipulation (source reduction), and habitat modification (habitat removal) in their community contexts. To foster meaningful dialogue, facilitators provided definitions of each LSM strategy and addressed participant questions before discussions commenced. Participants then shared their perspectives on the appropriateness and potential implementation of these approaches, offering specific recommendations on the contexts and conditions under which each method could be effectively applied.

Altogether, a total of nine FGD sessions were conducted; six with community members (three with males and three with females separately) and one each with local fishermen, pastoralists, and brick-makers. These groups were selected to represent major users of the water bodies. Each session consisted of eight to ten participants and lasted between one and two hours. Most discussions took place within the participants' communities (at the village leader's offices) and discussion with fishermen and brickmakers, participants were invited at Ifakara Health Institute's offices. All discussions were audio-recorded for further processing, and detailed notes were taken by at least two facilitators during each session.

## 7.3 Data processing and analysis

We integrated the analysis of quantitative survey data (done in R statistical software version 4.2.3 (R Core Team, 2019) and qualitative analysis using NVivo software version 12 [390]. Throughout the analysis process, data weaving and inferencing techniques were employed, integrating information from both components of the study to develop a comprehensive understanding from the viewpoint of the respondents.

For the quantitative data, descriptive statistics were used to summarize the mosquito aquatic habitats and the proportion of those utilized by communities. This included the proportions of all surveyed habitats, those containing mosquito larvae, and specifically, those containing *An. funestus*. The descriptive analysis was extended to categorize the habitats based on their usage by community members for different purposes.

For the qualitative data, the audio recordings of the FGDs were transcribed by Siaba Kinunda and Alfred Simfukwe, then reviewed by me and Felista Tarimo. During analysis, thematic coding was employed to identify key themes and patterns. Prior to analysis, a code book was developed using both deductive and inductive methods; whereby deductive codes were developed from the objectives of the study and the discussion guide, and inductive codes were developed through a thorough review of the transcripts. Similar codes were subsequently grouped into broader themes and categories that emerged from the data. The coding process was done by me and Felista Tarimo. Main themes identified included: i) community understanding about mosquito ovipositing behaviour and their aquatic habitats., ii) participants' views of the applicability, effectiveness and challenges associated with the three LSM approaches. Direct quotations from the participants were used to support and provide context to the themes.

## 7.4 Ethical considerations

Ethical approval for the study was obtained from the Ifakara Health Institute Institutional Review Board (Ref: IHI/ IRB/No: 26-2020) and the Medical Research Coordinating Committee (MRCC) at the National Institute for Medical Research-NIMR (Ref: NIMR/HQ/R.8a/Vol. IX/3495). Before commencing data collection, permission was obtained from the District Medical Officers (DMO) and subsequently from each village executive officer (VEO). The VEOs assisted in selecting participants for the focus group discussions (FGDs) based on our established criteria. Written informed consent was obtained from all participants prior to their involvement in the FGDs. Additionally, verbal consent for taking pictures of the community members during the observations, surveys, and FGDs were obtained.

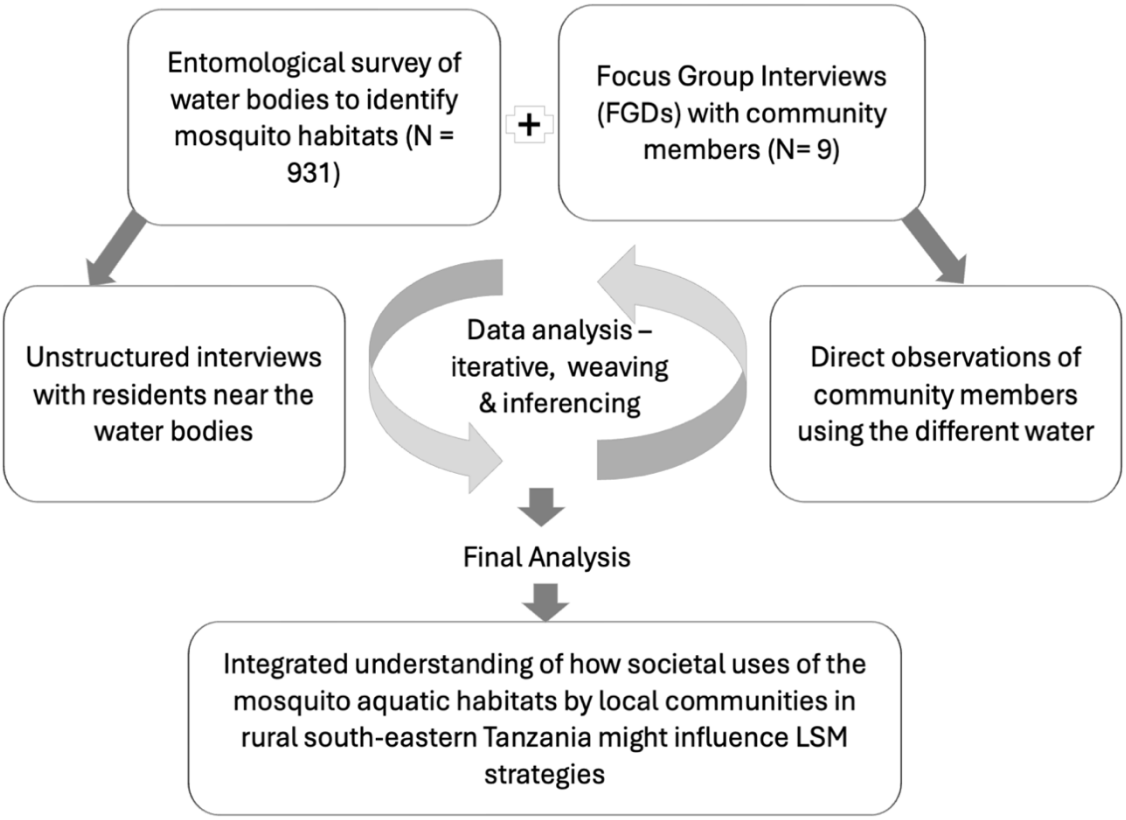

**Figure 7.2:** Mixed-Methods Approaches for data collection and analysis of how the societal uses of aquatic habitats by local communities in rural south-eastern Tanzania might influence LSM strategies, focusing on habitats frequented by *An. funestus*

## 7.5 Results

### 7.5.1 Survey of aquatic habitats in the study areas

The entomological survey identified 931 aquatic habitats of into six categories, namely: river streams, ground pools, dug pits, rice fields, ditches, and puddles (Figure 7.3 and Table 7.2). Nearly three quarters (73%, n = 612) of all the habitats contained mosquito larvae or pupae, and among these 23% (n = 213) contained *An. funestus*.

In the survey conducted, river streams were identified as the most prevalent aquatic habitats, accounting for 41% (n = 376) of the total, with 37% (n = 226) of these streams serving as larval habitats, and 52% (n = 112) of these larval habitats containing *An. funestus* larvae. Ground pools represented a smaller fraction, constituting 4% (n = 37) of all identified habitats, with 7% (n = 15) found to be containing *An. funestus* larvae. Dug pits constituted 22% (n = 208) of aquatic habitats, with 22% of these (n = 135) being larval sites, and 7% (n = 14) of which contained *An. funestus* larvae. On the other hand, rice fields comprised 7% (n = 65) of habitats, with 8% (n = 53) identified as larval sites and 6% (n = 12) harbouring *An. funestus* larvae. Another common habitat type was ditches, which accounted for 22% (n = 208) of all habitats and 23% (n = 139) of all larval habitats. About 57 (n = 27) ditches were found to have *An. funestus* larvae. Lastly, puddles formed 4% (n = 37) of habitats, with 5% (n = 28) serving as larval

**Figure 7.3:** Common types of aquatic habitat found in the study areas. The river stream image was captured using drone, it is approximately 2 meters in width and spanning over 100 meters in length. The ground pool was also captured by drone, has a surface area of approximately 60 square meters. The rice fields cover an area of about 0.1 hectares (1000 square meters). The ditch is a narrow, slow-moving water body approximately 1 meter wide and 15 meters long. The spring-fed well is a human-made pit, about 1.5 meters in diameter. The puddle is a small water

accumulation in a low-lying area with a surface area of approximately 2 square meters.

### 7.5.2 Community uses of the different water resources- results of the unstructured interviews and direct observations

Reported community uses of water bodies that contained mosquito larvae are presented in Table 7.1. Community members used the water from these aquatic habitats for various purposes – of the 931 habitats surveyed, our observations indicated that 90% (n = 837) were used by community members for one or more purposes (Table 7.1). Some of the common community uses for the habitats included as a source of water for drinking, cooking, washing dishes and clothes, and bathing (accounting for 37% (n = 306) of all observed use (crop irrigation (27%,n = 223) watering livestock (60%,n = 505); fishing a(37%,n = 311) and brick making (16% ,n = 132,Figure 7.4).

Nearly half of river streams were used for activities such as fishing, cattle grazing, and domestic needs. Ground pools, including those with *An. funestus* larvae, were commonly used for fishing and cattle grazing. On the other hand, human-made pits served multiple purposes, primarily domestic water uses and brick making. Approximately 30% (n = 154) of ditches were actively used by community members for cattle grazing and agriculture (Figure 7.4).

**Table 7.1:** Distribution of total habitat surveyed and habitats that had at least mosquito larvae and habitats that had at least one *An. funestus* mosquitoes.

| **Habitat type** | **Water bodies surveyed** | **Water identified used by community** | **Larval habitats** | **Habitats with *An. funestus*** |
|---|---|---|---|---|
| | **n (%)** | **n (%)** | **n (%)** | **n (%)** |
| **River streams** | 376 (41) | 353 (42) | 226 (37) | 112 (52) |
| **Ground pools** | 37 (4) | 33 (4) | 31 (5) | 15 (7) |
| **Dug pits** | 208 (22) | 188 (22) | 135 (22) | 14 (7) |
| **Rice fields** | 65 (7) | 64 (8) | 53 (8) | 12 (6) |
| **Ditches** | 208 (22) | 178 (21) | 139 (23) | 57 (27) |

| | | | | |
|---|---|---|---|---|
| **Puddles** | 37 (4) | 21 (3) | 28 (5) | 3 (1) |
| **Totals** | 931 (100) | 837 (100) | 612 (100) | 213 (100) |

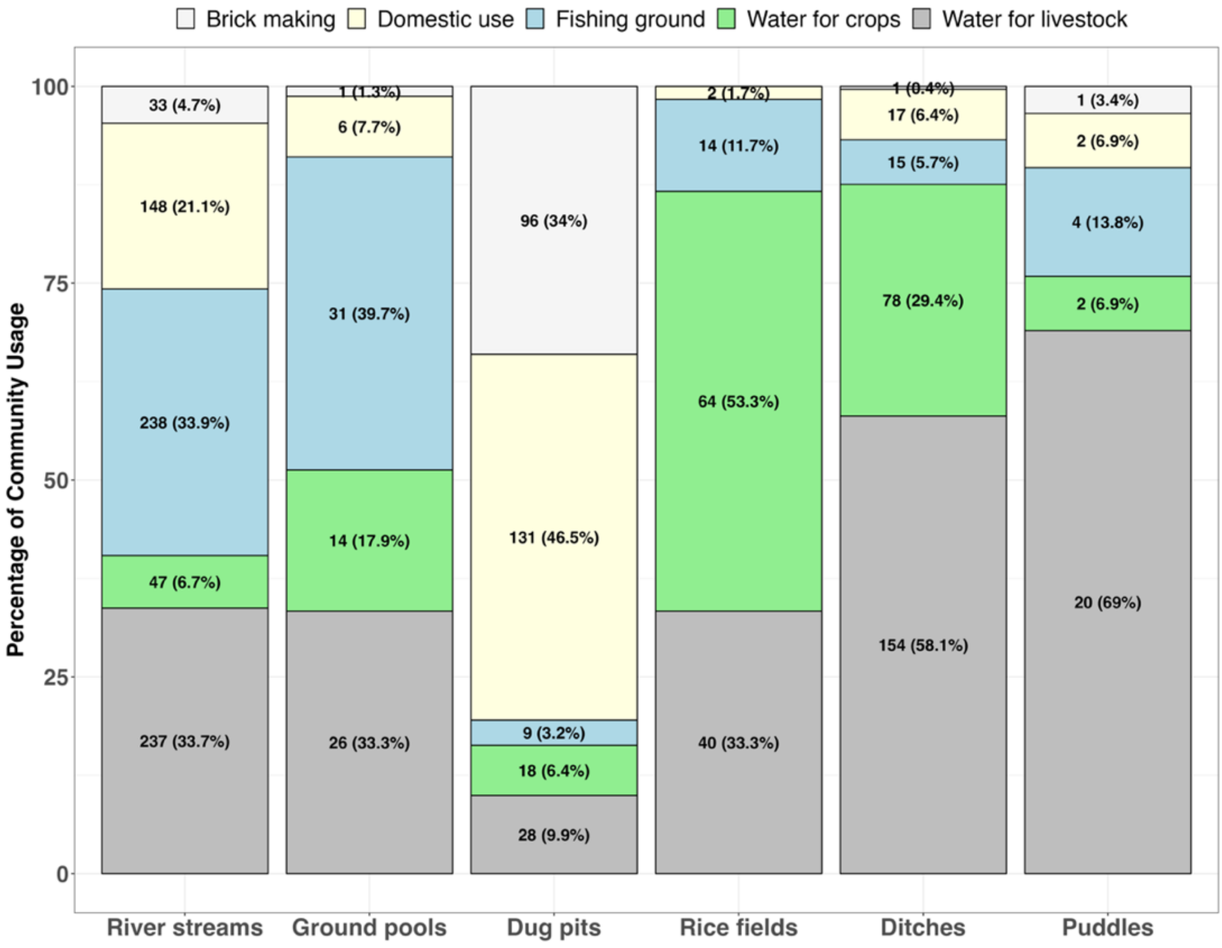

**Figure 7.4:** Distribution of different habitat types and those utilized by both *An. funestus* and the community. This figure provides a quantitative summary of the use of different aquatic habitats serving the community for various needs.

**Figure 7.5:** Photos showing examples of how community members use aquatic larval habitats for various purposes. This figure depicts various communal activities conducted in different aquatic environments, illustrating the interplay between daily life and potential mosquito breeding sites. Examples include (A) washing dishes beside river streams, (B) cleaning dishes within flooded rice fields, (C) laundering clothes by riverbanks, (D) fetching drinking water from dug pits, (E) providing water for livestock at river streams, and (F) collecting water from dug pits for household use.

### 7.5.3 Results of the Focus Group Discussions regarding community dependence on the different water sources

The focus group discussions comprised a total of 85 participants, consisting of 54 males and 31 females. The age range (determined for 80 of the 85 participants) was 19 to 71 years, with a mean age of 37.92. The majority of respondents had primary education (74%, n=63), while a smaller proportion had secondary education (19%, n=16) or no formal education (7%, n=6). In terms of marital status, 56% (n=45) were married, followed by 27% (n=23) who were not married, with smaller proportions being divorced (5%, n=4), widowed (7%, n=6), or unidentified (8%, n=7). In terms of occupation, 55% (n=47) were farmers, with others engaged as fishermen (12%, n=10), brick makers (12%, n=10), pastoralists (10.5%, n=9), and various other occupations (10.5%, n=9). The focus group discussions (FGDs) confirmed patterns of community dependence on various water sources, consistent with direct observation and unstructured interviews. During the FGDs, community members explained that they relied largely on river streams, dug pits and, in some cases, large pools to obtain water for different purposes including drinking, cooking, watering animals, bathing, and economic activities such as agriculture and construction activities. This dependence on specific water sources was driven by necessity and availability, as one participant voiced the lack of choice in their water source options:

> "*We use what we use because we must, not because it is what we would choose if we had other better and safer options.*" (Female farmer)

The availability and type of water sources varied by location, as revealed by participants in different areas. In some regions, community members utilized groundwater pumps, locally referred to as "*Mdundiko,*" installed by the government, to meet some of their water needs. In contrast, other areas predominantly relied on natural water sources such as rivers, streams and spring-fed wells for most domestic requirements. Participants noted that a key factor determining the type of water source used was proximity to the village centre or main roads. Communities closer to these areas generally accessed more reliable and cleaner water compared to those in more marginalized ones. One participant described their situation, stressing these disparities:

> *"Our lives in the remote farming area are different from those in the town [here referred to small towns], we in the interior village we dig our wells, and we rely on stream channels, but those in urban areas have pumped water "Mdudindiko" which they use for their domestic needs.*" (Female farmer)

### 7.5.4 Community understanding of mosquito reproduction and larval sites.

The majority of FGD participants understood that mosquito reproduction involves mating between males and females, followed by females laying eggs in water where they hatch into larvae, developing in aquatic habitats before emerging as adult mosquitoes. Most were familiar with common larval sites such as pits, river streams, and large water bodies. However, some participants lacked such understanding, and sometimes they would mention other unlikely places such as areas with dense vegetation like bushes, pit latrines (that are often not used by malaria vectors), dark and moist places, and corners of the houses. This confusion indicated a mix-up between mosquito breeding sites and the areas where adult mosquitoes are commonly found, as explained by a participant:

> "*From what I know, mosquitoes prefer dark and damp places, particularly those with vegetation. When we clear these areas, the mosquitoes find their preferred habitats disturbed, so they tend to move away, often relocating away from our homes*" (Male, farmer).

The majority of participants reported observing mosquito larvae in water bodies and associated the presence of larvae with adult mosquitoes. Many respondents noted that they frequently spotted larvae while performing daily chores and were able to identify them as mosquito larvae due to the abundance of adult mosquitoes around water sources, as explained by one of the participants:

> "*I've seen mosquito larvae while fetching water from the river. I've seen them attached to grasses along the river's edge, when you disturb the grasses, a group of mosquitoes will fly, these are the same mosquitoes that come to our homes to look for blood.*" (Female farmer)

### 7.5.5 Perceptions and recommendations on LSM for malaria control

Discussions on the potential of LSM for malaria control focused on the participants' views of the applicability, effectiveness, challenges, and recommendations associated with the three LSM approaches. Generally, the participants expressed varying levels of interest for the different LSM approaches (Table 7.2).

#### *i. Larviciding*

Regarding larviciding, the majority of participants expressed enthusiasm, viewing it as a practical option for their communities. They recognized its potential benefits in reducing malaria by linking the use of larvicides to a decrease in mosquito populations. Participants understood that fewer mosquitoes would likely lead to reduced malaria transmission. This proactive approach to lowering malaria risk was widely acknowledged, as said by one of the participants:

> *"Now, there are many puddles and mosquitoes, suppose we apply the larvicide in this area and then we decide to promote its use in other areas too, as we continue to do this, the number of mosquitoes will decrease, and then there will be a reduction in malaria cases."* (Male farmer)

Many participants believed that addressing the mosquito problem at its source, by preventing larvae from emerging as adults, would be a more effective way of controlling malaria compared to ITNs. This viewpoint was commonly relayed in association with the phrase of "*prevention is better than cure*"; highlighting that stopping mosquitoes from maturing into adults, is a preferable strategy. Community members deemed larviciding to be an appropriate approach for targeting mosquito in their habitats, provided that the chemicals used were safe for humans and animals as this participant said:

> *"In my opinion, it is suitable, indeed suitable, to treat mosquito habitats and that is how we could benefit. The mosquito will not be able to emerge, and we will not have malaria, as people say, 'prevention is better than cure', it is just like that."* (Female farmer)

During the discussions, participants expressed concerns about managing larval habitats in remote or forested areas that are often hard to reach and overlooked in community cleaning efforts, such as those organized by local government authorities. This shows the need for strategies that can identify and treat all habitats, including those that are inaccessible. Participants also noted the limitations of localized measures in addressing all larval habitats and emphasized the potential of larviciding to cover more extensive geographic areas.

> *"If we apply the larvicide here, but in the forest, there is another unseen pond! What do we do? The chemical's efficacy will end, but then the mosquitoes will move and start new habitats there, right? so, the advantage of chemical can be applied in every habitat at the same time."* (Female farmer)

Participants preferred larviciding partly because they viewed complete removal of mosquito habitats (source reduction) and habitat manipulation as impractical, given the community reliance on these water sources. They appreciated larviciding for its ability to control mosquito populations while maintaining access to essential water resources, highlighting this balance as a significant advantage, as described by one of the participants:

> *"Some of these water habitats we created ourselves because we need them for our daily livelihood, if they keep the mosquito, that was not our intention and so the mosquitoes have to be killed while ensuring the water remains safe and usable for our various purposes."* (Female farmer)

Despite the general acceptance of larviciding, the focus group discussions (FGDs) revealed some concerns among participants, particularly regarding the use of chemicals to control mosquitoes. First, they questioned whether treated water would still be safe for domestic use and agricultural activities, or their livestock or fish as these participants said:

> *"In our current environment, I don't think it's possible because that's a chemical, but those same water bodies we mentioned are the primary water sources for the community, and then the same water bodies are oviposition*

> *sites for the mosquitoes. If the larvicide harms livestock, people might refuse to use it.*" (Female, farmer).
>
> "*Because we don't know if the chemicals, even if they are brought to target mosquito breeding sites, will kill the fish or pose risks to humans, because we lack knowledge and understanding.*" (Male fishermen).

Secondly, expressed concerns about the feasibility of implementing larviciding in low-income communities due to the required technical expertise and financial resources. In particular, they noted that where multiple larviciding treatments are required, this would be challenging due to lack of continuous financial resources:

> *"The method of applying chemicals is technical and requires financial resources, this method is more realistic, even the larvae of mosquitoes would decrease very fast. However, consistent application is essential, as mosquito populations grow, and the power of the chemical diminishes over time. To prevent mosquitoes from returning, regular reapplication of the chemical is necessary, which needs funds."* (Male farmer).

Lastly, the discussions also revealed a general scepticism towards larvicides brought from outside the country, especially fuelled by the aftermath of COVID-19, and the scepticism towards COVID vaccines. The participants wondered how communities would be convinced to accept this intervention, as this participant said:

> *"Just like with the coronavirus vaccine, many of us, including myself, were hesitant... How will communities accept these chemicals in the water we drink?"* (Male, farmer).

#### ii. Habitat manipulation

Habitat manipulation for controlling larval habitats received mixed reactions from study participants. Some saw it as a practical measure, particularly getting rid of useless stagnant water near residential areas, while others raised concerns about its feasibility and legality, especially near protected natural water bodies. Supporters of this approach suggested initiatives like clearing tall grass around water sources and homes, noting these actions were practical in their communities and offered additional benefits beyond mosquito control:

> *"We can manage to clear the stagnant water around our homes. It's something within our power to do, and it helps reduce the mosquito problem in our immediate surroundings."* (Female farmer)
>
> *"When you clean and remove the grass, even snakes do not stay, thereby creating a safer environment."* (Female farmer)

The primary concern regarding habitat manipulation, as voiced by most participants, centred on the inaccessibility of certain water bodies, especially those in government-protected areas or regions with land development restrictions. Participants note that there were legal prohibitions against altering vegetation within 60 meters of a river stream to avoid ecological disturbances, emphasizing the regulatory challenges associated with this approach:

> "*We have a nearby river Luli. It has reeds and dense vegetation. Now, that vegetation hosts numerous organisms like snakes, chameleons, and lizards.* Tanzania National Parks Authority (TANAPA) *cannot allow you to clear vegetation 60 meters around the river streams because it will chase away these animals from their natural habitats.*" (Male farmer)

Moreover, habitat manipulation was also considered impractical during the rainy season when there was often flooding and water everywhere, making it impossible to clear the water or to keep up with vegetation growth as this participant elaborated:

> *"During the rainy season vegetation grows fast around water bodies, if you clear it today, it quickly grows back in just days.* (Female farmer)

This approach was especially opposed by fishermen who feared that it could disrupt breeding habitats for the fish. The fishermen explained that vegetation alongside riverbanks provided calm waters and safe havens for fish to lay their eggs. Disrupting this, therefore, could interfere with their livelihoods as this fisherman explained:

> *"Fish prefer to lay their eggs in parts of the river that are calm and have plenty of vegetation. You won't find fish eggs in fast-flowing waters. If you tell me to clear the grass along the riverbanks today, I will also be disturbing breeding habitats for the fish. This will likely reduce fish reproduction and ultimately harm our income."* (Male fishermen)

One particular exception was that the pastoralists who participated in these discussions expressed their support for this approach as they deemed it would not have negative impact on their livestock. Reducing vegetation alongside water sources was also perceived as beneficial as it opened up the water for their livestock. However, they too were concerned about whether or not they would have time to do such work, given their nomadic nature and busy schedules as this participant said:

> *"I wonder when we, as pastoralists, would find the time for this task. Every day we're up early to go grazing and don't return until evening. If the government decides to undertake this exercise, we will agree, because our animals only go to these places for water."*

### *iii. Source reduction through habitat modification or removal.*

This approach was the least favoured due to multiple reasons: i) most water bodies are utilized for domestic or livelihood purposes, ii) concerns that filling these water bodies would require creating other pits to obtain sand, which could potentially become new larval habitats, and iii) the impracticality of altering natural water bodies (Table 7.2). Participants noted that it might be feasible for smaller, unused breeding sites like puddles within the community. The potential for habitat modification to completely eliminate water sources was a significant concern, especially given the multifunctional nature of these resources in the community. Consequently, participants deemed this approach both impractical and inapplicable.

Another concern raised regarding habitat modification was that some habitats are too large to modify or remove without creating new potential breeding sites. Other than the river streams and large ponds, other examples of these were the pit holes resulting from brickmaking and mining activities; study participants explained that it would be impossible to find landfills to cover these without creating more pits in the process as this participant elaborated (Table 7.3). Participants noted the impracticality of finding adequate landfill materials to fill these large pits without the need to excavate additional areas. Additionally, the approach was deemed unsuitable during the rainy season due to frequent flooding that enlarges water bodies, complicating any efforts to control habitats.

Furthermore, participants emphasized the practical challenges, and the effort required to engage in habitat modification amidst their busy agricultural schedules. Brickmakers, in particular, opposed this strategy because it threatened their livelihood. They rely on pits filled with water for brickmaking and create new pits annually, suggesting that filling these would directly impact their income. Similarly, pastoralists expressed concerns about the adverse effects on their livestock, emphasizing their reliance on these water sources and preferring to maintain them for animal use.

**Table 7.2:** Community perspectives on different approaches to larval source management, for malaria vector control.

| Approach | Perceived Benefits | Concerns | Specific recommendations |
|---|---|---|---|
| **Larviciding** | ▪ Reduces mosquito populations and malaria cases,<br>▪ Targets roots of the problem,<br>▪ Can provide broad area coverage,<br>▪ Balances mosquito control with water needs. | ▪ May have health and environmental safety concerns,<br>▪ May affect aquatic life and livestock,<br>▪ Requires significant cost and labour,<br>▪ Could be limited by community scepticism. | ▪ Educate and engage the community,<br>▪ Involve locals<br>▪ Provide safe use guidelines,<br>▪ Plan and monitor strategically,<br>▪ Ensure effective communication between authorities, communities, and scientists. |
| | | | |
| **Habitat Manipulation** | ▪ Directly controls mosquito breeding sites,<br>▪ Practical and enhances cleanliness,<br>▪ Deters dangerous animals. | ▪ There may be legal and environmental concerns,<br>▪ Is impractical during rainy seasons,<br>▪ Might impact the livelihoods of specific groups, e.g. fishermen. | ▪ Promote community education and participation,<br>▪ Collaborate with government agencies,<br>▪ Establish specific cleanup times and calendar of activity,<br>▪ Adapt methods to seasons and geographical areas, for feasibility. |
| | | | |
| **Source Reduction/ Habitat Removal** | ▪ Reduces mosquito populations sustainably<br>▪ Leads to a cleaner environment,<br>▪ Decreases malaria cases,<br>▪ Can be implemented in designated areas. | ▪ Is a challenge because the water bodies also serve other purposes,<br>▪ Risks of new breeding sites,<br>▪ There might be community resistance,<br>▪ It is time and labour-intensive. | ▪ Promote education and active community involvement<br>▪ Designate activity areas and provide alternatives<br>▪ Implement regulations and get local leaders to participate<br>▪ Consider and preserve beneficial habitats. |

**Table 7.3:** Community concerns regarding source reduction through habitat modification or removal

| **Theme** | **Key Concerns** | **Example Quotes** |
|---|---|---|
| **Domestic uses of the water** | Water bodies are essential for domestic and livelihood activities. | *"Most of our water bodies are used for various purposes, from fishing to laundry. Removing them completely isn't just about mosquitoes; it affects our daily lives."* (Male farmer) |
| **Feasibility and practicality** | Natural water bodies cannot easily be altered. | *"Where would you even start to fill a natural water body? It is there because it has to be there, it's natural, even if mosquitoes are present, a different approach should be used, and not this one." (Male, fisherman)* |
| | Large habitats are difficult to modify without creating new ones. | *"The main challenge in filling the pits [is that it is] is difficult during the rainy season. You might say you'll fill a pit, but when it rains, not all places will drain off the water; there are many areas where water will accumulate… preventing water from stagnating during the rainy season is difficult".* (Female farmer) |
| | | *"For me, this approach is not possible because there are mining activities by small-scale miners in the village here. They dig large pits in the forests, when it rains, these pits fill with water. So, you can't ask people to go to those places and fill up those pits."* (Male farmer)<br>*"Another challenge I see is time, these pits are present during the rainy season, and we farmers are usually busy in the fields, so we don't have time to rest and fill these pits. Time is a real issue because as soon as we wake up, we are going to the fields, and by the time the rainy season ends, and the water is everywhere".* (Male farmer) |

|  |  |  |
|---|---|---|
| **Impact on livelihoods** | Modifying habitats could negatively impact livelihoods such as by hindering brickmaking. | "*In our area, brickmaking is an annual activity. Every year, we need to dig new pits for this purpose. So, if we fill the old ones, we will just end up creating new ones the following year*." (Male, brick maker)<br>"*I think that filling the pits we use for brickmaking would be economically counterproductive for us. We need the health institutions to work with us and the community to find other ways to control mosquitoes and manage malaria, without disrupting our brickmaking activities. If the government decides to fill these pits, we will have to dig new ones for making bricks and we will create new mosquito breeding sites*". (Male, brick maker) |
| **Water resources for livestock** | Modifying habitats could negatively impact resources for livestock. | *"If I'm told to fill up a pit that I regularly use, it would for sure affect me. Yes, there might be benefits, but on the other hand, I'll face consequences. For example, if that pit has enough water for my livestock and there's no alternative, then filling it up would significantly affect me and my livestock."* (Male, pastoralist) |

### 7.5.6 Broad recommendations by community members regarding LSM

Key recommendations made by participants during this study are summarized in Table 7.4. To ensure effectiveness of LSM strategies in the study communities, the participants members emphasized the importance of raising awareness about the techniques for malaria control. They suggested awareness campaigns, e.g. through community meetings, to address potential impacts that LSM might have on people, livestock, and the environment. Participants also advocated for clear understandable guidelines on the use of larvicides, including their frequency and safe application timing, as relevant to specific settings. Thirdly, they emphasized the importance of involving locals in program implementation to foster trust and ownership, noting that community support would increase if implementation were led by familiar faces.

There was a consensus on the need for careful planning of the deployment and timing of LSM to align with seasonal variations and readiness. Participants suggested that different LSM strategies might have different calendars of activities. For instance, while larviciding may be desirable during the rainy season when water sources are plentiful and vector populations are highest, habitat manipulation would be more feasible in the dry season when water bodies are fewer and reduced in size. To enhance the impact, participants suggested that local governments should mandate regular LSM activities, such as environmental clean-ups – for example, they suggested bi-weekly community cleaning days to encourage broad participation.

Additionally, if water sources are modified or removed, there was a strong recommendation for the government agencies to provide alternative sources and to reallocate activities like brickmaking to minimize environmental risks. The community emphasized that habitat modification should be limited to unused water sources to avoid disrupting local needs. This holistic approach highlights the community's concern for careful planning and local involvement in LSM initiatives.

**Table 7.4:** Recommendations from community members regarding larval source management

| Theme | Recommendations | Example Quotes |
|---|---|---|
| **Awareness and Education** | Conduct awareness campaigns to educate and answer questions about the impacts of LSM on people, livestock, and the environment. | *"Community members might reject [the idea] initially due to a lack of understanding. Education should be provided first, then once people understand, they will accept it."* (Female farmer) |
| **Guidelines for Larviciding** | Provide clear guidelines on the dosage and timing of larvicides to ensure safe usage. | *“We need guidance to inform us on the appropriate time for spraying and the correct dosage to avoid unintentional harm to other beings."* (Male farmer) |
| **Local Involvement** | Train and involve local community members in LSM implementation to build trust and ensure program ownership. | *“If I see a local person, someone from our own village, doing the larviciding, I will have more faith than if it were strangers who I don’t know their intentions.”* (Male farmer) |
| **Timing of Implementation** | Carefully plan the timing of LSM to align with seasonal variations and community readiness. | *"The rainy season is the best time for spraying chemical, as many puddles and mosquito breeding places are at its peak. That's when the larvicide should be applied."* (Female farmer) |
| **Government Mandate** | Advocate for government-mandated LSM activities, including environmental cleaning and removal of stagnant water. | *“In my view, for people to engage in this exercise, the government should set a specific day for cleaning, like the 'Magufuli Saturday', where everyone knows they should be cleaning around their premises."* (Male farmer) |
| **Alternative Water Sources** | If water sources are modified or removed, provide alternative sources for community use. | *"Abandoned pits near homes can be filled, but those used for community activities, like domestic purposes, watering animals, construction activities like building clinics or schools, can be difficult for the community to agree."* (Female farmer) |

## 7.6 Discussion

Effective larval source management (LSM) necessitates a nuanced understanding and targeted approach to water bodies that are essential for mosquito larval development. However, since local communities often depend on the same water bodies for various other purposes, targeting these habitats for vector control requires careful considerations of local societal practices and expectations. The overall goal of this study was to explore how local communities in rural southeastern Tanzania use these water bodies and how this might influence LSM strategies. We focused primarily on habitats frequented by *An. funestus,* first because this is the primary malaria vector in rural south-eastern Tanzania, and second, because the vector species prefers a set of unique habitats that often remain as the sole water supplies during the dry season. Overall, our results illustrate a dual challenge: the critical need for water resources for various community purposes on one hand, and the simultaneous need to effectively manage these resources effectively to limit mosquito breeding.

It was observed that a vast majority of aquatic habitats used by malaria vectors, notably river streams, ground pools, dug pits, rice fields, ditches, and puddles, are integral to the daily lives of local communities; where they are used for washing, fishing, cattle grazing, and even as sources of drinking water. This linking of community life with potential mosquito vector larval habitats underlines the importance of engaging with communities to tailor LSM approaches that will respect their reliance on these habitats [75]. While the community members acknowledged the need for effective malaria vector control measures, there was a clear call for these measures to be applied thoughtfully considering the multiple uses of aquatic habitats by local communities. The need for more information about the safety and impact of LSM approaches on daily life was emphasized, pointing towards a gap in communication and education regarding LSM strategies.

The survey revealed that aquatic habitats, such as river streams, ground pools, and dug pits, play crucial multifunctional roles in community life, serving as essential resources for domestic and agricultural activities as well as breeding grounds for *An. funestus* larvae. This complexity presents significant challenges for malaria control efforts, such as habitat manipulation or larviciding, which must balance ecological impacts with community needs. Community interactions with these water sources varied widely, including uses for drinking, irrigation, brick making, livestock watering, and rice farming. In higher altitude areas, people intentionally planted rice around or within ground pools, and exclusively relied on rainfall to support these practices. Notably, river streams and ground pools were frequently used for washing dishes and clothes, reflecting their accessibility and utility, which aligns with findings from Kenya highlighting similar dependencies on aquatic habitats for daily chores [391]. Dug pits and ditches were commonly associated with brick making and agriculture, indicating their importance in economic and food production activities. This diverse use cases underline the significance of water bodies to community livelihood and underscores the need for LSM strategies that effectively control malaria vectors while being culturally and practically acceptable to the communities they serve [72,303,392].

Building on the varied use of aquatic habitats, it was noted that community members had a solid understanding of the mosquito lifecycle including ability to distinguish between aquatic stages and adult life stages. They could identify mosquito larvae and understood the direct relationship between the presence of larvae and the subsequent increase in adult mosquito populations. This community knowledge is particularly valuable for LSM, as it can empower local populations to actively participate in identifying and reporting potential breeding sites, thereby enhancing the efficiency and success of LSM interventions [76]. This level of awareness is supported by findings from other research, which has consistently shown a considerable understanding of malaria transmission dynamics within malaria endemic communities [391]. For example, research conducted in similar settings have reported that, local communities are often aware of mosquito breeding sites and their link to the risk of malaria [393,394]. However, these studies also indicate variation in the depth of knowledge and its application towards preventive practices; suggesting that while awareness is widespread, its effective interpretation to reduce malaria risk may differ from one community to another.

The study underscored significant challenges in LSM strategies, particularly habitat manipulation and source reduction, due to their potential to disrupt community livelihoods. Modifying water bodies used for brick making and livestock could adversely affect local economies and animal welfare, emphasizing the need for a careful balance between effective vector control and community sustainability. Larviciding, although favoured for its perceived straightforwardness and effectiveness in controlling mosquito populations, raised concerns about the safety of water post-treatment, impacting livestock and aquatic life. These concerns about the safety of larvicides and their potential impact on human, animal, and environmental health, including aquatic life, echo broader challenges previously documented [388,395]. However, studies in regions with similar living conditions indicate that people generally accept the use of larvicides, provided they do not negatively impact the environment or their way of life [74,387]. Nonetheless, the persistence of environmental and health safety concerns, which have also been observed in other studies highlights the importance of community education and involvement in LSM to ensure acceptance and understanding of these methods [74,387,388,396].

Habitat manipulation, recognized under initiatives like the "*Jumamosi ya Magufuli*" campaign in Tanzania, an initiative started by Tanzania former President, which promotes environmental cleanliness, was also seen as a viable LSM approach [397]. This highlights how existing policy and government information campaigns can be harnessed to promote LSM based on habitat management. However, its application is limited near natural water sources that are legally protected, stressing the need to consider environmental regulations in LSM planning [303]. Moreover, concerns from some sections of the communities, for example fishermen who were concerned about the potential negative impact on fish breeding habitats, illustrate additional complexities in applying LSM approaches to river streams, which comprise a significant water source in rural communities. These findings suggest that while LSM strategies are essential, they must be adaptable and sensitive to both ecological and community contexts.

While source reduction approaches are considered the most effective strategy for mosquito control because it completely removes larval habitats [60,102], this study suggests it was also the least preferred method among community members. The main concern was its potential impact on livelihoods and daily activities. Nearly all identified larval habitats were also used by community members for different purposes. Farmers rely on these water sources for both irrigation and domestic purposes; pastoralists need them for their livestock; brick makers use them in their brick-making processes, and fishermen depend on them for their catch. Similar patterns were observed in Malawi, where community dependence on mosquito larval habitats for various activities was reported [395]. This highlights the need for a careful balance between implementing public health measures to combat malaria and ensuring the well-being of communities, especially in areas where livelihood and daily activities are connected to the environment [70,303].

Community members emphasized the importance of raising awareness and providing education about the potential risks and benefits of all LSM approaches. They advocated for open communication and active community engagement in mosquito control efforts to ensure broad understanding and involvement in the implementation process [303]. Additionally, they pointed out the need of carefully scheduling these activities, selecting time periods for implementation both when the intervention will be more effective and when the majority of the community can actively participate. This concurs with the wider body of evidence indicating that vector control initiatives are more successful and relevant to local needs when the community is well-informed and directly involved in the mosquito control efforts [75]. One challenge that could arise with regard to this is balancing the timing of LSM implementation.

Most importantly, community members voiced that if habitat manipulation or modification-based LSM would be pursued as part of mosquito control efforts, the government should ensure the provision of alternative water sources. This demand highlights the communities' concern over the potential negative impacts such interventions could have on their daily lives, stressing the importance of mitigating these effects through thoughtful planning and the establishment of support systems. It is increasingly recognized that environmental management for malaria control must be integrated with local development needs [70,387,388]. Our findings add to this by proving the importance of not only addressing the public health aspects of malaria control but considering the broader implications on community access to water, agricultural practices, and overall economic well-being. Successful LSM interventions require a holistic understanding of local ecosystems and socio-economic dynamics; ensuring that efforts to combat malaria do not inadvertently compromise the resources upon which communities depend.

Therefore, a trade-off between mosquito control measures and community use of water sources can be significantly mitigated through better investment in water infrastructure. Improving water infrastructure could create a "win-win" scenario by simultaneously addressing malaria control and enhancing other areas of health, such as Water, Sanitation, and Hygiene (WASH), while supporting economic livelihoods [398]. By providing reliable and safe alternative water sources, communities would be less dependent on natural habitats that serve as mosquito breeding grounds, allowing for more effective LSM strategies without compromising community needs [75,395]. Moreover, better water infrastructure can improve overall public health by reducing waterborne diseases and providing essential resources for agriculture and livestock, thus boosting local economies [399]. Integrating LSM efforts with broader development initiatives focused on enhancing water infrastructure would not only facilitate sustainable malaria control but also promote long-term community well-being and resilience [388,400].

While this study has been the first to extensively explore the interaction between mosquito aquatic habitats and community needs in south-eastern Tanzania, it has some limitations in the methodological approach. The study primarily collected data through direct observations and FGDs with communities from selected villages, purposely chosen for their observable use of water sources that also serve as mosquito larval habitats. This approach was taken to facilitate understanding of the importance of aquatic habitats for human activities, and how this could impact the acceptance of mosquito control measures. However, by focusing on these specific settings, the study may have overlooked areas where such habitats play a smaller role in the community's daily life. Future research should include more diverse locations, especially those where reliance on mosquito larval habitats for water is not a significant aspect for daily living.

## 7.7 Conclusions

This study provides valuable insights into community perspectives on LSM for malaria control and elimination efforts. Additionally, it shows the complexities that might arise during the planning and implementation of LSM given the dual role of aquatic habitats as both important community resources and breeding sites for malaria vectors. In settings such as south-eastern Tanzania, where the dominant malaria vector, *An. funestus* primarily breeds in permanent and semi-permanent habitats l, our study reveals a clear preference for strategies like larviciding and habitat manipulation, which can more easily be aligned to daily activities and have minimal disruption to local livelihoods. These findings emphasize the importance of community engagement and the need for LSM strategies to be both culturally and environmentally sensitive to achieve community acceptance and sustainability. Furthermore, findings emphasize the need for balanced approaches that respects community practices and environmental considerations. Indeed, engaging communities in the design and implementation of LSM, along with providing education on the safety and efficacy of such interventions, is vital to ensure these strategies do not negatively impact local water resources. Finally, it is important to consider the socio-economic and regulatory constraints, especially regarding protected natural water sources. This calls for adaptable, community-informed strategies that maximize public health benefits while preserving community well-being and environmental integrity. Ultimately, vector control approaches should be designed in a holistic manner, ensuring to integrate the needs, perspectives, and daily lives of the communities it aims to protect.

# Chapter 8: General Discussion

## 8.1 Overview of the main findings

The overarching aim of this thesis was to improve our understanding of the ecology of *An. funestus*, a primary vector of malaria in many regions of Africa, particularly in southeastern Tanzania. While my work occasionally included adult mosquitoes, the main focus was on aquatic stages of the vector. Insights gained through this research are intended to inform and improve vector control strategies, contributing to malaria elimination efforts in Tanzania and other areas where *An. funestus* is a predominant vector. Despite significant advancements in malaria control, transmission remains high in Tanzania, necessitating additional approaches to strengthen intervention impact by exploiting the ecological characteristics of *An. funestus*. This general discussion chapter synthesizes the key findings from each chapter, highlights the broader implications for vector control and malaria elimination, reflects on overarching limitations, and highlights arising questions for future research.

### 8.1.1 Ecological vulnerabilities and control strategies

This thesis began with an extensive literature review to explore the available evidence on ecological traits of *An. funestus* and how these can be leveraged for targeted vector control. The findings suggest that integrated strategies combining larval source management (LSM) and insecticide-based interventions could be highly effective. The high levels of insecticide resistance observed in *An. funestus* populations underscores the necessity for diversified control strategies. Understanding the ecology of the vector is crucial to exploit specific vulnerabilities, potentially leading to the local elimination of *An. funestus* and a significant reduction in malaria transmission [92].

I argued that LSM could have specific value for *An. funestus* because in contrast to other African malaria vectors, it tends to use perennial and large aquatic habitats such as river streams, ground pools, and spring-fed pools [23,24,154]. These stable environments enable the species to maintain year-round populations, making it a resilient vector capable of sustaining malaria transmission even during dry seasons [244,255].

This distinct habitat preference underscores the need for targeted LSM strategies focusing on these permanent and semi-permanent water bodies [312]. While previous studies have investigated the aquatic ecology of *An. funestus*, their findings suggest that the stability and size of *An. funestus* habitats make them relatively easy to locate. This aligns well with the WHO’s paradigm of 'few, fixed, and findable,' highlighting that LSM could be particularly effective for *An. funestus*. Therefore, effective control measures must be habitat-specific and environmentally sustainable, leveraging these features for targeted interventions [23,24,154].

This review also summarized key aspects of adult *An. funestus* ecology including that it predominantly rests and feeds indoors and is strongly anthropophilic and endophilic. However, there are reports of outdoor biting and zoophagy, particularly in areas with high livestock populations [401]. This behavioural plasticity complicates control efforts but also provides multiple intervention points. Indoor residual spraying (IRS) and insecticide-treated nets (ITNs) are effective against indoor populations, while additional measures, such as outdoor spraying and spatial repellents, may be necessary to address outdoor biting behaviours [304,401].

Insights into the ecology of *An. funestus* ecology highlighted by this review indicated that effective Integrated Vector Management (IVM) strategy for this vector should combine LSM with insecticide-based methods targeting adult mosquitoes in human and animal shelters. Consistent implementation of such strategies, complemented by community engagement and environmental improvements, can lead to significant reductions in *An. funestus* populations and malaria transmission. This approach is supported by historical successes in malaria control, which have demonstrated the effectiveness of integrated and community-based interventions [69,72].

### 8.1.2 Using remote sensing data for geospatial modelling of habitat suitability

Chapter 3 presented a study that combined extensive field surveys and geospatial analysis to investigate the aquatic habitats of *An. funestus* during the dry season in southeastern Tanzania. The study began with an extensive field survey conducted across 18 villages, where water bodies were systematically inspected for mosquito larvae and characterized by their physio-chemical attributes and surrounding environmental features. Following this, geospatial modelling was employed to identify key predictors of aquatic habitat availability and use.

Key findings were that first, *An. funestus* larvae in the study area predominantly inhabit river streams and ground pools during the dry season, particularly those with clear, perennial waters and shading. These habitats are crucial for sustaining vector populations through the dry season. The presence of *An. funestus* larvae was strongly positively associated with tree covered areas and reduced in built-up areas. This suggests that natural environments with dense tree cover provide favourable microclimates for this vector's development and survival. Surprisingly, human population densities were not significantly associated with the positivity of *An. funestus* larvae, indicating that the species' habitat preferences are more strongly driven by environmental and ecological factors than by proximity to human settlements [22].

The detailed characterization and spatial mapping of *An. funestus* habitats in this study provides a robust framework for guiding larval source management (LSM) efforts. By focusing on the specific habitat characteristics and environmental factors that were associated with *An. funestus* larval populations, malaria control programs can deploy more precise and effective interventions [102]. For instance, larvicides could be specifically deployed in river streams and ground pools in forested areas, which were here identified as high-risk habitats for *An. funestus* larvae in the dry season.

### 8.1.3 Seasonal adaptability and aquatic habitat utilization

In Chapter Four, I examined the seasonal habitat use of *An. funestus* larvae to understand how it responded to the changing availability of different aquatic habitat types. The study revealed significant seasonal shifts in the availability and types of aquatic habitats in southeastern Tanzania. The total area covered by water contracted from the wet to the dry season, leading to a decline in the number of aquatic habitats. Consequently, a higher proportion of the few remaining habitats were occupied by *An. funestus* during the dry season. River streams, ground pools, and ditches were prominent in the wet season, whereas only river streams and ground pools persisted significantly into the dry season. The abundance of larvae was generally higher in these persistent habitats during the dry season, reflecting the reduced availability of standing water. The interaction between season and habitat type was significant, indicating that habitat use varied with seasonal changes.

The diversity of mosquito species was greater in the wet season, with higher species richness and evenness observed in river streams and ground pools. The spatial distribution of *An. funestus* habitats showed significant clustering during the dry season, suggesting that LSM efforts could be more effectively targeted when fewer but more spatially distinct habitats are present. For larviciding, which typically has a short-term impact, targeting all available positive habitats during both the wet and dry seasons is recommended, aiming for as much coverage as logistically feasible. However, for other forms of LSM, such as habitat modification or removal, which have long-term impacts, it is more effective to conduct these interventions during the dry season. This strategy helps in identifying the actual habitats or areas where habitats form during rains and makes removal or modification more accessible and manageable.

Mathematical models show that targeting habitats in the wet season has a greater impact on malaria due to the higher number of malaria cases during this period. Therefore, for *An. funestus*, which perpetuates year-long malaria transmission, maintaining manageable levels of LSM during the dry season is valuable, especially since bed net use tends to be lower. During the wet season, we should use approaches that achieve the greatest possible coverage despite reduced accessibility, such as using drones for spraying. These findings suggest that LSM strategies need to be adaptive, targeting persistent habitats like river streams and ground pools in the dry season and a broader range of habitats in the wet season. This approach enhances malaria vector control by aligning interventions with the seasonal ecology of *An. funestus*, ensuring both immediate and sustained impacts on malaria transmission.

### 8.1.4 Influence of aquatic habitats on *An. funestus* fitness

Building on the findings from Chapters Three and Four, in Chapter Five, I explored the potentially variable significance of different types of aquatic habitats for *An. funestus* adult population dynamics and transmission potential. The primary focus was to test for differences in the "quality" of larval habitats, as defined in terms of the fitness and insecticide resistance traits of the *An. funestus* adults that emerged from them. While the importance of larval habitats is often defined in terms of their productivity (number of larvae/adults produced), here I wanted to test for differences in the survival, reproductive potential, and resistance traits in larvae emerging from different habitat types. To do this, I examined the larval productivity of different habitats and the mean body size, survival rates, and insecticide resistance of adult *An. funestus* emerging from them.

The study found clear evidence that the adult fitness and resistance traits of *An. funestus* vary significantly between larval habitat types. Ground pools were identified as the most productive habitat, followed by river streams, ditches, and rice fields. Notably, while ground pools had the highest productivity and also produced adult mosquitoes with the highest survival and insecticide resistance, other habitats like rice fields, which had moderate larval productivity, produced mosquitoes with high lipid and glycogen reserves. A surprising finding was that the higher levels of lipids in *An. funestus* emerging from rice fields did not translate into longer survival, which contrasts with previous work showing lipids as a key determinant of mosquito long-term survival [402,403]. This variability in larval habitat associations between different mosquito fitness traits (e.g., energetic reserves, survival) highlights the complex nature of environmental influences on mosquito fitness [351].

Ground pools and river streams gave rise to *An. funestus* adults with significantly different resistance profiles to various insecticides, with mosquitoes emerging from ground pools showing higher resistance to permethrin and deltamethrin compared to those from river streams. Both habitats produced mosquitoes that would be classified as 'resistant' by WHO criteria. This variation suggests that specific habitat characteristics might influence the selection pressure for resistance traits, potentially due to the presence of different *Anopheles* species or varying exposure to insecticides or other chemical pollutants [404].

The findings from this chapter indicate that LSM strategies should focus on the most productive habitats, such as ground pools, as these also produce the most fit and resistant mosquitoes. Targeting these habitats, which generate highly resistant and long-lived mosquitoes, could enhance the effectiveness of malaria control efforts. However, this information should not translate to decide which habitats to treat versus which to leave untreated. Instead, it provides insight into malaria risk in general. In cases where resources are very limited, ensuring full LSM coverage of ground pools should be the first priority, followed by targeting other habitats to achieve as high coverage as possible. Additionally, where larvicides are used, there is a needy in using long-lasting larvicides in the wet season to reduce the need for frequent reapplications.

### 8.1.5 Seasonal and habitat-specific use variations in both aquatic and adult

Building on the findings from the cross-sectional seasonal surveys of A*n. funestus* larvae in Chapter Four, this sixth chapter presented results of an in-depth longitudinal survey conducted over 12 months to provide fine-scale temporal data on larval and adult *An. funestus* dynamics throughout the year. This was done by carrying out monthly and bi-weekly surveys of *An. funestus* in larval habitats and houses in two villages of the Kilombero Valley. The aims were to assess the habitat use of *An. funestus* at the larval (aquatic habitat types) and adult (house types) stages across a year and to test for seasonal shifts in habitat use in response to varying microclimatic conditions and habitat availability. The longitudinal survey identified marked variation in the availability of larval habitats across the year, with the pattern of seasonality varying both between habitat types and villages.

These variations, in turn, influenced the distribution and abundance of larvae and adult mosquitoes in different house types [311,365].

This finer-scale longitudinal study validated results from the cross-sectional larval surveys by showing that river streams and ground pools were the most likely to remain permanent throughout the year, providing continuous oviposition sites for *An. funestus*. However, the productivity of these habitats exhibited seasonal trends, with higher larval presence and densities observed during transitional periods between the wet and dry season. This is attributed to the reduced availability of standing water, which concentrates mosquito oviposition in the fewer available habitats during these transitional months.

Additionally, geographic discrepancies should be considered when drawing conclusions. For example, while Ikungua village showed variations in habitat in the use and availability during the dry season, river streams were predominantly used by *An. funestus* for oviposition in Chikuti. This highlights the necessity for seasonally and geographically adaptive LSM strategies. During the dry season, when larval habitats are few, but highly productive, targeted LSM should be intensified to reduce the emergence of adult mosquitoes. Furthermore, the types of habitats targeted should be specific to each location.

Seasonal variation in habitat use was also evident for adult *An. funestus*, with a significant season habitat interaction for adults. Comparing different house types, *An. funestus* adult density was generally higher in traditional houses characterized by thatch roofs and mud walls. Importantly, the seasonal trend varied significantly between house types. Mud thatch houses exhibited a more pronounced increase in adult mosquito density compared to modern houses (unplastered iron and plastered iron). This seasonal house type interaction suggests that traditional houses provide more entry points and favourable conditions for mosquitoes during periods of increased rainfall and humidity, likely due to their poorer structural integrity [99,369,379,405].

This chapter reveals the significant interaction between season and habitat for both larvae and adults. Although there were variations in mosquito densities across different house types and seasons, the overall pattern of seasonality was consistent.

Therefore, it may be more effective to focus control efforts on improving housing quality, particularly in traditional houses with thatch roofs and mud walls, which consistently show higher mosquito densities. This targeted approach, rather than varying control measures month-by-month, could enhance the effectiveness of malaria vector control throughout the year.

### 8.1.6 Societal uses of aquatic habitats and implications for LSM

During the field data collection for my previous chapters, I observed that communities were utilizing the water bodies identified as *An. funestus* habitats for various domestic purposes such as drinking, washing, fishing, and livestock watering. This dual role of aquatic habitats as both mosquito oviposition sites and essential community resources highlighted the need to understand more about the control options available to these communities and to provide appropriate recommendations.

Community members highlighted the importance of raising awareness and educating about the potential risks and benefits of all LSM approaches. They advocated for open communication and active community engagement in mosquito control efforts to ensure a broad understanding and involvement in the implementation process [70,75]. Additionally, they stressed the importance of scheduling these activities at times when interventions would be most effective and when the majority of the community could actively participate. This adaptive approach is vital, given the dynamic nature of mosquito larval habitats and human activities. This perspective aligns with the broader body of evidence indicating that vector control initiatives are more successful and relevant to local needs when the community is well-informed and directly involved.

One challenge identified is balancing the timing of LSM implementation with community activities. Effective LSM requires careful planning to ensure that interventions do not disrupt essential community activities or ecological balances. For instance, larviciding might be most effective during the rainy season when mosquito breeding sites are plentiful, whereas habitat manipulation might be more feasible during the dry season when water bodies are fewer and smaller.

Moreover, community members emphasized the necessity of providing alternative water sources if existing ones were to be modified or removed. This recommendation highlights concerns over the potential negative impacts on daily lives, stressing the importance of mitigating these effects through thoughtful planning and support systems. Successful LSM interventions require a holistic understanding of local ecosystems and socio-economic dynamics, ensuring that malaria control efforts do not inadvertently compromise the resources upon which communities depend. Investing in water infrastructure presents a potential "win-win" scenario, simultaneously addressing malaria control and enhancing overall public health. Reliable and safe alternative water sources would reduce community dependence on natural habitats that serve as mosquito breeding grounds, facilitating more effective LSM strategies without compromising community needs. Improved water infrastructure can also reduce waterborne diseases and support agricultural and livestock activities.

The use of water bodies as mosquito breeding sites and community resources presents unique challenges and opportunities for LSM [74,76]. This study's findings emphasize the necessity of integrating community perspectives to ensure the acceptability of LSM strategies. Effective malaria control programs hinge on community engagement and education. The community's preference for larviciding and habitat manipulation over habitat removal, coupled with concerns about the safety and environmental impact of larvicides, shows the need for culturally sensitive and community-integrated approaches to LSM.

## 8.2 Questions arising

This thesis generated several questions that need further investigation. First, in investigating associations between *An. funestus* larval habitats and land cover (Chapter 3), occurrence was positively associated with forest cover and negatively with built-up areas in the dry season. This finding is somewhat unexpected given the strong anthropophilic behaviours of *An. funestus*, which typically suggests a preference for human-modified environments. This discrepancy raises the question of why *An. funestus* would be less associated with built-up areas in the dry season. One possibility is that the resolution of the remote sensing images used in the study was insufficient to capture finer-scale habitat features accurately. Therefore, the question remains: How can higher-resolution remote sensing data, such as UAV imagery, potentially improve the accuracy of habitat suitability models for *An. funestus*?

A common feature across all studies of *An. funestus* larvae (Chapters 3, 4, and 6) was that the availability and use of different habitat types varied geographically between study villages. For example, in Chapter 6, Ikungua village showed variations in the availability and use of aquatic habitats during the dry season, while Chikuti village predominantly utilized river streams. These geographic discrepancies could be attributed to several factors. There might be true differences in the behaviour or ecology of mosquito populations in different locations, although this is less likely if the sites are close together and involve the same species. Alternatively, variations might be caused by differences in elevation, microclimatic conditions, and hydrology, such as groundwater levels and river flow rates, which can affect the persistence of aquatic habitats. Human activities also play a role; for instance, variations in agricultural practices can influence habitat availability, with some areas using river streams for irrigation while others rely exclusively on rainfall. Soil composition, including differences in soil types and their water retention capabilities, can also affect the formation of pools suitable for mosquito oviposition. Understanding these factors is crucial for determining how different geographic regions affect the overall effectiveness of LSM strategies. Identifying the specific environmental, climatic, and microclimatic factors that most significantly influence the spatial distribution of mosquito breeding sites is essential for developing targeted and effective control measures.

The fitness and insecticide resistance observed in Chapter 5 highlighted the need for understanding the specific genetic and environmental factors contributing to insecticide resistance in *An. funestus* populations. A critical question arising from this study is: What mechanisms or factors result in some habitat types producing more resistant and fitter individuals than others? Notably, density dependence does not appear to be the primary factor, as mosquitoes emerging from the most productive habitats were among the fittest, contrary to the expectation that higher larval competition would lead to smaller body size and lower survival rates. One hypothesis that could explain this is differences in nutrient levels, agrochemicals or predator presence between habitats types. For example, ground pools were observed to produce mosquitoes with higher resistance to insecticides. Investigating whether these habitats have higher nutrient levels or the concentration of sub-lethal exposure to agricultural pesticides or other chemicals compared to other habitats could provide insights into the mechanisms behind this resistance.

Future research should focus on measuring nutrient levels, sub-lethal exposure to agricultural pesticides, and other relevant ecological factors across different larval habitats to test these hypotheses. Additionally, it is important to explore the genetic basis of resistance traits to understand how environmental factors and genetics interact to produce the observed resistance phenotypes.

The longitudinal survey of larval and adult mosquito populations across different house types presented another set of open questions. The observed time lag between peak larval densities and peak adult mosquito densities suggests a complex relationship between environmental conditions and mosquito life stages. This study identified a significant interaction between house type and seasonality, with different house types showing varying patterns of mosquito abundance throughout the year. Factors such as microclimatic conditions within houses, their proximity to larval habitats, and structural features may explain these variations. Understanding these interactions is crucial for optimizing mosquito control strategies, particularly in tailoring interventions to specific house types during peak mosquito seasons.

Analysis from Chapter 7 indicated that more than 90% of aquatic habitats were used by the community for various purposes. This dual use of habitats poses a critical question: How can community daily activities be integrated to reduce mosquito populations effectively? Chapters 3 and 4 indicated that *An. funestus* tends to thrive less in disturbed habitats and uses clear water, suggesting that human activities in these areas could be beneficial for mosquito larvae control. Understanding how these human activities impact mosquito productivity is crucial. Do habitats used by the community produce more or fewer mosquitoes? For instance, agricultural practices that change water quality or remove vegetation might reduce larval habitats' suitability for *An. funestus*. Also, how could pastoralists and brick makers be engaged in LSM activities such as applying larvicides to water bodies where cattle are taken for watering?

## 8.3 Limitations of the study

This study has provided a detailed understanding of the ecology of dominant malaria vector *An. funestus* in southeastern Tanzania. However, there are some limitations which need to be considered when considering implications.  Limitations applying to specific studies are highlighted in the associated chapters, and here I will reflect on a few additional cross-cutting issues.  Firstly, my research was conducted in only a few villages (between 2 to 18) in southeastern Tanzania, which may limit the generalizability of the findings to other locations. The environmental conditions, habitat types, and mosquito behaviours observed in this region may not be representative of other malaria-endemic regions. Therefore, further studies in varied geographic locations are necessary to confirm the broader applicability of these findings.

Another significant limitation is the reliance on a one-year observational period. This timeframe may not capture long-term trends and variations in mosquito population dynamics and environmental conditions. Long-term studies are required to understand these patterns more comprehensively over multiple years.

The accuracy of larval and adult mosquito counts, and habitat measurements depends on the methodologies employed. Potential biases in sampling techniques, identification errors, and incomplete data collection could affect the reliability of the findings. Additionally, the absence of parallel data on mosquito infection rates, Entomological Inoculation Rate (EIR), or human malaria prevalence within the communities studied is a significant limitation. This lack of epidemiological data makes it difficult to directly assess the implications of my mosquito ecology results for malaria transmission. While my thesis aimed to tie results toward the implications for malaria control, it largely assumed transmission impacts can be inferred from vector densities rather than direct transmission variables.

Another limitation is the uncertainty about the feasibility of implementing Larval Source Management (LSM) in these areas. Although community perspectives were considered, the viewpoints of other key stakeholders, such as the National Malaria Control Program (NMCP), were not fully engaged. Their participation would be important for determining if, how, and when LSM could be effectively implemented in the studied areas.

## 8.4 Conclusion

My thesis has covered several critical aspects of *An. funestus* ecology and community perspectives on malaria control which are of direct value for understanding this species' behaviour, stability, and response to interventions. This work, encompassing geospatial modelling of larval habitats, seasonal adaptability, fitness and insecticide resistance, the impact of habitat characteristics (on both aquatic and adult) on mosquito populations across different habitat and house types, and community engagement, significantly enhances our understanding of *An. funestus*. The findings emphasize the need for seasonally adaptive LSM strategies, also emphasize the importance of housing improvements in mosquito control, and the critical role of community involvement. Future research should address the questions emerging from this work, including exploring the complex interactions between microclimatic variables, vector ecology, and community practices and perceptions. Overall, this comprehensive understanding of *An. funestus* ecology, combined with community engagement, is very important for optimizing the existing vector control strategies.

## 8.5 Policy Implication for Improving malaria control strategies through targeted interventions on *Anopheles funestus*

This section summarizes the key findings from each chapter and the practical recommendation that can be used by National Malaria Control Programme (NMCP), Ministry of Heath, technical working groups supporting NMCP.

### Overview

Malaria remains a major cause of mortality, morbidity, and economic burden in Tanzania. This study offers key insights into the ecological and behavioural patterns of *An. funestus*, specifically focusing on their larval habitat use. It highlights opportunities to refine malaria control strategies, especially through **sustainable Larval Source Management (LSM)**. The central focus was on the seasonal variations in larval ecology and adult mosquito habitat use, aiming to assess the potential benefits of targeting interventions to specific aquatic habitats, house types, and seasons. This policy brief outlines these key recommendations, guiding NMCP in developing more **effective and sustainable interventions**.

## 8.6 Key findings and recommendations

### 8.6.1 Exploiting ecological vulnerabilities for targeted control

**Findings:** *An. funestus* exhibits specific ecological preferences, such as the use of larger and stable aquatic habitats for oviposition such as river streams, ground pools, dug pits and spring fed wells. Additionally, this species predominant feeds and rests inside houses, and has high resistance to pyrethroid insecticides.

**Recommendations**

- Implement integrated vector management (IVM): combine larval source management (LSM) with indoor residual spraying (IRS) and insecticide-treated nets (ITNs) to target both larval and adult stages of *An. funestus* in areas where *funestus* is dominant.
- **For LSM focus on sustainable interventions:** Prioritize interventions in **perennial habitats**, such as streams and ground pools, that are also used by communities for daily activities. These interventions should be **designed with community input**, ensuring that the use of aquatic habitats for domestic purposes is respected.
- **Provide alternative water sources:** Where LSM interventions may disrupt community access to water sources, **invest in alternative water supply systems** to minimize conflict and improve community support for LSM.

### 8.6.2 Using geospatial models to identify and target aquatic habitats of dominant malaria vectors

**Findings:** GIS and remote sensing techniques can be used to predict and map the distribution of *An. funestus* habitats. This approach can guide the targeting of control measures to specific locations.

**Recommendations**

- Train local personnel: Equip the local health workers with training in GIS and remote sensing to be able to identify and map *An. funestus* larval habitats. enhance the precision and effectiveness of malaria control efforts.
- Invest in field surveillance: While remote sensing is valuable, it cannot completely substitute the ground surveillance. The NMCP should continue to invest in technical field staff capable of conducting the intensive larval surveillance.

### 8.6.3 Adapting strategies to seasonal variations

**Findings:** *An. funestus* adapts to seasonal changes by utilizing a broader range of habitats during the wet season, maintaining year-round transmission. There is also an opportunity to align the control strategies for *An. funestus* with other malaria vectors such as *An. gambiae*, as they can sometimes co-exist in the same water bodies

**Recommendations**

- Seasonal adjustment of interventions: Modify control strategies seasonally, focusing on permanent habitats during dry months and a wider range of habitats during wet months.
- Continuous monitoring: Implement continuous ecological monitoring to track changes in habitat availability and vector behaviour, allowing for timely adjustments in control measures.

### 8.6.4 Addressing larval habitat-specific fitness traits

**Findings:** Different aquatic habitats influence the fitness traits of *An. funestus*, including energy reserves, survival, and insecticide resistance.

**Recommendations**

- Target productive habitats: Prioritize interventions in the most productive habitats, such as ground pools and river streams. Although differences in fitness and resistance traits exist between habitats, these differences are relatively moderate and generally align with productivity. Therefore, targeting the most productive sites should be effective in controlling *An. funestus* populations.

- Diversify control methods: Use a combination of biological, chemical, and environmental management strategies to reduce the development of resistance and improve overall control efficacy. Implementing a variety of control measures will help manage mosquito populations more effectively and prevent the buildup of resistance.

### 8.6.5 Integrating community engagement and education

**Findings:** Community practices and perceptions significantly impact the effectiveness of LSM. Communities prefer larviciding and habitat manipulation over habitat removal, with concerns about the safety and environmental impact of interventions.

**Recommendations**

- Understand community lifestyles: Understand people's way of life, their environments, and how they use water resources.
- Engage communities: Actively involve local communities in the planning and implementation of LSM such as habitat removal and habitat manipulation to reduce mosquito’s aquatic habitats. This can be achieved by conducting education campaigns to address concerns and promote the benefits and safety of LSM.
- Culturally sensitive approaches: Develop and implement culturally sensitive LSM strategies that align with community practices and needs.

## 8.7 Policy Implications

In order to implement these recommendations a coordinated effort between NMCP, local health authorities, researchers, and communities is required. By leveraging ecological insights, a country can enhance its malaria control strategies, reduce transmission rates, and move closer to malaria elimination. Key policy actions include:

- Investment in research and technology: Support ongoing research on ecological studies to refine intervention strategies. While technology such as GIS and remote sensing can significantly improve the identification and mapping of larval habitats, there will always be a critical need for empirical ground-truthing and validation.

- Capacity building: Provide training and resources to local health workers and communities to ensure effective implementation and sustainability of control measures.
- Integrated health programs: Foster collaboration between malaria control programs and other health initiatives to maximize impact and resource utilization.
- Enhanced focus on Larval Source Management (LSM): This work indicates that LSM should be considered for control in areas where *An. funestus* dominates transmission. Implementing LSM during the dry season, when habitats are fewer and easier to find, aligns with the WHO criteria of ‘few, fixed, and findable.’ Although there are challenges with this concept, the findings suggest that *An. funestus* habitats in the dry season meet these criteria, providing a strong argument for using LSM as a supplementary tool in areas like Kilombero.
- Community engagement: Actively involve local communities in malaria control efforts. Train and engage communities before the implementation of LSM to ensure their active participation and enhance the effectiveness of the interventions. Conduct education campaigns to address concerns about the safety and environmental impact of larvicides. Culturally sensitive approaches that align with community practices and needs can enhance participation and effectiveness.

# 9. Appendices

## Appendix 1: Codebook

People's perspectives about mosquito's aquatic habits and different LSM approach to control their breeding sites

| CODE | Brief description | When to use |
|---|---|---|
| Current malaria prevention | Comments on current control practices, experiences, challenges, and recommendations | Anytime bed net, repellants, insecticide, LSM, and any tradition methods used in context of the current practices |
| | | Any time participants speak of the challenges or hardship or barriers in the context of the current practices provide |
| | | Any time mentioned the recommendation of the current practices provide |
| | | Any time mention vulnerable group for the transmission |
| Mating behaviors | Comments on mosquito mating behaviors | Anytime mosquitoes mating/ breeding is mentioned |
| | | Comments related to personal observations of mosquito mating habits |
| Breeding habitats | Comments related to where mosquitoes breed/ rest/ hide | Any time breeding/ resting habitats are mentioned such as water, dark places, grass |
| | | Comments related to personal observations of mosquito breeding habitats |
| Domestic water sources and breeding habitats | Comments on the type of water sources and their potential as breeding habitats | Anytime they mention where they get water sources |
| | | Mosquitoes and larvae near/ or in the water sources |
| | | Any mentioned of the knowledge/ awareness of the breeding in water source for domestic uses |
| Larviciding | Comments on knowledge, awareness, | Any mention of larviciding, |
| | | Any mention of challenges associated with implementation of larviciding approaches |

| | | |
|---|---|---|
| | perception, recommendations, opinions about different LSM practices | Any mention of concerns of larviciding |
| | | Any mention of potential benefits of larviciding |
| | | Any recommendations of where and when to use any of the larviciding |
| | | Any recommendations of where and when not use larviciding approaches |
| Habitat manipulation | Comments on knowledge, awareness, perception, recommendations, opinions about different LSM practices | Any mention of cleaning habitats |
| | | Any mention of challenges associated with implementation of manipulation |
| | | Any mention of concerns of manipulation |
| | | Any mention of potential benefits of manipulation |
| | | Any recommendations of where and when to use any of the manipulation |
| | | Any recommendations of where and when not use manipulation |
| Habitat modification | Comments on knowledge, awareness, perception, recommendations, opinions about different LSM practices | Any mention of modification |
| | | Any mention of challenges associated with implementation of modification, filling habitats |
| | | Any mention of concerns of modification |
| | | Any mention of potential benefits of modification |
| | | Any recommendations of where and when to use any of modification |
| | | Any recommendations of where and when not use modification |

## Appendix 2: Field photo gallery

*These early moments started with training my research team.*

## *Data were collected in multiple ways*

*Despite the challenges of crossing rivers, long walks, thirst, and sometimes fear and tears, my journey was still possible through resilience, dedication, and teamwork*

## *Communities were part of the journey*

***Together in the field, we learn, grow, and make a difference. Our teamwork turns challenges into opportunities***

# References

1. Coleman-Jones, E. Ronald Ross and the great malaria problem: historical reference in the biological sciences. *J Biol Educ* **33**, 181–184 (1999).
2. Cox, F. E. G. History of the discovery of the malaria parasites and their vectors. *Parasit Vectors* **3**, 1-9 (2010).
3. Barber, M. A. & Hayne, T. B. Arsenic as a larvicide for anopheline larvae. *Public Health Reports (1896-1970)* 3027–3034 (1921).
4. Keiser, J., Singer, B. H. & Utzinger, J. Reducing the burden of malaria in different eco-epidemiological settings with environmental management: a systematic review. *Lancet Infect Dis* **5**, 695–708 (2005).
5. Nájera, J. A., González-Silva, M. & Alonso, P. L. Some lessons for the future from the Global Malaria Eradication Programme (1955–1969). *PLoS Med* **8**, e1000412 (2011).
6. Douglas, N. M., Burkot, T. R. & Price, R. N. Malaria eradication revisited. *Int J Epidemiol* **51**, 382–392 (2022).
7. Nosten, F., Richard-Lenoble, D. & Danis, M. A brief history of malaria. *Presse Med* **51**, 104130 (2022).
8. Trape, J.-F., Pison, G., Spiegel, A., Enel, C. & Rogier, C. Combating malaria in Africa. *Trends Parasitol* **18**, 224–230 (2002).
9. Trape, J.-F. The public health impact of chloroquine resistance in Africa. *The Intolerable Burden of Malaria: A New Look at the Numbers: Supplement to Volume 64 (1) of the American Journal of Tropical Medicine and Hygiene* (2001).
10. Trape, J.-F. *et al.* Impact of chloroquine resistance on malaria mortality. *Comptes Rendus de l'Académie des Sciences-Series III-Sciences de la Vie* **321**, 689–697 (1998).
11. Litsios, S. *The Tomorrow of Malaria.* (1996).
12. Nabarro, D. N. & Tayler, E. M. The" roll back malaria" campaign. *Science* vol. 280 2067–2068 Preprint at (1998).
13. Force, W. Bank. M. T. *Rolling Back Malaria: The World Bank Global Strategy & Booster Program.* (World Bank Publications, 2005).
14. Bhatt, S. *et al.* The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015. *Nature* **526**, 207–211 (2015).

15. World Health Organization. *World Malaria Report 2023*. (World Health Organization, 2023).
16. Takken, W. & Koenraadt, C. J. M. *Ecology of Parasite-Vector Interactions Edited By* : vol. 3.
17. Beier, J. C. Malaria parasite development in mosquitoes. *Annu Rev Entomol* **43**, 519–543 (1998).
18. Tuteja, R. Malaria− an overview. *FEBS J* **274**, 4670–4679 (2007).
19. Basu, S. & Sahi, P. K. Malaria: an update. *The Indian Journal of Pediatrics* **84**, 521–528 (2017).
20. Dondorp, A. M. *et al*. Artemisinin resistance in Plasmodium falciparum malaria. *New England journal of medicine* **361**, 455–467 (2009).
21. Al-Quraishy, S., Abdel-Maksoud, M. A., Al-Shaebi, E. M. & Dkhil, M. A. Botanical candidates from Saudi Arabian flora as potential therapeutics for Plasmodium infection. *Saudi J Biol Sci* **28**, 1374–1379 (2021).
22. Kahamba, N. F. *et al*. Geospatial modelling of dry season habitats of the malaria vector, *Anopheles funestus*, in south-eastern Tanzania. *Parasit Vectors* **17**, 38 (2024).
23. Nambunga, I. H. *et al*. Aquatic habitats of the malaria vector *Anopheles funestus* in rural south-eastern Tanzania. *Malar J* **19**, 1–11 (2020).
24. Omondi, C., Nonoh, J. & Ntabo, R. Characterization of *Anopheles funestus* Larval Habitats in Fiyoni, Kwale County, Kenya: Insights into Malaria Vector Ecology and Control. *Eur J Ecol* **9**, (2023).
25. Ngowo, H. *et al*. Using Bayesian state-space models to understand the population dynamics of the dominant malaria vector, *Anopheles funestus* in rural Tanzania. (2022).
26. Paaijmans, K. P. *et al*. Influence of climate on malaria transmission depends on daily temperature variation. *Proceedings of the National Academy of Sciences* **107**, 15135–15139 (2010).
27. Jacques Derek Charlwood. *The Ecology of Malaria Vectors*. *Journal of Chemical Information and Modeling* vol. 53 (2020).
28. Spielman, A. Ecology of Insect Vector Populations. *Am J Trop Med Hyg* **18**, 638–639 (1969).

29. Rejmánková, E., Grieco, J., Achee, N. & Roberts, D. R. Ecology of larval habitats. *Anopheles mosquitoes-New insights into malaria vectors* 397–446 (2013).

30. Gallichotte, E. N. *et al.* Towards a method for cryopreservation of mosquito vectors of human pathogens. *Cryobiology* **99**, 1–10 (2021).

31. Olotu, A. *et al.* Seven-year efficacy of RTS, S/AS01 malaria vaccine among young African children. *New England Journal of Medicine* **374**, 2519–2529 (2016).

32. Laurens, M. B. RTS, S/AS01 vaccine (Mosquirix$^{TM}$): an overview. *Hum Vaccin Immunother* **16**, 480–489 (2020).

33. White, M. T. *et al.* Immunogenicity of the RTS, S/AS01 malaria vaccine and implications for duration of vaccine efficacy: secondary analysis of data from a phase 3 randomised controlled trial. *Lancet Infect Dis* **15**, 1450–1458 (2015).

34. Moiroux, N. *et al.* Human exposure to early morning *Anopheles funestus* biting behavior and personal protection provided by long-lasting insecticidal nets. *PLoS One* **9**, (2014).

35. World Health Organization & Organization, W. H. *Global Malaria Programme Operational Strategy 2024-2030.* (World Health Organization, Geneva:World Health Organization, 2024).

36. Balter, M. Can WHO roll back malaria? Preprint at (2000).

37. Hakizimana, E. *et al.* Monitoring long-lasting insecticidal net (LLIN) durability to validate net serviceable life assumptions, in Rwanda. *Malar J* **13**, 1–8 (2014).

38. Organization, W. H. *Global Malaria Programme Operational Strategy 2024-2030.* (World Health Organization, 2024).

39. Pryce, J., Richardson, M. & Lengeler, C. Insecticide-treated nets for preventing malaria. *Cochrane Database of Systematic Reviews* (2018).

40. Barker, T. H. *et al.* Effectiveness of dual active ingredient insecticide-treated nets in preventing malaria: A systematic review and meta-analysis. *PLoS One* **18**, e0289469 (2023).

41. Okumu, F. The fabric of life: what if mosquito nets were durable and widely available but insecticide-free? *Malar J* **19**, (2020).

42. Mbuba, E. *et al.* Multi-country evaluation of the durability of pyrethroid plus piperonyl-butoxide insecticide-treated nets: study protocol. *Malar J* **22**, 30 (2023).
43. Pinchoff, J. *et al.* Targeting indoor residual spraying for malaria using epidemiological data: a case study of the Zambia experience. *Malar J* **15**, 11 (2016).
44. Choi, L., Pryce, J. & Garner, P. The combination of indoor residual spraying with insecticide-treated nets versus insecticide-treated nets alone for preventing malaria. *Cochrane Database Syst Rev* **2017**, (2017).
45. Mabaso, M. L. H., Sharp, B. & Lengeler, C. Historical review of malarial control in southern African with emphasis on the use of indoor residual house-spraying. *Tropical Medicine & International Health* **9**, 846–856 (2004).
46. Kamuliwo, M. *et al.* The changing burden of malaria and association with vector control interventions in Zambia using district-level surveillance data, 2006-2011. *Malar J* **12**, 1–9 (2013).
47. Taffese, H. S. *et al.* Malaria epidemiology and interventions in Ethiopia from 2001 to 2016. *Infect Dis Poverty* **7**, 1–9 (2018).
48. Sharp, B. L., Ridl, F. C., Govender, D., Kuklinski, J. & Kleinschmidt, I. Malaria vector control by indoor residual insecticide spraying on the tropical island of Bioko, Equatorial Guinea. *Malar J* **6 (no pagi**, (2007).
49. WHO. *World Malaria Report 2018*. (2018).
50. Coetzee, M. & Koekemoer, L. L. Molecular Systematics and Insecticide Resistance in the Major African Malaria Vector *Anopheles funestus*. *Annu Rev Entomol* **58**, 393–412 (2013).
51. Cuamba, N., Morgan, J. C., Irving, H., Steven, A. & Wondji, C. S. High Level of Pyrethroid Resistance in an *Anopheles funestus* Population of the Chokwe District in Mozambique. *PLoS One* **5**, e11010 (2010).
52. Afrane, Y. A., Bonizzoni, M. & Yan, G. *Secondary Malaria Vectors of Sub-Saharan Africa: Threat to Malaria Elimination on the Continent?* (IntechOpen, 2016).
53. Kreppel, K. S. *et al.* emergence of behavioural avoidance strategies of malaria vectors in areas of high LLin coverage in tanzania. **10**, 14527 (2020).

54. Meza, F. C. *et al*. Mosquito electrocuting traps for directly measuring biting rates and host-preferences of *Anopheles arabiensis* and *Anopheles funestus* outdoors. *Malar J* **18**, 1–11 (2019).
55. Msangi, S., Lyatuu, E., Masenga, C. & Kihumo, E. The effects of washing and duration of use of long-lasting insecticidal nets (PermaNets) on insecticidal effectiveness. *Acta Trop* **107**, 43–47 (2008).
56. World malaria report 2016. Preprint at (2016).
57. Corbel, V. & N'Guessan, R. Distribution, Mechanisms, Impact and Management of Insecticide Resistance in Malaria Vectors: A Pragmatic Review. in *Anopheles mosquitoes - New insights into malaria vectors* (InTech, 2013). doi:10.5772/56117.
58. Matiya, D. J., Philbert, A. B., Kidima, W. & Matowo, J. J. Dynamics and monitoring of insecticide resistance in malaria vectors across mainland Tanzania from 1997 to 2017: a systematic review. *Malar J* **18**, 102 (2019).
59. Killeen, G. F. Characterizing, controlling and eliminating residual malaria transmission. *Malar J* **13**, (2014).
60. Killeen, G. F., Seyoum, A. & Knols, B. G. J. Rationalizing Historical Successes of Malaria Control in Africa in Terms of Mosquito Resource Availability Management. (2004).
61. World Health Organization. *Larval Source Management – a Supplementary Measure for Malaria Vector Control. An Operational Manual*. (2018).
62. Fillinger, U. & Lindsay, S. W. Larval source management for malaria control in Africa: myths and reality. *Malar J* **10**, 353 (2011).
63. Tusting, L. S. *et al*. Mosquito larval source management for controlling malaria. *Cochrane Database of Systematic Reviews* (2013) doi:10.1002/14651858.CD008923.pub2.
64. Jacques Derek Charlwood. *The Ecology of Malaria Vectors*. *Journal of Chemical Information and Modeling* vol. 53 (2020).
65. World Health Organization, 2018. *Larval Source Management – a Supplementary Measure for Malaria Vector Control. An Operational Manual.*
66. World Health Organization, 2019. *Guidelines for Malaria Vector Control*. (World Health Organization, 2019).
67. Deane, L. M. Malaria studies and control in Brazil. *Am J Trop Med Hyg* **38**, 223–230 (1988).

68. Kitron, U. & Spielman, A. Suppression of transmission of malaria through source reduction: antianopheline measures applied in Israel, the United States, and Italy. *Rev Infect Dis* **11**, 391–406 (1989).
69. Soper, F. L. & Wilson, D. B. *Anopheles Gambiae in Brazil, 1930 to 1940.* (Rockefeller Foundation, 1943).
70. Koenraadt, C. J. M. Larval source management for malaria control: prospects for new technologies and community involvement. in *Ecology and Control of Vector-borne Diseases* 683–693 (Wageningen Academic Publishers, 2021).
71. Ferguson, H. M. *et al.* Ecology: A Prerequisite for Malaria Elimination and Eradication. *PLoS Med* **7**, e1000303 (2010).
72. Fillinger, U. & Lindsay, S. W. Larval source management for malaria control in Africa: myths and reality. *Malar J* **10**, 353 (2011).
73. Chaki, P. P. *et al.* Institutional evolution of a community-based programme for malaria control through larval source management in Dar es Salaam, United Republic of Tanzania. *Malar J* **13**, (2014).
74. Gowelo, S. *et al.* Community Participation in Habitat Management and Larviciding for the Control of Malaria Vectors in Southern Malawi. *Am J Trop Med Hyg* **108**, 51 (2023).
75. van den Berg, H. *et al.* Community-based malaria control in southern Malawi: a description of experimental interventions of community workshops, house improvement and larval source management. *Malar J* **17**, 266 (2018).
76. Gowelo, S. A. A participatory approach for malaria control in southern Malawi: Effects of the environment and community on larval source management. Preprint at (2020).
77. Sinka, M. E. *et al.* A global map of dominant malaria vectors. *Parasit Vectors* **5**, 1–11 (2012).
78. Vezenegho, S. B. *et al.* Characterization of the *Anopheles funestus* group, including *Anopheles funestus*-like, from Northern Malawi. *Trans R Soc Trop Med Hyg* **107**, 753-762 (2013).
79. Coetzee, M. *et al.* *Anopheles coluzzii* and *Anopheles amharicus*, new members of the *Anopheles gambiae* complex. *Zootaxa* **3619**, 246–274 (2013).
80. Smith, D. L., Dushoff, J., Snow, R. W. & Hay, S. I. The entomological inoculation rate and *Plasmodium falciparum* infection in African children. *Nature* **438**, 492–495 (2005).

81. Kaindoa, E. W. *et al.* Interventions that effectively target *Anopheles funestus* mosquitoes could significantly improve control of persistent malaria transmission in south-eastern Tanzania. *PLoS One* **12**, e0177807 (2017).
82. Sougoufara, S. *et al.* Biting by *Anopheles funestus* in broad daylight after use of long-lasting insecticidal nets: A new challenge to malaria elimination. *Malar J* **13**, 1–7 (2014).
83. Mwangangi, J. M. *et al.* The role of *Anopheles arabiensis* and *Anopheles coustani* in indoor and outdoor malaria transmission in Taveta District, Kenya. *Parasit Vectors* (2013) doi:10.1186/1756-3305-6-114.
84. Swellengrebel, N. H., Annecke, S. & De Meillon, B. Malaria investigations in some parts of the Transvaal and Zululand. *Malaria Investigations in some Parts of the Transvaal and Zululand.* (1931).
85. Heisch, R. B. & Harper, J. O. An epidemic of malaria in the Kenya highlands transmitted by *Anopheles funestus*. *Journal of tropical medicine and hygiene* **52**, 187–190 (1949).
86. Kaindoa, E. W. *et al.* Interventions that effectively target *Anopheles funestus* mosquitoes could significantly improve control of persistent malaria transmission in south-eastern Tanzania. *PLoS One* **12**, e0177807 (2017).
87. Finda, M. F. *et al.* Dramatic decreases of malaria transmission intensities in Ifakara , south - eastern Tanzania since early 2000s. *Malar J* 1–18 (2018) doi:10.1186/s12936-018-2511-2.
88. Matowo, N. S. *et al.* An increasing role of pyrethroid-resistant *Anopheles funestus* in malaria transmission in the Lake Zone, Tanzania. *Sci Rep* **11**, 1–13 (2021).
89. Costantini, C., Sagnon, N. F., Ilboudo-Sanogo, E., Coluzzi, M. & Boccolini, D. Chromosomal and bionomic heterogeneities suggest incipient speciation in *Anopheles funestus* from Burkina Faso. *Parassitologia* **41**, 595–612 (1999).
90. Dia, I., Diop, T., Rakotoarivony, I., Kengne, P. & Fontenille, D. Bionomics of *Anopheles gambiae* Giles, *Anopheles arabiensis* Patton, *Anopheles funestus* Giles and *Anopheles nili* (Theobald)(Diptera: Culicidae) and transmission of Palsmodium falciparum in a sudano-Guinean zone (Ngari-Senegal). *J Med Entomol* **40**, 279–283 (2003).

91. Lwetoijera, D. W. *et al*. Increasing role of *Anopheles funestus* and *Anopheles arabiensis* in malaria transmission in the Kilombero Valley, Tanzania. *Malar J* **13**, 1-10 (2014).

92. Kahamba, N. F. *et al*. Using ecological observations to improve malaria control in areas where *Anopheles funestus* is the dominant vector. *Malar J* **21**, 1–15 (2022).

93. Ramsdale, C. D., Fontaine, R. E. & Organization, W. H. Ecological investigations of *Anopheles gambiae* and *Anopheles funestus*. Preprint at https://apps.who.int/iris/handle/10665/65589 (2010).

94. Doucoure, S. *et al*. *Anopheles arabiensis* and *Anopheles funestus* biting patterns in Dielmo, an area of low level exposure to malaria vectors. *Malar J* **19**, 1–8 (2020).

95. Finda, M. F. *et al*. Dramatic decreases of malaria transmission intensities in Ifakara , south - eastern Tanzania since early 2000s. *Malar J* 1–18 (2018) doi:10.1186/s12936-018-2511-2.

96. Charlwood, J. D., Vij, R. & Billingsley, P. F. Dry season refugia of malaria-transmitting mosquitoes in a dry savannah zone of east Africa. *American Journal of Tropical Medicine and Hygiene* **62**, 726–732 (2000).

97. Kaindoa, E. W. *et al*. New evidence of mating swarms of the malaria vector, Anopheles arabiensis in Tanzania. *Wellcome Open Res* **2**, 1–19 (2017).

98. Nambunga, I. H. *et al*. Wild Populations of Malaria Vectors Can Mate Both Inside and Outside Human Dwellings. (2021).

99. Msugupakulya, B. J. *et al*. Preferred resting surfaces of dominant malaria vectors inside different house types in rural south-eastern Tanzania. *Malar J* **19**, 1–15 (2020).

100. Sattler, M. A. *et al*. Habitat characterization and spatial distribution of Anopheles sp. mosquito larvae in Dar es Salaam (Tanzania) during an extended dry period. *Malar J* **4**, 1–15 (2005).

101. Nambunga, I. H. *et al*. Aquatic habitats of the malaria vector *Anopheles funestus* in rural south-eastern Tanzania. *Malar J* **19**, 1-11 (2020).

102. Tusting, L. S. *et al*. Mosquito larval source management for controlling malaria. *Cochrane Database of Systematic Reviews* (2013) doi:10.1002/14651858.CD008923.pub2.

103. Derek Charlwood, J. *et al.* ‘Nature or nurture’: survival rate, oviposition interval, and possible gonotrophic discordance among South East Asian anophelines. *Malar J* **15**, 1–12 (2016).

104. Russell, T. L. *et al.* Linking individual phenotype to density-dependent population growth: the influence of body size on the population dynamics of malaria vectors of malaria vectors dynamics growth: the influence of body size on the population Linking individual phenotype to density-dependent population ‘Data Supplement’ Subject collections Linking individual phenotype to density-dependent population growth: the influence of body size on the population dynamics of malaria vectors. 3142–3151 (2011) doi:10.1098/rspb.2011.0153.

105. Mahgoub, M. M., Kweka, E. J. & Himeidan, Y. E. Characterisation of larval habitats, species composition and factors associated with the seasonal abundance of mosquito fauna in Gezira, Sudan. *Infect Dis Poverty* **6**, 1–10 (2017).

106. Venter, N. *et al.* Benchmarking insecticide resistance intensity bioassays for Anopheles malaria vector species against resistance phenotypes of known epidemiological significance. *Parasit Vectors* **10**, (2017).

107. Charlwood, J. D. *et al.* Density independent feeding success of malaria vectors (Diptera: Culicidae) in Tanzania. *Bull Entomol Res* **85**, 29–35 (1995).

108. Kaindoa, E. W. *et al.* Housing gaps, mosquitoes and public viewpoints: a mixed methods assessment of relationships between house characteristics, malaria vector biting risk and community perspectives in rural Tanzania. *Malar J* **17**, 1–16 (2018).

109. Lwetoijera, D. W. *et al.* Increasing role of *Anopheles funestus* and *Anopheles arabiensis* in malaria transmission in the Kilombero Valley, Tanzania. *Malar J* **13**, 1–10 (2014).

110. Lwetoijera, D. W. *et al.* A need for better housing to further reduce indoor malaria transmission in areas with high bed net coverage. *Parasit Vectors* **6**, 1–9 (2013).

111. World Health Organisation. World malaria report 2021. https://www.who.int/publications/i/item/9789240040496.

112. Bhatt, S. *et al.* The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015. **526**, 207–211 (2015).

113. Coetzee, M. & Koekemoer, L. L. Molecular Systematics and Insecticide Resistance in the Major African Malaria Vector *Anopheles funestus*. *Annu Rev Entomol* **58**, 393–412 (2013).

114. Sherrard-Smith, E. *et al*. Mosquito feeding behavior and how it influences residual malaria transmission across Africa. *Proc Natl Acad Sci U S A* **116**, 15086–15095 (2019).

115. Durnez, L. & Coosemans, M. *Residual Transmission of Malaria: An Old Issue for New Approaches*. (InTech, 2013).

116. Kiszewski, A. *et al*. A global index representing the stability of malaria transmission. *Am J Trop Med Hyg* **70**, 486–498 (2004).

117. Kelly-Hope, L. A., Hemingway, J. & McKenzie, F. E. Environmental factors associated with the malaria vectors *Anopheles gambiae* and *Anopheles funestus* in Kenya. *Malar J* **8**, 1–8 (2009).

118. Mbogo, C. M. *et al*. Spatial and temporal heterogeneity of *Anopheles* mosquitoes and Plasmodium falciparum transmission along the Kenyan coast. *Am J Trop Med Hyg* **68**, 734–742 (2003).

119. McCann, R. S. *et al*. Reemergence of *Anopheles funestus* as a vector of *Plasmodium falciparum* in Western Kenya after long-term implementation of insecticide-treated bed nets. *American Journal of Tropical Medicine and Hygiene* **90**, 597–604 (2014).

120. Okello, P. E. *et al*. Variation in malaria transmission intensity in seven sites throughout Uganda. *American Journal of Tropical Medicine and Hygiene* **75**, 219–225 (2006).

121. Matowo, N. S. *et al*. An increasing role of pyrethroid-resistant *Anopheles funestus* in malaria transmission in the Lake Zone, Tanzania. *Sci Rep* **11**, 1–13 (2021).

122. Zengenene, M. P., Munhenga, G., Chidumwa, G. & Koekemoer, L. L. Characterization of life-history parameters of an *Anopheles funestus* (Diptera: Culicidae) laboratory strain. *Journal of Vector Ecology* **46**, 24–29 (2021).

123. Woolhouse, M. E. J. *et al*. Heterogeneities in the transmission of infectious agents: Implications for the design of control programs. *Proc Natl Acad Sci U S A* **94**, 338–342 (1997).

124. Ngowo, H. S., Hape, E. E., Matthiopoulos, J. & Okumu, F. O. Fitness Characteristics of the Malaria Vector, *Anopheles funestus*, During an Attempted Laboratory Colonization. (2020).

125. Ngowo, H. *et al.* Using Bayesian state-space models to understand the population dynamics of the dominant malaria vector, *Anopheles funestus* in rural Tanzania. (2022).

126. Coetzee, M. Key to the females of Afrotropical Anopheles mosquitoes (Diptera: Culicidae). *Malar J* **19**, 1–20 (2020).

127. Coetzee, M. & Fontenille, D. Advances in the study of *Anopheles funestus*, a major vector of malaria in Africa. *Insect Biochem Mol Biol* **34**, 599–605 (2004).

128. Spillings, B. L. *et al.* A new species concealed by Anopheles funestus Giles, a major malaria vector in Africa. *Am J Trop Med Hyg* **81**, 510–515 (2009).

129. Vezenegho, S. B. *et al.* Characterization of the *Anopheles funestus* group, including Anopheles funestus-like, from Northern Malawi. *Trans R Soc Trop Med Hyg* **107**, 753–762 (2013).

130. Garros, C. *et al.* Restriction fragment length polymorphism method for the identification of major African and Asian malaria vectors within the *Anopheles funestus* and *An. minimus* groups. *Am J Trop Med Hyg* **70**, 260–265 (2004).

131. Koekemoer, L. L. *et al.* Impact of the Rift Valley on restriction fragment length polymorphism typing of the major African malaria vector *Anopheles funestus* (Diptera: Culicidae). *J Med Entomol* **43**, 1178–1184 (2006).

132. Gillies, M. T., & Coetzee, M. A supplement to the *Anophelinae* of Africa south of the Sahara (Afrotropical region. (1987).

133. Meillon, B. De. On *Anopheles funestus* and its allies in the Transvaal. *Ann Trop Med Parasitol* **27**, 83–97 (1933).

134. Gillies, M. T. & De Meillon, B. The *Anophelinae* of Africa south of the Sahara (Ethiopian zoogeographical region). *The Anophelinae of Africa south of the Sahara (Ethiopian Zoogeographical Region)*. (1968).

135. Pinda, P. G. *et al.* Comparative assessment of insecticide resistance phenotypes in two major malaria vectors, *Anopheles funestus* and *Anopheles arabiensis* in south-eastern Tanzania. *Malar J* **19**, 408 (2020).

136. Das, S., Muleba, M., Stevenson, J. C., Pringle, J. C. & Norris, D. E. Beyond the entomological inoculation rate: characterizing multiple blood feeding

behavior and Plasmodium falciparum multiplicity of infection in Anopheles mosquitoes in northern Zambia. *Parasit Vectors* **10**, (2017).

137. Andrianaivolambo, L. *et al*. Anthropophilic mosquitoes and malaria transmission in the eastern foothills of the central highlands of Madagascar. *Acta Trop* **116**, 240–245 (2010).
138. Tchouassi, D. P. *et al*. Characterization of malaria transmission by vector populations for improved interventions during the dry season in the Kpone-on-Sea area of coastal Ghana. *Parasit Vectors* **5**, (2012).
139. Diakité, N. R. *et al*. Spatial and temporal variation of malaria entomological parameters at the onset of a hydro-agricultural development in central Côte d'Ivoire. *Malar J* **14**, 1–11 (2015).
140. Ossè, R. A. *et al*. Involvement of Anopheles nili in Plasmodium falciparum transmission in North Benin. *Malar J* **18**, 1–10 (2019).
141. Ogola, E. O. *et al*. Insights into malaria transmission among *Anopheles funestus* mosquitoes, Kenya. *Parasit Vectors* **11**, 1–10 (2018).
142. Gillies, M. T. & Wilkes, T. J. A study of the age-composition of populations of *Anopheles gambiae* Giles and *An. funestus* Giles in North-Eastern Tanzania. *Bull Entomol Res* **56**, 237–262 (1965).
143. Burke, A. *et al*. *Anopheles parensis* contributes to residual malaria transmission in South Africa. *Malar J* **18**, (2019).
144. Kamau, L., Koekemoer, L. L., Hunt, R. H. & CoETZEE, M. *Anopheles parensis*: the main member of the *Anopheles funestus* species group found resting inside human dwellings in Mwea area of central Kenya toward the end of the rainy season. *J Am Mosq Control Assoc* **19**, 130–133 (2003).
145. Temu, E. A., Minjas, J. N., Tuno, N., Kawada, H. & Takagi, M. Identification of four members of the *Anopheles funestus* (Diptera: Culicidae) group and their role in *Plasmodium falciparum* transmission in Bagamoyo coastal Tanzania. *Acta Trop* **102**, 119–125 (2007).
146. Mutuku, F. M. *et al*. Impact of insecticide-treated bed nets on malaria transmission indices on the south coast of Kenya. *Malar J* **10**, 356 (2011).
147. Degefa, T. *et al*. Indoor and outdoor malaria vector surveillance in western Kenya: implications for better understanding of residual transmission. *Malar J* **16**, (2017).

148. Hakizimana, E. *et al*. Spatio-temporal distribution of mosquitoes and risk of malaria infection in Rwanda. *Acta Trop* **182**, 149–157 (2018).

149. Daygena, T. Y., Massebo, F. & Lindtjorn, B. Variation in species composition and infection rates of Anopheles mosquitoes at different altitudinal transects, and the risk of malaria in the highland of Dirashe Woreda, south Ethiopia. *Parasites & Vectors [Electronic Resource]* **10**, 343 (2017).

150. Kibret, S., Wilson, G. G., Ryder, D., Tekie, H. & Petros, B. Malaria impact of large dams at different eco-epidemiological settings in Ethiopia. *Trop Med Health* **45**, 1–14 (2017).

151. Kerah-Hinzoumbé, C. *et al*. Malaria vectors and transmission dynamics in Goulmoun, a rural city in south-western Chad. *BMC Infect Dis* **9**, 1–11 (2009).

152. Antonio-Nkondjio, C. *et al*. Malaria vectors and urbanization in the equatorial forest region of south Cameroon. *Trans R Soc Trop Med Hyg* **99**, 347–354 (2005).

153. Oyewole, I. O. *et al*. Species composition and role of Anopheles mosquitoes in malaria transmission along Badagry Axis of Lagos Lagoon, Lagos, Nigeria. *Int J Insect Sci* **2**, IJIS-S4698 (2010).

154. Debrah, I. *et al*. Larval ecology and bionomics of *Anopheles funestus* in highland and lowland sites in western Kenya. *PLoS One* **16**, e0255321 (2021).

155. Larval habitats of *Anopheles funestus*: mosquito vector of malaria - Numerisud. https://numerisud.ird.fr/documents-et-films/photos/Gites-larvaires-d-*Anopheles-funestus*-moustique-vecteur-de-paludisme2.

156. Kweka, E. J., Munga, S., Himeidan, Y., Githeko, A. K. & Yan, G. Assessment of mosquito larval productivity among different land use types for targeted malaria vector control in the western Kenya highlands. *Parasit Vectors* **8**, (2015).

157. Symes, C. B. *Anopheles funestus* (Giles) as a ‘domestic’breeder. *Ann Trop Med Parasitol* **30**, 361–364 (1936).

158. Ramsdale, C. D., Fontaine, R. E. & Organization, W. H. Ecological investigations of *Anopheles gambiae* and *Anopheles funestus*.

159. Kweka, E. J. *et al*. Anopheline Larval Habitats Seasonality and Species Distribution: A Prerequisite for Effective Targeted Larval Habitats Control Programmes. *PLoS One* **7**, (2012).

160. Diuk-Wasser, M. A. *et al.* Mapping rice field anopheline breeding habitats in Mali, West Africa, using Landsat ETM+ sensor data. *Int J Remote Sens* **25**, 359–376 (2004).

161. Ngowo, H. S., Kaindoa, E. W., Matthiopoulos, J., Ferguson, H. M. & Okumu, F. O. Variations in household microclimate affect outdoor-biting behaviour of malaria vectors. *Wellcome Open Res* **2**, (2017).

162. Omukunda, E., Githeko, A., Ndong'a, M. F., Mushinzimana, E. & Yan, G. Effect of swamp cultivation on distribution of anopheline larval habitats in Western Kenya. *J Vector Borne Dis* **49**, 61 (2012).

163. Minakawa, N., Sonye, G., Dida, G. O., Futami, K. & Kaneko, S. Recent reduction in the water level of Lake Victoria has created more habitats for Anopheles funestus. *Malar J* **7**, 1–6 (2008).

164. Antonio-Nkondjio, C. *et al.* Distribution and larval habitat characterization of Anopheles moucheti, Anopheles nili, and other malaria vectors in river networks of southern Cameroon. *Acta Trop* **112**, 270–276 (2009).

165. Koekemoer, L. L. *et al.* Larval salinity tolerance of two members of the Anopheles funestus group. *Med Vet Entomol* **28**, 187–192 (2014).

166. Barreaux, A. M. G., Stone, C. M., Barreaux, P. & Koella, J. C. The relationship between size and longevity of the malaria vector Anopheles gambiae (s.s.) depends on the larval environment. *Parasit Vectors* **11**, 485 (2018).

167. Takken, W. *et al.* Larval nutrition differentially affects adult fitness and Plasmodium development in the malaria vectors Anopheles gambiae and Anopheles stephensi. *Parasit Vectors* **6**, 1–10 (2013).

168. Lyons, C. L., Coetzee, M. & Chown, S. L. Stable and fluctuating temperature effects on the development rate and survival of two malaria vectors, Anopheles arabiensis and Anopheles funestus. *Parasit Vectors* **6**, 1–9 (2013).

169. Garrett-Jones, C., Ferreira Neto, J. A. & Organization, W. H. *The Prognosis for Interruption of Malaria Transmission through Assessment of the Mosquito's Vectorial Capacity*. (1964).

170. Mayagaya, V. S. *et al.* The impact of livestock on the abundance, resting behaviour and sporozoite rate of malaria vectors in southern Tanzania. *Malar J* **14**, 17 (2015).

171. Killeen, G. F. Characterizing, controlling and eliminating residual malaria transmission. *Malar J* **13**, (2014).

172. Takken, W. & Verhulst, N. O. Host Preferences of Blood-Feeding Mosquitoes. in *Annual Review of Entomology, Vol 58* (ed. Berenbaum, M. R.) vol. 58 433-+ (2013).
173. Kreppel, K. S. *et al*. emergence of behavioural avoidance strategies of malaria vectors in areas of high LLin coverage in tanzania. **10**, 14527 (2020).
174. Meza, F. C. *et al*. Mosquito electrocuting traps for directly measuring biting rates and host-preferences of Anopheles arabiensis and Anopheles funestus outdoors. *Malar J* **18**, 1–11 (2019).
175. Kaindoa, E. W. *et al*. New evidence of mating swarms of the malaria vector, Anopheles arabiensis in Tanzania. *Wellcome Open Res* **2**, 1–19 (2017).
176. Zawada, J. W. *et al*. Molecular and physiological analysis of Anopheles funestus swarms in Nchelenge, Zambia. *Malar J* **17**, 1–7 (2018).
177. Charlwood, J. D., Thompson, R. & Madsen, H. *Observations on the Swarming and Mating Behaviour of Anopheles Funestus from Southern Mozambique*. (2003).
178. Charlwood, J. D., Cuamba, N., Tomás, E. V. E., Jt Briët, O. & Briët, O. J. T. Living on the edge: a longitudinal study of Anopheles funestus in an isolated area of Mozambique. *Malar J* **12**, 1–10 (2013).
179. Nambunga, I. H. *et al*. Wild Populations of Malaria Vectors Can Mate Both Inside and Outside Human Dwellings. (2021).
180. Lehmann, T. *et al*. Aestivation of the African malaria mosquito, Anopheles gambiae in the Sahel. *American Journal of Tropical Medicine and Hygiene* **83**, 601–606 (2010).
181. Hunt, R. H., Brooke, B. D., Pillay, C., Koekemoer, L. L. & Coetzee, M. Laboratory selection for and characteristics of pyrethroid resistance in the malaria vector Anopheles funestus. *Med Vet Entomol* **19**, 271–275 (2005).
182. Hargreaves, K. *et al*. Anopheles funestus resistant to pyrethroid insecticides in South Africa. *Med Vet Entomol* **14**, 181–189 (2000).
183. Pringle, G. Malaria in the pare area of Tanzania III The course of malaria transmission since the suspension of an experimental programme of residual insecticide spraying. *Trans R Soc Trop Med Hyg* **61**, 69–79 (1967).
184. Mapua, S. A. *et al*. Exceedingly high proportions of Plasmodium-infected Anopheles funestus mosquitoes in two villages in the Kilombero valley, south-eastern Tanzania. *medRxiv* (2021).

185. Agyekum, T. P. *et al.* A systematic review of the effects of temperature on Anopheles mosquito development and survival: Implications for malaria control in a future warmer climate. *Int J Environ Res Public Health* **18**, 7255 (2021).
186. Dambach, P. *et al.* Reduction of malaria vector mosquitoes in a large-scale intervention trial in rural Burkina Faso using Bti based larval source management. *Malar J* **18**, 311 (2019).
187. Derua, Y. A., Kweka, E. J., Kisinza, W. N., Githeko, A. K. & Mosha, F. W. Bacterial larvicides used for malaria vector control in sub-Saharan Africa: review of their effectiveness and operational feasibility. *Parasit Vectors* **12**, (2019).
188. Hardy, A., Makame, M., Cross, D., Majambere, S. & Msellem, M. Using low-cost drones to map malaria vector habitats. *Parasit Vectors* **10**, (2017).
189. Hardy, A. *et al.* Mapping hotspots of malaria transmission from pre-existing hydrology, geology and geomorphology data in the pre-elimination context of Zanzibar, United Republic of Tanzania. *Parasit Vectors* **8**, 1–15 (2015).
190. Hardy, A., Makame, M., Cross, D., Majambere, S. & Msellem, M. Using low-cost drones to map malaria vector habitats. *Parasit Vectors* **10**, (2017).
191. Pérez-Pacheco, R., Rodríguez-Hernández, C., Lara-Reyna, J., Montes-Belmont, R. & Ruiz-Vega, J. Control of the mosquito Anopheles pseudopunctipennis (Diptera: Culicidae) with Romanomermis iyengari (Nematoda: Mermithidae) in Oaxaca, Mexico. *Biological Control* **32**, 137–142 (2005).
192. Macdonald, G. Epidemiological basis of malaria control. *Bull World Health Organ* **15**, 613 (1956).
193. Soper, F. L. & Wilson, D. B. *Anopheles Gambiae in Brazil, 1930 to 1940*. (Rockefeller Foundation, 1943).
194. Chaki, P. P. *et al.* Institutional evolution of a community-based programme for malaria control through larval source management in Dar es Salaam, United Republic of Tanzania. *Malar J* **13**, (2014).
195. Fillinger, U. *et al.* A tool box for operational mosquito larval control: preliminary results and early lessons from the Urban Malaria Control Programme in Dar es Salaam, Tanzania. *Malar J* **7**, 1–25 (2008).

196. Minakawa, N., Seda, P. & Yan, G. Influence of host and larval habitat distribution on the abundance of African malaria vectors in western Kenya. *American Journal of Tropical Medicine and Hygiene* **67**, 32–38 (2002).

197. Venter, N. *et al.* Benchmarking insecticide resistance intensity bioassays for Anopheles malaria vector species against resistance phenotypes of known epidemiological significance. *Parasit Vectors* **10**, (2017).

198. Okia, M. *et al.* Insecticide resistance status of the malaria mosquitoes: Anopheles gambiae and Anopheles funestus in eastern and northern Uganda. *Malar J* **17**, 157 (2018).

199. Nkemngo, F. N. *et al.* Multiple insecticide resistance and Plasmodium infection in the principal malaria vectors Anopheles funestus and Anopheles gambiae in a forested locality close to the Yaoundé airport, Cameroon. *Wellcome Open Res* **5**, (2020).

200. Dabiré, K. R. *et al.* Anopheles funestus (Diptera: Culicidae) in a humid savannah area of western Burkina Faso: bionomics, insecticide resistance status, and role in malaria transmission. *J Med Entomol* **44**, 990–997 (2007).

201. Wondji, C. S. *et al.* Identification and distribution of a GABA receptor mutation conferring dieldrin resistance in the malaria vector Anopheles funestus in Africa. *Insect Biochem Mol Biol* **41**, 484–491 (2011).

202. Tchigossou, G. *et al.* Molecular basis of permethrin and DDT resistance in an Anopheles funestus population from Benin. *Parasit Vectors* **11**, 1–13 (2018).

203. Hancock, P. A. *et al.* Mapping trends in insecticide resistance phenotypes in African malaria vectors. *PLoS Biol* **18**, e3000633 (2020).

204. Tchouakui, M. *et al.* A marker of glutathione S-transferase-mediated resistance to insecticides is associated with higher Plasmodium infection in the African malaria vector Anopheles funestus. *Sci Rep* **9**, 1–12 (2019).

205. Okoye, P. N., Brooke, B. D., Koekemoer, L. L., Hunt, R. H. & Coetzee, M. Characterisation of DDT, pyrethroid and carbamate resistance in Anopheles funestus from Obuasi, Ghana. *Trans R Soc Trop Med Hyg* **102**, 591–598 (2008).

206. Odero, J. O. *et al.* Genetic Markers Associated with the Widespread Insecticide Resistance in Malaria Vector Anopheles funestus Populations across Tanzania. (2024).

207. Wangrawa, D. W., Odero, J. O., Baldini, F., Okumu, F. & Badolo, A. Distribution and insecticide resistance profile of the major malaria vector

Anopheles funestus group across the African continent. *Med Vet Entomol* (2024).

208. Programme, W. H. Organization. G. M. Global plan for insecticide resistance management in malaria vectors.
209. Churcher, T. S., Lissenden, N., Griffin, J. T., Worrall, E. & Ranson, H. The impact of pyrethroid resistance of the efficacy and effectiveness of bednets for malaria control in Africa. *Elife* **5**, (2016).
210. Gleave, K., Lissenden, N., Chaplin, M., Choi, L. & Ranson, H. Piperonyl butoxide (PBO) combined with pyrethroids in insecticide-treated nets to prevent malaria in Africa. *Cochrane Database of Systematic Reviews* (2021).
211. World Health Organization. WHO Guidelines for Malaria. (2021).
212. Mosha, J. F. *et al.* Effectiveness and cost-effectiveness against malaria of three types of dual-active-ingredient long-lasting insecticidal nets (LLINs) compared with pyrethroid-only LLINs in Tanzania: a four-arm, cluster-randomised trial. *The Lancet* **399**, 1227–1241 (2022).
213. World Health Organization (WHO). Welcome to Vector Control Product Prequalification | WHO - Prequalification of Medical Products (IVDs, Medicines, Vaccines and Immunization Devices, Vector Control). https://extranet.who.int/pqweb/vector-control-products.
214. Choi, L., Pryce, J. & Garner, P. The combination of indoor residual spraying with insecticide-treated nets versus insecticide-treated nets alone for preventing malaria. *Cochrane Database Syst Rev* **2017**, (2017).
215. Smith, A. & Pringue, G. Malaria in the Taveta area of Kenya and Tanzania. Part V. Transmission eight years after the spraying period. *East Afr Med J* **44**, 469–474 (1967).
216. Mabaso, M. L. H., Sharp, B. & Lengeler, C. Historical review of malarial control in southern African with emphasis on the use of indoor residual house-spraying. *Tropical Medicine & International Health* **9**, 846–856 (2004).
217. Coetzee, M. *et al.* Malaria in South Africa: 110 years of learning to control the disease. *South African Medical Journal* **103**, 770–778 (2013).
218. Msugupakulya, B. J. *et al.* Preferred resting surfaces of dominant malaria vectors inside different house types in rural south-eastern Tanzania. *Malar J* **19**, 1–15 (2020).

219. Kirby, M. J. *et al*. Effect of two different house screening interventions on exposure to malaria vectors and on anaemia in children in The Gambia: a randomised controlled trial. *The Lancet* **374**, 998–1009 (2009).

220. Kaindoa, E. W., Mmbando, A. S., Shirima, R., Hape, E. E. & Okumu, F. O. Insecticide-treated eave ribbons for malaria vector control in low-income communities. *Malar J* **20**, 1–12 (2021).

221. Killeen, G. F. *et al*. Control of Malaria Vector Mosquitoes by Insecticide-Treated Combinations of Window Screens and Eave Baffles. *Emerg Infect Dis* **23**, 782–789 (2017).

222. Sternberg, E. D. *et al*. Impact and cost-effectiveness of a lethal house lure against malaria transmission in central Côte d'Ivoire: a two-arm, cluster-randomised controlled trial. *The Lancet* **397**, 805–815 (2021).

223. Killeen, G. F., Govella, N. J., Lwetoijera, D. W. & Okumu, F. O. Most outdoor malaria transmission by behaviourally-resistant Anopheles arabiensis is mediated by mosquitoes that have previously been inside houses. *Malar J* **15**, 225 (2016).

224. Okumu, F. O., Govella, N. J., Moore, S. J., Chitnis, N. & Killeen, G. F. Potential Benefits, Limitations and Target Product-Profiles of Odor-Baited Mosquito Traps for Malaria Control in Africa. *PLoS ONE [Electronic Resource]* **5**, e11573 (2010).

225. Nolan, T. Control of malaria-transmitting mosquitoes using gene drives. *Philosophical Transactions of the Royal Society B* **376**, 20190803 (2021).

226. James, S. *et al*. Pathway to deployment of gene drive mosquitoes as a potential biocontrol tool for elimination of malaria in sub-Saharan Africa: recommendations of a scientific working group. *Am J Trop Med Hyg* **98**, 1 (2018).

227. Quinn, C., Anthousi, A., Wondji, C. & Nolan, T. CRISPR-mediated knock-in of transgenes into the malaria vector Anopheles funestus. *G3 Genes|Genomes|Genetics* **11**, (2021).

228. Thizy, D. *et al*. Guidance on stakeholder engagement practices to inform the development of area-wide vector control methods. *PLoS Negl Trop Dis* **13**, e0007286 (2019).

229. Resnik, D. B. Two unresolved issues in community engagement for field trials of genetically modified mosquitoes. *Pathog Glob Health* **113**, 238–245 (2019).

230. Mwangungulu, S. P. *et al.* Crowdsourcing Vector Surveillance: Using Community Knowledge and Experiences to Predict Densities and Distribution of Outdoor-Biting Mosquitoes in Rural Tanzania. *PLoS One* **11**, e0156388 (2016).

231. Sawadogo, S. P. *et al.* Targeting male mosquito swarms to control malaria vector density. *PLoS ONE [Electronic Resource]* **12**, (2017).

232. Finda, M. F. *et al.* Linking human behaviours and malaria vector biting risk in south-eastern Tanzania. *PLoS One* **14**, 1–23 (2019).

233. Lupenza, E. T. *et al.* Using pastoralist community knowledge to locate and treat dry-season mosquito breeding habitats with pyriproxyfen to control Anopheles gambiae s.l. and Anopheles funestus s.l. in rural Tanzania. *Parasitol Res* (2021).

234. Urio, N. H. *et al.* Effects of agricultural pesticides on the susceptibility and fitness of malaria vectors in rural south-eastern Tanzania. *Parasit Vectors* **15**, 1–14 (2022).

235. World Health Organisation. World malaria report 2021. https://www.who.int/publications/i/item/9789240040496 (2021).

236. Hemingway, J. & Ranson, H. Insecticide Resistance in Insect Vectors of Human Disease. *Annu Rev Entomol* **45**, 371–391 (2000).

237. Hemingway, J. *et al.* Tools and strategies for malaria control and elimination: what do we need to achieve a grand convergence in malaria? *PLoS Biol* **14**, e1002380 (2016).

238. Msugupakulya, B. J. *et al.* Changes in contributions of different Anopheles vector species to malaria transmission in east and southern Africa from 2000 to 2022. *Parasit Vectors* **16**, 408 (2023).

239. Takken, W. & Koenraadt, C. J. M. *Ecology of Parasite-Vector Interactions*. vol. 3 (2013).

240. Pinda, P. G. *et al.* Comparative assessment of insecticide resistance phenotypes in two major malaria vectors, Anopheles funestus and Anopheles arabiensis in south-eastern Tanzania. *Malar J* **19**, 408 (2020).

241. Kweka, E. J., Munga, S., Himeidan, Y., Githeko, A. K. & Yan, G. Assessment of mosquito larval productivity among different land use types for targeted malaria vector control in the western Kenya highlands. *Parasit Vectors* **8**, (2015).

242. Debrah, I. *et al.* Larval ecology and bionomics of Anopheles funestus in highland and lowland sites in western Kenya. *PLoS One* **16**, e0255321 (2021).

243. Nambunga, I. H. *et al.* Aquatic habitats of the malaria vector Anopheles funestus in rural south-eastern Tanzania. *Malar J* **19**, 1–11 (2020).

244. Mala, A. O. *et al.* Dry season ecology of Anopheles gambiae complex mosquitoes at larval habitats in two traditionally semi-arid villages in Baringo, Kenya. *Parasit Vectors* **4**, (2011).

245. WorldData.info. Climate in Morogoro, Tanzania. https://www.worlddata.info/africa/tanzania/climate-morogoro.php.

246. Mapua, S. A. *et al.* Persistently high proportions of Plasmodium-infected Anopheles funestus mosquitoes in two villages in the Kilombero valley, south-eastern Tanzania. *medRxiv* (2021).

247. Finda, M. F. *et al.* Linking human behaviours and malaria vector biting risk in south-eastern Tanzania. *PLoS One* **14**, 1–23 (2019).

248. Swai, J. K. *et al.* Protecting migratory farmers in rural Tanzania using eave ribbons treated with the spatial mosquito repellent, transfluthrin. *Malar J* **18**, (2019).

249. Gillies, M. T. & Wilkes, T. J. A study of the age-composition of populations of Anopheles gambiae Giles and A. funestus Giles in North-Eastern Tanzania. *Bull Entomol Res* **56**, 237–262 (1965).

250. Gillies, M. T. & De Meillon, B. The Anophelinae of Africa south of the Sahara (Ethiopian zoogeographical region). *The Anophelinae of Africa south of the Sahara (Ethiopian Zoogeographical Region).* (1968).

251. Gillies, M. T. & Coetzee, M. A supplement to the Anophelinae of Africa South of the Sahara. *Publ S Afr Inst Med Res* **55**, 1–143 (1987).

252. NASA, M. AIST, Japan Spacesystems, US/Japan ASTER Science Team, 2019. ASTER Global Digital Elevation Model V003. *NASA EOSDIS Land Processes DAAC*.

253. Esri Releases New 2020 Global Land Cover Map - Esri. https://www.esri.com/about/newsroom/announcements/esri-releases-new-2020-global-land-cover-map/.

254. Brown, C. F. *et al.* Dynamic World, Near real-time global 10 m land use land cover mapping. *Sci Data* **9**, 1–17 (2022).

255. Charlwood, J. D., Vij, R. & Billingsley, P. F. Dry season refugia of malaria-transmitting mosquitoes in a dry savannah zone of east Africa. *Am J Trop Med Hyg* **62**, 726–732 (2000).

256. Verdonschot, P. F. M. & Besse-Lototskaya, A. A. Flight distance of mosquitoes (Culicidae): A metadata analysis to support the management of barrier zones around rewetted and newly constructed wetlands. *Limnologica* **45**, 69–79 (2014).

257. Schiavina, M., Freire, S. & MacManus, K. GHS-POP R2022A—GHS Population Grid Multitemporal (1975–2030). *European Commission, Joint Research Centre* (2022).

258. R Core Team. R: A Language and Environment for Statistical Computing. Preprint at (2019).

259. QGIS Development Team, A. E. QGIS geographic information system. *Open source geospatial foundation project* (2018).

260. Chang, W., Cheng, J., Allaire, J. J., Xie, Y. & McPherson, J. Shiny: web application framework for R.(R package version 1.5. 0)[Computer software]. Preprint at (2018).

261. Hosmer Jr, D. W., Lemeshow, S. & Sturdivant, R. X. *Applied Logistic Regression*. vol. 398 (John Wiley & Sons, 2013).

262. Dia, I., Guelbeogo, M. W. & Ayala, D. Advances and Perspectives in the Study of the Malaria Mosquito Anopheles funestus. *Anopheles mosquitoes - New insights into malaria vectors* (2013) doi:10.5772/55389.

263. Mwangangi, J. M. *et al. Mwangangi Joseph et al 2007 Spatial Distribution and Habitat Characterisation of Anopheles Larvae along the Kenyan Coast.Pdf*. *Journal of Vector Borne Diseases* vol. 44 44–51 (2007).

264. Omukunda, E., Githeko, A., Ndong'a, M. F., Mushinzimana, E. & Yan, G. Effect of swamp cultivation on distribution of anopheline larval habitats in Western Kenya. *J Vector Borne Dis* **49**, 61 (2012).

265. Minakawa, N., Sonye, G., Dida, G. O., Futami, K. & Kaneko, S. Recent reduction in the water level of Lake Victoria has created more habitats for Anopheles funestus. *Malar J* **7**, 1–6 (2008).

266. Mendis, C. *et al*. Anopheles arabiensis and An. funestus are equally important vectors of malaria in Matola coastal suburb of Maputo, southern Mozambique. *Med Vet Entomol* **14**, 171–180 (2000).

267. Minakawa, N., Mutero, C. M., Githure, J. I., Beier, J. C. & Yan, G. Spatial distribution and habitat characterization of anopheline mosquito larvae in western Kenya. *American Journal of Tropical Medicine and Hygiene* **61**, 1010–1016 (1999).

268. Munga, S. *et al.* Land use and land cover changes and spatiotemporal dynamics of anopheline larval habitats during a four-year period in a highland community of Africa. *American Journal of Tropical Medicine and Hygiene* **81**, 1079–1084 (2009).

269. Lupenza, E. T. *et al.* Using pastoralist community knowledge to locate and treat dry-season mosquito breeding habitats with pyriproxyfen to control Anopheles gambiae s.l. and Anopheles funestus s.l. in rural Tanzania. *Parasitol Res* (2021).

270. Silver, J. B. *Mosquito Ecology: Field Sampling Methods*. (springer science & business media, 2007).

271. Zengenene, M. P. *et al.* Anopheles species composition and breeding habitat characterisation in Chiredzi District, Zimbabwe. *African Entomology* **28**, 84–94 (2020).

272. Ferguson, H. M. *et al.* Ecology: A Prerequisite for Malaria Elimination and Eradication. *PLoS Med* **7**, e1000303 (2010).

273. Service, M. W. Agricultural development and arthropod-borne diseases: a review. *Rev Saude Publica* **25**, 165–178 (1991).

274. Kar, N. P., Kumar, A., Singh, O. P., Carlton, J. M. & Nanda, N. A review of malaria transmission dynamics in forest ecosystems. *Parasit Vectors* **7**, 1–12 (2014).

275. Burkett-Cadena, N. D. & Vittor, A. Y. Deforestation and vector-borne disease: Forest conversion favors important mosquito vectors of human pathogens. *Basic Appl Ecol* **26**, 101–110 (2018).

276. Yasuoka, J. & Levins, R. Impact of deforestation and agricultural development on anopheline ecology and malaria epidemiology. *Am J Trop Med Hyg* **76**, 450–460 (2007).

277. Nicholas, K. *et al.* Abundance and Distribution of Malaria Vectors in Various Aquatic Habitats and Land Use Types in Kakamega County, Highlands of Western Kenya. *Ethiop J Health Sci* **31**, (2021).

278. Editors, W. A. C. G. *Ecological Modelling Applied to Entomology*. *Ecological Modelling Applied to Entomology* (2014). doi:10.1007/978-3-319-06877-0.

279. Organization, W. H. Larval source management: a supplementary malaria vector control measure: an operational manual. (2013).

280. Paaijmans, K. P. *et al*. Observations and model estimates of diurnal water temperature dynamics in mosquito breeding sites in western Kenya. *Hydrological Processes: An International Journal* **22**, 4789–4801 (2008).

281. Smith, M. W. *et al*. Incorporating hydrology into climate suitability models changes projections of malaria transmission in Africa. *Nat Commun* **11**, (2020).

282. Hardy, A. J. *et al*. Habitat Hydrology and Geomorphology Control the Distribution of Malaria Vector Larvae in Rural Africa. *PLoS ONE [Electronic Resource]* **8**, (2013).

283. Byrne, I. *et al*. Environmental and spatial risk factors for the larval habitats of Plasmodium knowlesi vectors in Sabah, Malaysian Borneo. *Sci Rep* **11**, 11810 (2021).

284. Minakshi, M. *et al*. High-accuracy detection of malaria mosquito habitats using drone-based multispectral imagery and Artificial Intelligence (AI) algorithms in an agro-village peri-urban pastureland intervention site (Akonyibedo) in Unyama SubCounty, Gulu District, Northern . *J Public Health Epidemiol* **12**, 202–217 (2020).

285. Kweka, E. J. *et al*. Anopheline Larval Habitats Seasonality and Species Distribution: A Prerequisite for Effective Targeted Larval Habitats Control Programmes. *PLoS One* **7**, (2012).

286. Mwangangi, J. M., Muturi, E. J. & Mbogo, C. M. Seasonal mosquito larval abundance and composition in Kibwezi, lower eastern Kenya. *J Vector Borne Dis* **46**, 65–71 (2009).

287. Makuwaza, A. *et al*. Characterization of Potential Larval Habitats and Spatial Distribution of Anopheles Species Larvae During the Dry Season in Mutare City, Zimbabwe: a Cross-sectional Study. *Res Sq* (2021) doi:10.21203/rs.3.rs-178393/v1.

288. Walker, M. *et al*. Temporal and micro-spatial heterogeneity in the distribution of Anopheles vectors of malaria along the Kenyan coast. *Parasit Vectors* **6**, 1–14 (2013).

289. Diakité, N. R. *et al*. Spatial and temporal variation of malaria entomological parameters at the onset of a hydro-agricultural development in central Côte d'Ivoire. *Malar J* **14**, 1–11 (2015).

290. Shaman, J., Stieglitz, M., Stark, C., Le Blancq, S. & Cane, M. Using a dynamic hydrology model to predict mosquito abundances in flood and swamp water. *Emerg Infect Dis* **8**, 8 (2002).

291. Mahenge, H. H. *et al*. Common predators and factors influencing their abundance in Anopheles funestus aquatic habitats in rural south-eastern Tanzania. *PLoS One* **18**, e0287655 (2023).

292. Mutuku, F. M. *et al*. Pupal habitat productivity of Anopheles gambiae complex mosquitoes in a rural village in western Kenya. (2006).

293. Kudom, A. A., Mensah, B. A. & Agyemang, T. K. Characterization of mosquito larval habitats and assessment of insecticide-resistance status of Anopheles gambiae senso lato in urban areas in southwestern Ghana. *Journal of Vector Ecology* **37**, 77–82 (2012).

294. Mshani, I. H. *et al*. Comparison of Fine-Scale Malaria Strata Derived from Population Survey Data Collected Using mRDTs, Microscopy and qPCR in South-Eastern Tanzania. *medRxiv* 2006–2024 (2024).

295. Roy, D. P., Huang, H., Houborg, R. & Martins, V. S. A global analysis of the temporal availability of PlanetScope high spatial resolution multi-spectral imagery. *Remote Sens Environ* **264**, 112586 (2021).

296. Ghuffar, S. DEM generation from multi satellite PlanetScope imagery. *Remote Sens (Basel)* **10**, 1462 (2018).

297. Gao, B.-C. NDWI—A normalized difference water index for remote sensing of vegetation liquid water from space. *Remote Sens Environ* **58**, 257–266 (1996).

298. Therneau, T., Atkinson, B., Ripley, B. & Ripley, M. B. Package 'rpart'. *Available online: cran. ma. ic. ac. uk/web/packages/rpart/rpart. pdf (accessed on 20 April 2016)* (2015).

299. Kuhn, M. *et al*. Package 'caret'. *R J* **223**, (2020).

300. Albertsen, C. M., Whoriskey, K., Yurkowski, D., Nielsen, A. & Flemming, J. M. Fast fitting of non-Gaussian state-space models to animal movement data via Template Model Builder. Preprint at (2015).

301. Bivand, R. Implementing spatial data analysis software tools in R. *Geogr Anal* **38**, 23–40 (2006).

302. Dida, G. O. *et al*. Spatial distribution and habitat characterization of mosquito species during the dry season along the Mara River and its tributaries, in Kenya and Tanzania. *Infect Dis Poverty* **7**, 1–16 (2018).

303. Walker, K. & Lynch, M. Contributions of Anopheles larval control to malaria suppression in tropical Africa: Review of achievements and potential. *Med Vet Entomol* **21**, 2–21 (2007).

304. Coetzee, M. & Fontenille, D. Advances in the study of Anopheles funestus, a major vector of malaria in Africa. *Insect Biochem Mol Biol* **34**, 599–605 (2004).

305. Godfray, H. C. J. Mosquito ecology and control of malaria. *Journal of Animal Ecology* **82**, 15–25 (2013).

306. Tsegaye, A. *et al*. Anopheles larval habitats seasonality and environmental factors affecting larval abundance and distribution in Arjo-Didessa sugar cane plantation, Ethiopia. *Malar J* **22**, 1–12 (2023).

307. Mahgoub, M. M., Kweka, E. J. & Himeidan, Y. E. Characterisation of larval habitats, species composition and factors associated with the seasonal abundance of mosquito fauna in Gezira, Sudan. *Infect Dis Poverty* **6**, 1–10 (2017).

308. Ondiba, I. M., Oyieke, F. A., Athinya, D. K., Nyamongo, I. K. & Estambale, B. B. A. Larval species diversity, seasonal occurrence and larval habitat preference of mosquitoes transmitting Rift Valley fever and malaria in Baringo County, Kenya. *Parasit Vectors* **12**, 1–14 (2019).

309. Coalson, J. E. *et al*. The complex epidemiological relationship between flooding events and human outbreaks of mosquito-borne diseases: a scoping review. *Environ Health Perspect* **129**, 96002 (2021).

310. Keno, H. *et al*. Characterization of Anopheles mosquito larval habitats and species composition in Bambasi District, Northwestern Ethiopia. *Int J Trop Insect Sci* **42**, 2325–2336 (2022).

311. Charlwood, J. D., Cuamba, N., Tomás, E. V. E., Jt Briët, O. & Briët, O. J. T. Living on the edge: a longitudinal study of Anopheles funestus in an isolated area of Mozambique. *Malar J* **12**, 1–10 (2013).

312. Fillinger, U., Sonye, G., Killeen, G. F., Knols, B. G. J. & Becker, N. The practical importance of permanent and semipermanent habitats for controlling aquatic stages of Anopheles gambiae sensu lato mosquitoes:

Operational observations from a rural town in western Kenya. *Tropical Medicine and International Health* **9**, 1274–1289 (2004).

313. Sattler, M. A. *et al.* Habitat characterization and spatial distribution of Anopheles sp. mosquito larvae in Dar es Salaam (Tanzania) during an extended dry period. *Malar J* **4**, 1-15 (2005).
314. Beck, L. R. *et al.* Remote sensing as a landscape epidemiologic tool to identify villages at high risk for malaria transmission. *Am J Trop Med Hyg* **51**, 271–280 (1994).
315. Kelly-Hope, L. A., Hemingway, J. & McKenzie, F. E. Environmental factors associated with the malaria vectors Anopheles gambiae and Anopheles funestus in Kenya. *Malar J* **8**, 1–8 (2009).
316. Tokarz, R. E. *Spatial-Temporal Distribution of Mosquito Larval Hot Spots in Papoli, Uganda: A Community-Based Approach to Mosquito Control.* (University of South Florida, 2017).
317. Cator, L. J. *et al.* The role of vector trait variation in vector-borne disease dynamics. *Front Ecol Evol* **8**, 189 (2020).
318. Dear, N. F. *et al.* Household-level and surrounding peri-domestic environmental characteristics associated with malaria vectors Anopheles arabiensis and Anopheles funestus along an urban–rural continuum in Blantyre, Malawi. *Malar J* **17**, 1–14 (2018).
319. Agyekum, T. P. *et al.* A systematic review of the effects of temperature on Anopheles mosquito development and survival: Implications for malaria control in a future warmer climate. *Int J Environ Res Public Health* **18**, 7255 (2021).
320. Moreira, L. A., Wang, J., Collins, F. H. & Jacobs-Lorena, M. Fitness of anopheline mosquitoes expressing transgenes that inhibit Plasmodium development. *Genetics* **166**, 1337–1341 (2004).
321. Carvajal-Lago, L., Ruiz-López, M. J., Figuerola, J. & Martínez-de la Puente, J. Implications of diet on mosquito life history traits and pathogen transmission. *Environ Res* **195**, 110893 (2021).
322. Shapiro, L. L. M., Murdock, C. C., Jacobs, G. R., Thomas, R. J. & Thomas, M. B. Larval food quantity affects the capacity of adult mosquitoes to transmit human malaria. *Proceedings of the Royal Society B: Biological Sciences* **283**, 20160298 (2016).

323. Andreazza, F., Oliveira, E. E. & Martins, G. F. Implications of sublethal insecticide exposure and the development of resistance on mosquito physiology, behavior, and pathogen transmission. *Insects* **12**, 917 (2021).

324. Carrasco, D. *et al*. Behavioural adaptations of mosquito vectors to insecticide control. *Curr Opin Insect Sci* **34**, 48–54 (2019).

325. Chandrasegaran, K., Lahondère, C., Escobar, L. E. & Vinauger, C. Linking mosquito ecology, traits, behavior, and disease transmission. *Trends Parasitol* **36**, 393–403 (2020).

326. Russell, T. L. *et al.* Linking individual phenotype to density-dependent population growth: the influence of body size on the population dynamics of malaria vectors of malaria vectors dynamics growth: the influence of body size on the population Linking individual phenotype to density-dependent population 'Data Supplement' Subject collections Linking individual phenotype to density-dependent population growth: the influence of body size on the population dynamics of malaria vectors. 3142–3151 (2011) doi:10.1098/rspb.2011.0153.

327. Msugupakulya, B. J. *et al.* Influence of larval growth and habitat shading on retreatment frequencies of biolarvicides against malaria vectors. *Sci Rep* **14**, 1002 (2024).

328. Moller-Jacobs, L. L., Murdock, C. C. & Thomas, M. B. Capacity of mosquitoes to transmit malaria depends on larval environment. *Parasit Vectors* **7**, 1–12 (2014).

329. Tsurim, I., Silberbush, A., Ovadia, O., Blaustein, L. & Margalith, Y. Inter-and intra-specific density-dependent effects on life history and development strategies of larval mosquitoes. *PLoS One* **8**, e57875 (2013).

330. Oliver, S. V & Brooke, B. D. The effect of larval nutritional deprivation on the life history and DDT resistance phenotype in laboratory strains of the malaria vector Anopheles arabiensis. *Malar J* **12**, 44 (2013).

331. Takken, W. *et al.* Larval nutrition differentially affects adult fitness and Plasmodium development in the malaria vectors Anopheles gambiae and Anopheles stephensi. *Parasit Vectors* **6**, 1–10 (2013).

332. Gimnig, J. E. *et al.* Density-dependent development of Anopheles gambiae (Diptera: Culicidae) larvae in artificial habitats. *J Med Entomol* **39**, 162–172 (2002).

333. Wang, Y. *et al*. Investigation of mosquito larval habitats and insecticide resistance in an area with a high incidence of mosquito-borne diseases in Jining, Shandong Province. *PLoS One* **15**, e0229764 (2020).
334. Araújo, M. da-S., Gil, L. H. S. & e-Silva, A. de-A. Larval food quantity affects development time, survival and adult biological traits that influence the vectorial capacity of Anopheles darlingi under laboratory conditions. *Malar J* **11**, 1–9 (2012).
335. Pinda, P. G. *et al*. Relationships between biological age, distance from aquatic habitats and pyrethroid resistance status of Anopheles funestus mosquitoes in south-eastern Tanzania. *Malar J* **21**, 1–12 (2022).
336. Mulamba, C. *et al*. Widespread Pyrethroid and DDT Resistance in the Major Malaria Vector Anopheles funestus in East Africa Is Driven by Metabolic Resistance Mechanisms. *PLoS One* **9**, e110058 (2014).
337. Yadouleton, A. W. *et al*. Insecticide resistance status in Anopheles gambiae in southern Benin. *Malar J* **9**, 83 (2010).
338. Orondo, P. W. *et al*. Insecticide resistance status of Anopheles arabiensis in irrigated and non-irrigated areas in western Kenya. *Parasit Vectors* **14**, 335 (2021).
339. Tchigossou, G. *et al*. Molecular basis of permethrin and DDT resistance in an Anopheles funestus population from Benin. *Parasit Vectors* **11**, 1–13 (2018).
340. Djouaka, R., Irving, H., Tukur, Z. & Wondji, C. S. Exploring Mechanisms of Multiple Insecticide Resistance in a Population of the Malaria Vector Anopheles funestus in Benin. *PLoS One* **6**, e27760 (2011).
341. Matowo, N. S. *et al*. Fine-scale spatial and temporal heterogeneities in insecticide resistance profiles of the malaria vector, Anopheles arabiensis in rural south-eastern Tanzania. *Wellcome Open Res* **2**, 96 (2017).
342. Huho, B. J. *et al*. Nature beats nurture: a case study of the physiological fitness of free-living and laboratory-reared male Anopheles gambiae sl. *Journal of Experimental Biology* **210**, 2939–2947 (2007).
343. Takken, W., Klowden, M. J. & Chambers, G. M. Effect of body size on host seeking and blood meal utilization in Anopheles gambiae sensu stricto (Diptera: Culicidae): the disadvantage of being small. *J Med Entomol* **35**, 639–645 (1998).

344. Koekemoer, L. L., Kamau, L., Hunt, R. H. & Coetzee, M. A cocktail polymerase chain reaction assay to identify members of the Anopheles funestus (Diptera: Culicidae) group. *Am J Trop Med Hyg* **66**, 804–811 (2002).

345. Van Handel, E. Rapid determination of total lipids in mosquitoes. *J Am Mosq Control Assoc* **1**, 302–304 (1985).

346. Van Handel, E. Rapid determination of total lipids in mosquitoes. *J Am Mosq Control Assoc* **1**, 302–304 (1985).

347. World Health Organization. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes: Second edition. *World Health Organisation Technical Report Series* 1–22 (2016) doi:10.1021/jp052915b.

348. Pinheiro, J., Bates, D., DebRoy, S. & Sarkar, D. R Core Team. 2019 linear and nonlinear mixed effects models. R package version 3.1-141. *Available at h Ttp://CRAN. R-Project. Org/Package= Nlme* (2019).

349. Brooks, M. E. *et al.* glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R J* **9**, 378–400 (2017).

350. Therneau, T. M. & Lumley, T. Package 'survival'. *R Top Doc* **128**, 28–33 (2015).

351. Kassambara, A., Kosinski, M. & Biecek, P. Package survminer Type Package Title Drawing Survival Curves using "ggplot2." Preprint at (2017).

352. Bolker, B. M. *et al.* Generalized linear mixed models: a practical guide for ecology and evolution. *Trends Ecol Evol* **24**, 127–135 (2009).

353. World Health Organization. *Manual for Monitoring Insecticide Resistance in Mosquito Vectors and Selecting Appropriate Interventions*. (World Health Organization, 2022).

354. Gimmg, J. E. *et al.* Characteristics of larval anopheline (Diptera: Culicidae) habitats in Western Kenya. *J Med Entomol* **38**, 282–288 (2001).

355. Fournet, F. *et al.* Diversity in anopheline larval habitats and adult composition during the dry and wet seasons in Ouagadougou (Burkina Faso). *Malar J* **9**, 1-10 (2010).

356. Maïga, H., Dabiré, R. K., Lehmann, T., Tripet, F. & Diabaté, A. Variation in energy reserves and role of body size in the mating system of Anopheles gambiae. *Journal of Vector Ecology* **37**, (2012).

357. Stone, C. M., Hamilton, I. M. & Foster, W. A. A survival and reproduction trade-off is resolved in accordance with resource availability by virgin female mosquitoes. *Anim Behav* **81**, 765–774 (2011).

358. Girard, M. *et al.* Microorganisms associated with mosquito oviposition sites: Implications for habitat selection and insect life histories. *Microorganisms* **9**, 1589 (2021).

359. Diabate, A. *et al.* The role of agricultural use of insecticides in resistance to pyrethroids in Anopheles gambiae sl in Burkina Faso. *Am J Trop Med Hyg* **67**, 617–622 (2002).

360. Dabiré, K. R. *et al.* Anopheles funestus (Diptera: Culicidae) in a humid savannah area of western Burkina Faso: bionomics, insecticide resistance status, and role in malaria transmission. *J Med Entomol* **44**, 990–997 (2007).

361. Riveron, J. *et al.* Insecticide resistance in malaria vectors: an update at a global scale. Preprint at (2018).

362. World Health Organization. *Global Plan for Insecticide Resistance Management in Malaria Vectors*. (World Health Organization, 2012).

363. Sanou, A. *et al.* Insecticide resistance and behavioural adaptation as a response to long-lasting insecticidal net deployment in malaria vectors in the Cascades region of Burkina Faso. *Sci Rep* **11**, 17569 (2021).

364. Saleh, F. *et al.* Habitat Characteristics For Immature Stages of Aedes aegypti In Zanzibar City, Tanzania . *J Am Mosq Control Assoc* **34**, 190–200 (2018).

365. Imbahale, S. S. *et al.* A longitudinal study on Anopheles mosquito larval abundance in distinct geographical and environmental settings in western Kenya. *Malar J* **10**, 1–13 (2011).

366. Hakizimana, E. *et al.* Spatio-temporal distribution of mosquitoes and risk of malaria infection in Rwanda. *Acta Trop* **182**, 149–157 (2018).

367. Minakawa, N., Seda, P. & Yan, G. Influence of host and larval habitat distribution on the abundance of African malaria vectors in western Kenya. *American Journal of Tropical Medicine and Hygiene* **67**, 32–38 (2002).

368. Kaindoa, E. W., Mmbando, A. S., Shirima, R., Hape, E. E. & Okumu, F. O. Insecticide-treated eave ribbons for malaria vector control in low-income communities. *Malar J* **20**, 1–12 (2021).

369. Kirby, M. J. *et al.* Effect of two different house screening interventions on exposure to malaria vectors and on anaemia in children in The Gambia: a randomised controlled trial. *The Lancet* **374**, 998–1009 (2009).

370. Mbogo, C. M. *et al.* Spatial and temporal heterogeneity of Anopheles mosquitoes and Plasmodium falciparum transmission along the Kenyan coast. *Am J Trop Med Hyg* **68**, 734–742 (2003).
371. Zippenfenig, P. *Open-Meteo.Com Weather API.* (2023). doi:10.5281/ZENODO.7973572.
372. Bofu, R. M. *et al.* The needs and opportunities for housing improvement for malaria control in southern Tanzania. *Malar J* **22**, 1–15 (2023).
373. Wood, S. gamm4: Generalized additive mixed models using mgcv and lme4. *R Packag. version 0.2-3* (2014).
374. Wickham, H. ggplot2. *WIREs Computational Statistics* **3**, 180–185 (2011).
375. Koenraadt, C. J. M., Githeko, A. K. & Takken, W. The effects of rainfall and evapotranspiration on the temporal dynamics of Anopheles gambiae ss and Anopheles arabiensis in a Kenyan village. *Acta Trop* **90**, 141–153 (2004).
376. Haddow, A. J. Measurements of temperature and light in artificial pools with reference to the larval habitat of Anopheles (Myzomyia) gambiae, Giles, and A.(M.) funestus, Giles. *Bull Entomol Res* **34**, 89–93 (1943).
377. Lindsay, S. W. *et al.* Reduced mosquito survival in metal-roof houses may contribute to a decline in malaria transmission in sub-Saharan Africa. *Sci Rep* **9**, 7770 (2019).
378. Ngadjeu, C. S. *et al.* Influence of house characteristics on mosquito distribution and malaria transmission in the city of Yaounde, Cameroon. *Malar J* **19**, 53 (2020).
379. Atieli, H., Menya, D., Githeko, A. & Scott, T. House design modifications reduce indoor resting malaria vector densities in rice irrigation scheme area in western Kenya. *Malar J* **8**, 1–9 (2009).
380. Bhatt, S. *et al.* The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015. **526**, 207–211 (2015).
381. Russell, T. L. *et al.* Increased proportions of outdoor feeding among residual malaria vector populations following increased use of insecticide-treated nets in rural Tanzania. *Malar J* **10**, (2011).
382. Govella, N. J. & Ferguson, H. Why use of interventions targeting outdoor biting mosquitoes will be necessary to achieve malaria elimination. *Front Physiol* **3**, (2012).

383. World Health Organization. *WHO Guidelines for Malaria, 18 February 2022*. (2022).

384. Kandyata, A. *et al.* Impacts of Bacillus thuringiensis var. israelensis and Bacillus sphaericus insect larvicides on mosquito larval densities in Lusaka, Zambia. *Med J Zambia* **39**, 33–38 (2012).

385. Derua, Y. A., Kweka, E. J., Kisinza, W. N., Githeko, A. K. & Mosha, F. W. Bacterial larvicides used for malaria vector control in sub-Saharan Africa: review of their effectiveness and operational feasibility. *Parasit Vectors* **12**, (2019).

386. Fillinger, U. *et al.* A tool box for operational mosquito larval control: preliminary results and early lessons from the Urban Malaria Control Programme in Dar es Salaam, Tanzania. *Malar J* **7**, 1–25 (2008).

387. Berlin Rubin, N., Mboera, L. E. G., Lesser, A., Miranda, M. L. & Kramer, R. Process evaluation of a community-based microbial larviciding intervention for malaria control in rural Tanzania. *Int J Environ Res Public Health* **17**, 7309 (2020).

388. Hakizimana, E. *et al.* Community-based control of malaria vectors using Bacillus thuringiensis var. Israelensis (Bti) in Rwanda. *Int J Environ Res Public Health* **19**, 6699 (2022).

389. Peel, M. C., Finlayson, B. L. & McMahon, T. A. Updated world map of the Köppen-Geiger climate classification. *Hydrol Earth Syst Sci* **11**, 1633–1644 (2007).

390. NVivo - Lumivero. https://lumivero.com/products/nvivo/.

391. Imbahale, S. S., Fillinger, U., Githeko, A., Mukabana, W. R. & Takken, W. An exploratory survey of malaria prevalence and people's knowledge, attitudes and practices of mosquito larval source management for malaria control in western Kenya. *Acta Trop* **115**, 248–256 (2010).

392. Fillinger, U. *et al.* Identifying the most productive breeding sites for malaria mosquitoes in The Gambia. *Malar J* **8**, 1-14 (2009).

393. Finda, M. F., Kaindoa, E. W., Nyoni, A. P. & Okumu, F. O. 'The mosquitoes are preparing to attack us': knowledge and perceptions of communities in south-eastern Tanzania regarding mosquito swarms. *Malar J* **18**, 56 (2019).

394. Tarimo, F. S., Dillip, A., Kosia, E. M. & Lwetoijera, D. W. Community perception of the autodissemination of pyriproxyfen for controlling malaria vectors in south-eastern Tanzania. *Malar J* **22**, 333 (2023).
395. Gowelo, S. *et al.* Community factors affecting participation in larval source management for malaria control in Chikwawa District, Southern Malawi. *Malar J* **19**, 1–11 (2020).
396. Geissbühler, Y. *et al.* Microbial larvicide application by a large-scale, community-based program reduces malaria infection prevalence in urban Dar Es Salaam, Tanzania. *PLoS One* **4**, (2009).
397. Mvomero, H. ya wilaya ya. Siku ya USafi Kitaifa. (2023).
398. World Health Organization. *Burden of Disease Attributable to Unsafe Drinking-Water, Sanitation and Hygiene*. (World Health Organization, 2023).
399. Batterman, S. *et al.* Sustainable control of water-related infectious diseases: a review and proposal for interdisciplinary health-based systems research. *Environ Health Perspect* **117**, 1023–1032 (2009).
400. Whittaker, M. & Smith, C. Reimagining malaria: five reasons to strengthen community engagement in the lead up to malaria elimination. *Malar J* **14**, 410 (2015).
401. Mayagaya, V. S. *et al.* The impact of livestock on the abundance, resting behaviour and sporozoite rate of malaria vectors in southern Tanzania. *Malar J* **14**, 17 (2015).
402. Maïga, H. *et al.* Role of nutritional reserves and body size in Anopheles gambiae males mating success. *Acta Trop* **132**, S102–S107 (2014).
403. Preet, S. & Sneha, A. Fitness cost of malaria vector Anopheles stephensi induced with larvicidal and nutritional stress. *J Asia Pac Entomol* **21**, 97–105 (2018).
404. Oliver, S. V & Brooke, B. D. The effect of metal pollution on the life history and insecticide resistance phenotype of the major malaria vector Anopheles arabiensis (Diptera: Culicidae). *PLoS One* **13**, e0192551 (2018).
405. Okumu, F. The paradigm of eave tubes: scaling up house improvement and optimizing insecticide delivery against disease-transmitting mosquitoes. *Malar J* **16**, 207 (2017).