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Quantifying the ecology of *Anopheles funestus* and its implications for improved malaria control

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By

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Abstract

The malaria burden is highest in African countries where more than 95% of deaths and cases occur. There was a consistent decline in malaria deaths and cases in Africa between 2000 and 2015 but progress has since stalled. Due to biological changes in vector populations, notably insecticide resistance and behavioral adaptations such as outdoor-biting, the primary vector control measures are no longer as successful as they once were. In recent years, mosquito species considered to be the primary vector of malaria, (e.g. *Anopheles gambiae* s.s) have declined, and even disappeared from some communities. In settings such as rural south-eastern Tanzania, the residual transmission is now being maintained by *An. funestus* followed by *An. arabiensis*.

Currently, *An. funestus* mediates a high proportion of malaria transmission events in east and southern Africa. The resilience of this vector may be linked to its high insecticide resistance; though recent evidence suggests that it may also be capable of shifting its biting behaviors to avoid contact with insecticidal interventions. Yet, our ability to tackle this vector species is impeded by the limited knowledge of its basic ecology and population dynamics, the difficulties in colonizing it under laboratory conditions and the many uncertainties about appropriate surveillance approaches.

The overall aim of this PhD project was to quantify the ecology of *An. funestus* mosquitoes in Tanzania and assess the implications of its key attributes for improved malaria control in settings such as Tanzania where the vector species dominates. The work involved the following steps: 1) quantifying the fitness and behavioral attributes of wild *An. funestus* and their offspring during repeated colonization attempts under standard laboratory conditions, 2) developing and validating a framework for predicting human biting exposures from different exposure-free sampling methods, 3) developing and testing a population dynamics model to describe the ecology of the wild *An. funestus* populations, and 4) assessing the generalizability of the population dynamics model and its ability to reconstruct missing data.

To achieve the first objective, I attempted to colonize a local population of *An. funestus* s.s. from southeastern Tanzania and assessed the key barriers which

hinder laboratory establishment. Adult females (F_0) from three wild *An. funestus* populations were brought into the laboratory for rearing. Their fecundity, and the development, survival, body size and mating success of their F_1 offspring were measured to evaluate their fitness under laboratory conditions. While adult survival was relatively high, the mating success, poor hatching rate and poor larval survival and extended larval development periods were identified as key barriers to establishing a colony in the laboratory. Due to these factors, this colony was not sustained beyond the F_1 generation in the laboratory, but the lessons were deployed for a subsequent and more successful colonization effort.

To address the second objective, I analyzed data comparing the outdoor catch rates of *An. funestus* using six exposure-free trapping methods relative to the human landing catches (HLC), the gold standard method for estimating human exposures to mosquito bites. I tested different models for the relationship between HLC and other trapping methods while allowing flexibility for associations to be impacted by interspecific and intraspecific density dependence. This analysis indicated that that the association between catches in alternative traps and the HLC can best be explained by simple linear models; with minimal impact of intra and inter specific density dependence. A shiny app interface was developed to allow expanded use of this statistical calibration framework for future estimations of malaria vector biting risk in communities.

For the third objective, I used the demographic parameters generated from the colonization attempt (described above) and published literature, to develop the first population dynamics model of wild *An. funestus* in Tanzania. I used a Bayesian framework to develop a state-space model and reconstruct the observed population dynamics of this species. I then used this model to assess the strength of evidence for intrinsic (density dependence) and extrinsic (environmental covariates) drivers of *An. funestus* population dynamics and how they drive seasonality in abundance and demographic variables (development periods and survival). This analysis indicated that density dependence has a minimal contribution on the overall dynamics of *An. funestus* in these settings. Daily larval and adult survival probabilities were marginally affected by changes in environmental covariates (temperature and rainfall), suggesting there is little seasonality in these fitness parameters. This study also revealed that *An.*

funestus may be essential for sustaining year-round malaria transmission in settings such as rural south-eastern Tanzania.

Finally, I interrogated the generalizability and sensitivity of this modelling framework for *An. funestus* by assessing its ability to predict missing time series data. Here, I refitted the model to a single population and assessed any unexplained features of population dynamics which is causing the density dependence to have small contributions. I also omitted portions of the time series data to assess model prediction capability. For example I first removed 25% and then 50% of the data, then reconstructed the missing sections. The single population model indicated that *An. funestus* demographic variables were much more sensitive to changes in environmental covariates compared to the preceding hierarchical model; suggesting that clear signals of environmental drivers may be lost by fitting the model to multiple populations that may have distinct drivers. The model was able to reconstruct the observed population trajectory poorly when 50% of the data was removed as compared to when 25% was removed. Overall, the model was only able to predict for the missing data if the training set included some representation of data from both dry and wet seasons.

In conclusion, this PhD work contributes to a general understanding of the key barriers to colonization and the population dynamics of *An. funestus*. While An. funestus was not successfully colonised in this study, the lessons learned by documenting which fitness traits are impeded in the laboratory led to progress in further work at the Ifakara Health Institute, where a stable colony of An. funestus has now been established. Additionally, the model of *An. funestus* dynamics and demography developed here will underpin further research to evaluate and select optimal vector control packages for crashing these populations in southern Tanzania and other settings where they are the major source of residual malaria transmission.

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Author’s Declaration

I hereby declare that the work written in this thesis is entirely my own, except where otherwise stated. I honestly recognized the contributions of laboratory and field technicians, volunteers and the co-authorship of my supervisors, Professor Heather Ferguson, Professor Jason Matthiopoulos, Dr. Fredros Okumu and Dr. Luca Nelli. I further declare that, no part of this work has been submitted as part of any other degree.

List of Abbreviations

BGS: Biogents Sentinel Trap

CDC-LT: Centre for Disease Light Trap

DIC: Deviance Information Criterion

EIR: Entomological Inoculation Rate

FANG: Funestus Angola

FUMOZ: Funestus Mozambique

FUTAZ: Funestus Tanzania

GLMM: Generalized Linear Mixed Models

GUI: Graphical User Interface

HBR: Human Biting Rate

HDT: Host Decoy Trap

HLC: Human Landing Catch

IHI: Ifakara Health Institute

IRS: Indoor Residual Spray

ITN: Insecticide Treated Nets

ITT-C: Ifakara Tent Trap-Type C

JAGS: Just Another Gibbs Sampler

LRT: Likelihood Ratio Test

LSM: Larval Source Management

MCMC: Markov Chain Monte Carlo

MET: Mosquito Electrocuting Trap

MMX: Mosquito Magnet Trap

MTR: M-Trap

MTRC: M-Trap with CDC

NMCP: National Malaria Control Program

PCR: Polymerase Chain Reaction

s.l: *Sensu lato*

SNP: School Net Program

s.s: *Sensu stricto*

SSM: State-Space Model

SUN: Suna Trap

TMIS: Tanzania Malaria Indicator Survey

VCRL: Vector Control Research Laboratory

WHO: World Health Organization

1.0 Chapter 1: General Introduction

1.0 Global burden

In 2020, there were estimated to be around 241 million malaria cases and 627,000 deaths worldwide; with the burden spread across 85 countries [1]. The malaria burden is highest in African countries (Sub-Saharan) where 96% of total deaths and 95% of the total global cases occur [1]. Despite a significant decrease in malaria deaths over the last two decades (2000-2020), with annual deaths falling from 896,000 to 627,000, the disease continues to pose a significant public health problem [1]. In Africa, there has been a consistent decline in malaria deaths between 2000 and 2015 [2], but since then progress has flattened with cases even rising in some countries [3]. Several factors have been attributed to this slowing and reversal of progress, including a lack of sufficient resources (i.e. funding), insecticide resistance etc.

In combination, these factors are hindering progress towards elimination and control of malaria [4]. Overcoming this set back will require new funding and new tools to tackle malaria [5,6]. Recently a vaccine against malaria, RTS,S/AS01 (RTS,S), was approved for use by the World Health Organization (WHO) for the first time [7]. This vaccine was approved for use in children in places with moderate-to-high *Plasmodium falciparum* transmission [7]. This RTS,S is designed to target the sporozoites phase of the lifecycle. The vaccine is designed to prevent the parasite from infecting the liver, where it can mature, multiply, re-enter the bloodstream, and infect red blood cells, which can lead to disease symptoms [7]. While the availability of a vaccine marks an important step forward in the fight against malaria, this intervention is only partially protective give the modest efficacy of 30% [7,8] and will thus be insufficient on its own to eliminate malaria. Enhanced and expanded strategies for mosquito vector control, in combination with rapid diagnosis and treatment with effective artemisinin drug delivery, will be essential for further progress [2,9,10]. Current evidence indicates that malaria parasites are developing resistance to the artemisinin drug used for frontline treatment in Africa, which pose a challenge towards malaria control [11]. A range of new tools, including those targeting

mosquitoes and parasites, will be needed to overcome these current barriers and make further progress to elimination.

1.1 Malaria biology and pathology

Malaria is an infectious disease caused by protozoan parasites in the genus *Plasmodium*; of which *Plasmodium malariae, P. vivax, P. ovale, P. knowlesi* and *P. falciparum* cause most of the disease in humans [12]. The parasite which contributes the most morbidity and mortality in Africa is *P. falciparum* [13]. Female *Anopheles* mosquitoes are the vectors of malaria parasites and are widely distributed across the African continent [14,15]. Malaria is transmitted when a female *Anopheles* vector carrying *Plasmodium* parasites bites a person (host) while attempting to obtain a blood meal. Biting is obligatory in female *Anopheles* because they need vertebrate blood to develop eggs [16]. The parasite life cycle comprised of sexual stage that occurs in *Anopheles* mosquito vectors and an asexual stage that occurs inside vertebrates hosts [17]. When an infected female *Anopheles* mosquito bites a human, *Plasmodium* parasites in the form of sporozoites are injected into the blood stream. The parasite life cycle comprised of sexual stage that occurs in *Anopheles* mosquito vectors and an asexual stage that occurs inside vertebrates hosts [REF]. When a female *Anopheles* mosquito bites a human, Plasmodium parasites in the form of sporozoites are injected into the human dermis. The parasites then migrate to the liver through blood stream and invade the liver cells, hepatocytes. Sporozoites multiply asexually into merozoites in liver cells over the next 7 to 10 days, with single sporozoites producing more than 40,000 merozoites per cell [REF]. The parasites are later released into the bloodstream as merozoites, where they attack red blood cells and subsequently differentiate through ring-stage into trophozoites and eventually schizonts until the cells burst. Then they invade more blood cells causing fever each time the parasites break free and invade blood cells. Small fractions of these merozoites differentiate into gametocytes, which circulate in the peripheral bloodstream where they can infect mosquitoes during the next blood meal. Gametocytes mate sexually in the mosquito gut after which gametes transform into actively moving ookinetes that burrow through the mosquito's midgut wall to form oocysts on the exterior surface. Inside the oocyst, thousands of active sporozoites develop. The oocyst eventually bursts, releasing sporozoites that travel to the mosquito's salivary

glands. The cycle of human infection begins again when the mosquito bites another person during a subsequent blood meal [8]. Transmission can also occur through exposure to infected blood products and by congenital transmission [18]; however the majority of infections are due to mosquitoes.

After being bitten by an infectious female *Anopheles* mosquito, symptoms of malaria can arise six to ten days later. This duration varies depending on the parasite species (for example, non-*P. falciparum* takes 15-16 days) [18] and other factors such as prophylactic use, host immunity or anti-plasmodial treatment [19] and around 10-11 days in non-immunes. A number of symptoms with ranging severity have been described; with most patients reporting feeling cold and sweating, fever and headache [18,20]. The most severe symptoms that can lead to deaths include respiratory distress, coma, cerebral malaria, acute renal failure and even anaemia. Some other patients will experience nausea, vomiting or stomach discomfort [18,20]. The severity of the disease varies with host age and immune status. The most affected groups by malaria transmission globally are children under the age of 59 months and pregnant women [1].

1.2 Mosquito life cycle

The mosquito life cycle consists of two distinct phases: a juvenile (larval) stage which is aquatic and a terrestrial adult stage. The survival and development of mosquitoes in both stages are highly dependent on environmental conditions. The life cycle begins when eggs are laid into aquatic habitats. These eggs hatch into larvae within 2-4 days depending on environmental conditions [21]. Larvae develop into pupae (pupation) within 8–15 days; with the length of this period varying between Anopheline species and in response to environmental conditions (i.e. Temperature) [22,23]. The insect juvenile hormone regulates the process of entering (pupation) and exiting (emergence) from the pupal stage. Mosquito pupae are highly active and non-feeding during this stage. The pupae stage in Anopheline mosquitos lasts 24–48 hours [24,25], a time interval mostly determined by temperature [26,27]. When the weather is warmer, adult emergence occurs faster than when the weather is cooler [28,29].

After emergence, adult males and females search for energy via sugar sources while female mosquitoes will also require one or two blood meals to trigger egg production after mating [30]. Mating usually occurs in the first 1-2 days after emergence, and usually before females take their first blood meal [31,32]. Many Anopheline mosquitoes mate naturally in aerial swarms [33,34] or, to a smaller extent inside houses [35,36]. Eurygamic species do not exhibit natural mating behaviour such as swarming in confined spaces such as laboratory cages, thus making them difficult to colonize [37–39] while stenogamous species exhibit mating behaviour in confined spaces, this include *Anopheles gambiae sensu stricto*. Several research centres around the world have tried to colonize eurygamic species with limited success. *Anopheles funestus* is one of the eurygamous species. This has resulted in a lack of knowledge about the fundamental biology and ecology of such species and consideration of their quantitative predictions about how they might respond to different vector control interventions.

Adult female vectors usually take their first blood meal between 1-2 days after mating. This meal initiates the gonotrophic cycle, characterised by blood feed, blood meal digestion, egg development and finally oviposition. The length of this gonotrophic cycle varies among mosquito species and always depends on the access to blood hosts and ambient temperature [31,40]. When a host is available, *Anopheles* mosquito gonotrophic cycle length takes between 2-5 days [31,32,41]. Females will repeat this cycle until they die. The median lifespan of an adult mosquito in the wild population being approximately 10 days but with large variation between species and settings [41]. The survival and fecundity of adult mosquitoes are considered to be the most important factors for the stability of their populations (fecundity) and the force of malaria transmission (adult survival) [42–45].

The size and reproductive potential of adult mosquito populations are influenced by a range of environmental and intrinsic factors. Several factors during larval development have a large impact on the fitness of adult Anopheline mosquitoes [25,46]. The speed of larval development and survival through this period is highly dependent on temperature [23,25]. This relationship has a curvilinear nature, where survival will initially start to increase with temperature, then

decline sharply at hotter temperatures [47,48]. The speed of larval development and survival through this period is also affected by the presence of competitors or predators in larval habitats [49,50] as well as pathogens [51,52]. Intraspecific resource competition can also be a major cause of mortality during the larval development [22,45,48,53]. In general, there is a positive relationship between larval and adult abundance in the absence of intense resource competition and predation [54,55]. However, this relationship can be modified by density dependence as occurs when larvae compete for resources in aquatic habitats. This gives rise to negative density dependence, where larvae developing at higher densities often have slower development [26] and lower survival [56,57]. The effects of density during larval development can carry over to the adult stage; with adults developing from high density larval habitats often having reduced body size [58,59], survival [53,60], nutritional reserves and even mating success [61] compared to those from less crowded conditions. Cannibalism can also occur in larval habitats, with older larvae (fourth instar) eating younger larvae (first instar) [62,63]. Both intra-specific competition and predation during larval development influence the densities of adult mosquito populations [63]. In nature, some organisms are most likely to die due to senescence (old age), but it is hypothesized that most malaria vectors will be killed due to predation, diseases and other environmental hazards long before they reach old age [64]. This is the case for many insect species including mosquitoes; whose adult mortality is independent of age [65].

1.3 African malaria vectors

In Africa the major vectors of malaria transmission are the *Anopheles gambiae* complex and *An. funestus* groups [66–68], which are widely distributed across different countries Figure 1.1 [14,66,68]. These two vector groups contribute to almost all the malaria transmission in Africa. Vectors belonging to the *An. gambiae* complex are generally the most abundant, and include 8 morphologically identical species: *An. gambiae sensu stricto, An. coluzzi, An. arabiensis, An. quadriannulatus, An. amharicus, An. melas, An. merus,* and *An. bwambwae* [14,69,70]. *Anopheles gambiae* s.s. is commonly considered to be the most efficient malaria vector in this group, followed by *An. arabiensis* [14,71,72]. The higher efficiency of *An. gambiae* s.s is because it is more

anthropophilic than *An. arabiensis*; making it well suited to a human-specific pathogen. In many places these sister species are sympatric in larval habitats, with *An. gambiae* s.s. being more dominant in wetter settings, and *An. arabiensis* dominating in more arid environments [14,68,73,74]. They prefer breeding in shallow, small, and temporary larval habitats (i.e., puddles, hoof prints, tyre tracks, etc.), which are largely dependent on the rainfall [54]. The ecology of the *An. gambiae* complex has been extensively studied and described [66,67]. Within this group, *An. gambiae* s.s. has been most significantly impacted by Insecticide Treated Nets (ITNs) because of its greater tendency to feed on people indoors and during times they are using nets [75]. Consequently, this vector species has almost disappeared from some African settings where it used to be the dominant vector following sustained high coverage of ITNs [76,77]. *Anopheles arabiensis* has more flexible host choice than *An. gambiae* s.s, and often feeds on both human and livestock hosts [78,79], and can feed and rest outdoors as well as indoors [80]. These behaviours make *An. arabiensis* less susceptible to ITNs by reducing their contact with this intervention [81–83]. Detailed understanding of the ecology and behaviour of these species has enabled identification of more targeted approaches for each species (e.g. Cattle based vector control for *An. arabiensis* and new formulations for indoor insecticides for *An. gambiae* s.s).

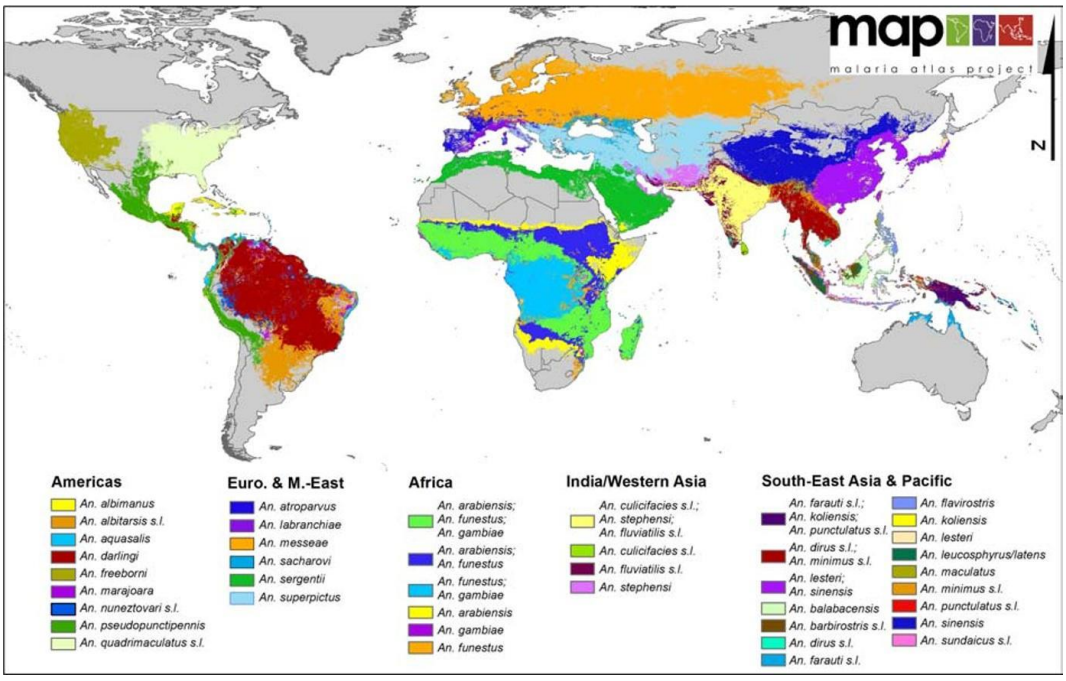

Figure 1.1: A global map of dominant vectors of malaria transmission [68].

In contrast to *An. gambiae s.l.*, relatively little is known about the ecology, behaviour and susceptibility of vectors within the *An. funestus* s.l group. *Anopheles funestus* s.l, 1900 is one of the most efficient vectors of malaria transmission world-wide [15]. This group includes at least 13 morphologically identical species: *Anopheles aruni, Anopheles brucei, Anopheles confusus, An. funestus s.s., Anopheles funestus*-like, *Anopheles fuscivenosus, Anopheles leesoni, Anopheles longipalpis* type A, *Anopheles longipalpis* type C, *Anopheles parensisis, Anopheles rivulorum, Anopheles rivulorum*-like, and *Anopheles vaneedeni* [66,84–86]. Only *An. funestus* s.s is considered to have a substantial role in malaria transmission due to its high vectorial capacity [87–89]. This vector has been largely neglected and is often considered a more minor vector of transmission than *An. gambiae* [67,90]. However, in recent years *An. funestus* there is increasing evidence to suggest this species is mediating a significant portion of the transmission in several African settings [89,90,92,93]. In particular, it has been highlighted as a major source of residual malaria transmission [91]. The transmission that remains in areas where high universal coverage with effective ITNs and/or IRS intervention has been achieved is defined as residual [93]. In contrast to the *An. gambiae* complex, *An. funestus s.s.* prefers permanent and semi-permanent larval habitats like swamps and large ponds [94,95]. These habitats are more likely to persist throughout the dry season and thus can sustain *An. funestus s.s.* populations and malaria transmission throughout the year [66,96]. These larval habitats can either be natural water bodies or artificial ones [54,97]. *Anopheles funestus* larvae are most abundant in aquatic habitats containing vegetation; as this feature is crucial for larvae survival [46,95]. This vector is also highly endophilic (feeding indoors) and anthropophilic (feeding on human beings) [98–100]. Although historically described as being an almost exclusive, late-night indoor biter, recent reports of *An. funestus s.s* switching to bite outdoors or in the morning indicate this vector may be changing its behaviour to avoid indoor interventions such as ITNs and IRS or after unsuccessful feeding indoor [101–103]. Another challenge with *An. funestus* s.s. is the inability to rear this species in standard laboratory conditions; meaning that there are very few stable colonies of this species [104]. The lack of stable colonies makes it even harder to study and conduct detailed ecological studies on this mosquito species. This combination

of attributes has allowed *An. funestus* to thrive in areas where other vector species have been more effectively suppressed, and indicates that new and additional control approaches will be required to tackle it. Consequently, identification of optimal vector control strategies is more difficult for *An. funestus s.l.* than *An. gambiae s.l.*; given the considerably larger knowledge gaps on its basic biology and ecology.

1.4 Vector Surveillance

Mosquito vector surveillance is considered a crucial component of malaria control [105]. Several surveillance tools have been designed and used to study different aspects of malaria vector populations ranging from trapping methods that target different life history ages, and molecular methods for identification of vector species and their infection status [71,106,107]. Regular and long-term surveillance is required to understand human exposure to vector populations and the impact of interventions. One of the primary goals of vector surveillance is to estimate the human biting rate (HBR); defined as the number of mosquito bites a person would be expected to receive in specific times and places. The HBR is the most important indicator of human exposure, and often used as a proxy for transmission intensity [108–110]. Since the development of the Ross MacDonald model of malaria transmission, the HBR has been recognized as a crucial predictor of transmission intensity [44,65,111]. This indicator can be directly estimated by sampling the number of mosquitoes attempting to bite a person using a gold standard method (Human Landing Catches-HLC). To perform an HLC, researchers typically expose part of their body (e.g. their legs) and collect any mosquitoes that land on it and try to bite [112,113]. While this method produces the most direct estimates of human exposure [114–116], it has several limitations. First, it puts those doing the collections at risk of infection [112,117]. While in the case of malaria, this risk may be mitigated by provision of prophylaxis [112,117], there remains a risk of exposure to other mosquito borne-diseases (i.e. Dengue, Zika, Rift Valley Fever) that could be circulating in the area [118–120]. Additionally, this approach has logistical challenges of being highly labour intensive and variable; making it ill-suited for mass surveillance. Consequently, there is a long history of exploring alternative trapping methods to provide exposure-free estimates of the HBR. Ideally such trapping methods

should provide consistent estimates with the HLC to be appropriate for quantifying malaria transmission intensity in a given area.

Several exposure-free traps have been developed and evaluated relative to the human landing catch in different African countries [114,121–125]. These studies have used various modelling and statistical approaches to estimate a single value [correction factor] which can be used to convert the catches by a given trap to HLCs [114,124,126]. The most notable and well evaluated trap for vector surveillance is the Centre for Disease and Control light trap (CDC-Light trap) [114,127]. This sampling tool was designed to be effective when sampling mosquitoes which prefer to bite human indoors [116,128,129]. The indoor bias of this trap leaves out mosquitoes which prefer to bite outdoors and on non-human hosts. Increased recognition of the importance of outdoor biting for residual transmission [130] necessitates development and evaluation of different outdoor traps for indirect estimation of HBR.

A limitation of current work is that it has generally focussed on trap validation for *An. gambiae s.l.*, and on indoor settings. It is often assumed that alternative traps will perform similarly well for *An. gambiae s.l.* and *An. funestus*, but this is rarely validated. Additionally, there is increasing evidence of *Anopheles funestus*, biting outdoors in areas of high, widespread usage of indoor interventions [89,101,102]. Given the growing recognition of the importance of outdoor exposure and transmission [77,101,131,132], for *An. funestus* and other vectors, there is a need to explicitly validate and expand range of the range of exposure-free sampling tools for exposure estimation of *An. funestus* HBR in outdoor settings.

1.5 Population dynamics model of malaria vectors

Numerous mathematical models have been developed and used for malaria control decision-making since Ross's original work [43,45,83,133–136]. Mathematical modelling has been used to simulate mosquito population dynamics and examine relationships between larval stages, adult mosquitoes and environmental covariates [133,137–140]. There are several examples of these models being used to explain vector population dynamics and the impact of

intervention at various resolutions [138–142]. These models have served to identify and explain the role of key environmental drivers such as precipitation and temperature on transmission through their impacts of mosquito population dynamics and transmission potential [29,79,143–146]. These meteorological variables are important explanatory variables of mosquito vector borne disease dynamics [140,147,148]. Additionally, annual cycles of rainfall can drive massive seasonal fluctuations in mosquito vector populations [47,149,150], which generally predict transmission dynamics. Rainfall is a key determinant of malaria transmission because it is directly linked to larval habitat availability and regulates the carrying capacity of the vector population [94,150–153]. Temperature also has a large influence on vector population dynamics and demography through its impacts on larval development, survival (adult and larvae) [47,154–156], and the length of gonotrophic cycles [154,157].

Modelling can make a crucial contribution to understanding vector population dynamics through estimating values of demographics and fitness traits, and the impact of environmental variables [150,158,159]. Models of mosquito population dynamics have been used to address a range of fundamental and applied problems with respect to vector ecology and control [133,135,139,160,161], and to predict knock-on impacts for transmission [43,141,162]. While a large body of work has been based on theoretical models under simulated scenarios, increasingly models are developed to address setting-specific issues in Africa, with model frameworks adapted to fit the local ecological context and needs. There is a growing appreciation of the value of incorporating models to support decision making within national malaria control programmes [163–165]. Having a detailed understanding of the dynamics and stability of target vector populations within specific settings is an essential first step to support such localized use of modelling.

Models of vector population dynamics can play a vital role in improving understanding of which additional interventions are likely to generate the most impact in particular ecological and epidemiological contexts. The impacts of a particular intervention approach may vary substantially between settings in relation to local vector ecology and resistance profiles. For example, differences in vector ecology and ambient environmental conditions between locations could alter the stability of vector populations and the effort needed to suppress or

destabilize them. A notable example of this could be differences in vector species. As reviewed above, the major African malaria vectors differ in several aspects of their ecology and behaviour that influence their environmental associations and seasonal dynamics [16,79,81,103,166–168]. Despite these differences, most models of African malaria vector population dynamics are based on parameters from *An. gambiae* s.l. [135,139,141,163,169]. There are few specific models of *An. funestus* dynamics because this species has been very difficult to study in the laboratory and only parts of its life cycle in the wild can be studied [24,170]. Given the unique ecology of *An. funestus*, and growing recognition of its importance in sustaining residual transmission, there is an urgent need to better understand the environmental drivers and determinants of its population stability.

A range of different approaches have been used to model the dynamics of malaria vector populations. These include deterministic models that describe changes in vector populations as a result of pre-defined mechanistic functions and parameters [131,148,150,171–173]. While very useful to outline the general properties of mosquito populations, these dynamic models can be difficult to create when crucial parameter values are unknown. This is often the case with *An. funestus* populations where crucial data on demographic and fitness parameters in the wild are unknown [174]. State-space models (SSM) provide an alternative approach to indirectly estimate these parameters by fitting a population dynamics model to observed time series data [159,175,176]. SSMs have been widely used in different areas of ecology, wildlife management and conservation biology to reconstruct otherwise hidden aspects of population dynamics [159,177–179] and influence management decisions [180]. However, these models have so far had limited application in medical entomology. This modelling approach holds particular promise for investigation of vector species like *An. funestus* whose demography is more challenging to directly monitor. Here, such models could be used to estimate feasible demographic rates that could explain the observed dynamics [159,179]; thus generating information on key parameters that could be used to model response to control.

1.6 Vector control interventions

Several vector control tools have been developed that target African malaria vectors [2,105,137,171,181–184]. These strategies can vary in the life history stages of the mosquito population they target (e.g. larvae versus adult) and their impact on mosquito population dynamics and malaria transmission [141]. The most widely used are Insecticide-Treated Nets (ITNS), Indoor Residual Spraying (IRS) and Larval Source Management (LSM) [2,91,105,137,185]. ITNs and IRS have been approved as core interventions by the WHO while LSM has been approved as complimentary intervention [105]. Vector control (IRS and ITNs) alone accounted for 78% of the total decline in malaria prevalence in African between 2000-2015 [2,3]. Improved vector control and case management, urbanization, improved health care, and higher living conditions, have all contributed to these gains [2,3]. Other vector control interventions include house screening, auto-dissemination of larvicides, space spraying, attractive target sugar baits, and genetically modified mosquitoes [181,186–189]. It is anticipated that vector control will continue to be a major component of malaria control and elimination strategies.

Conventionally, ITNs are delivered through mass campaigns targeting pregnant women during routine visits to health facilities, or through school age children programs (i.e. School Net program SNP) and mass distribution in communities as part of the National Malaria Control Program (NMCP) strategy [105,190–192]. ITNs have the dual advantage of first offering physical protection to users when they are fully intact and without holes, and second by providing a community effect by killing adult mosquitoes that contact insecticides while trying to feed; thus reducing their population [193,194]. The effectiveness of ITNs is determined by their residual efficacy and physical integrity. Consequently the WHO states that mass distribution should occur every three years based on ITN attrition projections. Furthermore, the effectiveness of the intervention is contingent on their consistent use during the transmission season.

Indoor residual spraying (IRS) is spraying of prequalified residual pesticides onto potential mosquito resting surfaces like interior walls, eaves, and ceilings of homes or structures such as domestic animal shelters [105]. The sort of

insecticides deployed and the characteristics of the sprayed wall influences IRS efficacy [105,195]. The WHO recommends that IRS products must have a residual efficacy of at least 6 months on the sprayed surface before being listed as a prequalified product [196]. Unlike ITNs which target host seeking females, IRS impacts resting mosquitoes (both female and males). The effectiveness of IRS depends on the spraying performance, the coverage and susceptibility of the vectors to the insecticide used [105,197]. In most countries, IRS is deployed right before the rainy season in which malaria transmission is concentrated [198,199]. IRS is recommended for application in high transmission areas, and ideally using a rotation of different insecticide classes to mitigate against resistance [105]. Due to the high cost and logistical challenges of implementing IRS, it is not as widely used in many countries as ITNs.

Larval Source Management targets the aquatic stages of the mosquitoes. LSM works by preventing the completion of development of the immature stages (eggs, larvae and pupae), which stops the emergence of adults [200,201]. This can be achieved either through habitat elimination, biological control, and habitat modifications or treating them with a pesticide that kills larvae [202,203]. Deployment of LSM is usually recommended during the driest period of the year when there is no rain or flowing water which might dilute the level of insecticide used in larviciding [105,202,203]. LSM is considered cost-effective and generally recommended in urban settings where the breeding habitats are “fixed, few and findable” [105]. There is evidence that it may also be effective when deployed in the rural setting under specific circumstance [105,201,202]. In African countries, LSM is not widely used in many settings due to its logistical challenges. Despite widespread use and relatively high coverage of ITNs and IRS, malaria vector populations remain widespread and abundant throughout the African continent. Vector abundance and infection rates vary considerably in space and time, leading to high heterogeneity in malaria transmission between regions [109,166,204–206]. The existence of residual transmission presents a number of obstacles for malaria elimination. Tackling residual transmission will require understanding of the mechanism through which vector population persists and additional new interventions to better target them at different life stages.

Key challenges to current vector control approaches include insecticide resistance [204,207–209] and shifts in mosquito behaviour including increased outdoor and early evening/late morning biting in major malaria vectors [77,102,130,169]. Resistance to pyrethroids, the primary insecticide class used in ITNs, has been widely documented in multiple vector species across Africa [104,209–214]. These and other constraints mean that ITNs and IRS alone will not be sufficient to achieve malaria elimination in Africa [215]. More effective strategies are required that can better target the sources of residual transmission; including vector populations with diverse behaviour and resistance profiles. This could include either the implementation of new approaches or a more optimal combination of existing methods (ITNs, IRS, LSM, etc.). Given that both ITNs and IRS target indoor vector populations, they may not be sufficient to disrupt population stability where a considerable portion of target populations feed and rest outdoors. Greater success may be achieved through integration of interventions targeting vectors at different life cycle stages (larvae and adults) and with different behaviours (indoor versus outdoor, etc.). Detailed knowledge of the ecology and behaviour of *An. gambiae s.l.* can facilitate identification of which combination of approaches could most effectively disrupt populations of this vector. However, similar identification of optimal intervention approaches or combinations will be harder for *An. funestus s.l.* without a much better understanding of the key drivers of their population dynamics and stability.

1.7 Research focus

In Tanzania, there has been a significant reduction in the malaria transmission since the scaling-up of ITNs [1,3]. Recent reports from the Tanzania Malaria Indicator Survey (TMIS) show an overall reduction in (national) malaria prevalence from 16.9% in 2007 [216] to 7.3% in 2017 [217]; indicative of an average 55% drop in transmission. Despite progress, more effort is required to tackle residual transmission. In Tanzania, residual transmission has been associated with the increasing in insecticide resistance [88] and changes in biting and resting preferences of the current vectors of malaria transmission [77,79,88,89,91].

In some parts of East Africa including Tanzania, populations of the formerly prolific malaria vector *An. gambiae* s.s., have significantly diminished, or completely vanished following mass ITN distribution leaving *An. arabiensis* and *An. funestus* as the remaining sources of transmission [182,190,218,219]. This scenario occurred In the Kilombero Valley, where the relative abundance of *An. gambiae* (compared to *An. arabiensis*) fell from about 90% to nearly 10% between 1997 and 2009 [77]. *Anopheles arabiensis* is now the most numerically abundant vector in this area [89,103], however, malaria infection rates are considerably higher in the co-occurring, lower density *An. funestus* populations. These *An funestus* populations account for the bulk of residual transmission (>85% of all infections) [88,89,91]. Studies show that *An. funestus* is not only widely resistant to pyrethroids in Tanzania [88,220,221], but may also have a survival advantage over *An. arabiensis* [100,222,223]. A difficulty with the surveillance of *An. funestus* is that although they are widespread, there is uncertainty about how well trapping methods can detect their relatively low density populations. There is a need to more rigorously validate existing and novel surveillance methods for *An. funestus*, particularly those used to detect "atypical" routes of exposure such as outdoor biting. Given the importance of *An. funestus* in Tanzania, there is a need to better understand how to colonize it, survey it, and assess its population dynamics and stability.

1.8 Objectives and outline

This thesis aims to strengthen knowledge on the ecology and population dynamics of *An. funestus;* the major vector of residual malaria transmission in Tanzania and other parts of Africa. The overall goal is to fill key knowledge gaps on its population dynamics as needed to predict the potential impact of different vector control interventions. The information used in this study was gathered through insectary and field surveys. Although all data collection occurred in the Kilombero Valley of south eastern Tanzania, the methodology developed is meant to provide baseline estimates for demographic parameters of *An. funestus* populations in other parts of Sub-Saharan Africa.

The objectives were:

1. ***To quantify the fitness and behaviour of wild Anopheles funestus from Tanzania and their offspring during repeated colonization attempts under standard laboratory conditions***. This objective is addressed in Chapter 2 which quantifies different fitness measures (survival, larval development period, mating, and body size) of *An. funestus* under laboratory conditions, with the aim of assessing barriers to successful colonization of this vector. This study also provided data on *An. funestus* demographic processes which provided prior values for the population dynamics models described in later chapters. The study described in this chapter has been peer reviewed and published in Malaria Journal [24].

2. ***To develop and validate a framework for predicting HLC-derived outdoor exposure rates from different exposure-free alternatives traps***. This objective is addressed in Chapter 3 which describes a field-based evaluation of different methods to predict the HBR of *An. funestus* in outdoor environments. This chapter also describes the development of a calibration tool which can be used to predict the number of *An. funestus* that would be expected in a standard Human Landing Catch (HLC) from the number collected by an alternative trap. This study has already been published in the Parasites & Vectors Journal [224].

3. ***To develop a population dynamics model that can be used to describe the ecology of the wild population of An. funestus***. This objective is addressed in Chapter 4 which describes the development of a State-Space Model in a Bayesian framework to infer the drivers of wild *An. funestus* populations in Tanzania. This model is used to assess the contribution of environmental variables (temperature and rainfall) and density dependence to *An. funestus* population growth. This framework has already been published in the Malaria Journal [153].

4. ***To assess the generalizability of this population dynamics model and its ability to reconstruct missing data***. This objective addressed in Chapter 5 which critically re-examines the population dynamics model

developed in the previous chapter (Chapter 4) through investigation of all potential limitations to its generalizability. The investigation provides new insights into the potential existence of hidden demographic processes which may be influencing the overall dynamics of these populations.

Chapter 6 provides a comprehensive discussion of all the previous chapters and their contributions to the overall objective of the PhD; a concise overview of general limitations, challenges, and suggestions for future work.

2.0 Chapter 2: Fitness characteristics of the malaria vector Anopheles funestus during an attempted laboratory colonization

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Abstract

Background

The malaria vector *Anopheles funestus* is increasingly recognized as a dominant vector of residual transmission in many African settings. Efforts to better understand its biology and control are significantly impeded by the difficulties of colonizing it under laboratory conditions. To identify key bottlenecks in colonization, this study compared the development and fitness characteristics of wild *An. funestus* from Tanzania (FUTAZ) and their F_1 offspring during colonization attempts. The demography and reproductive success of wild FUTAZ offspring were compared to that of individuals from one of the only *An. funestus* strains that has been successfully colonized (FUMOZ, from Mozambique) under similar laboratory conditions.

Methods

Wild *An. funestus* (FUTAZ) were collected from three Tanzanian villages and maintained in an insectary at 70-85% RH, 25-27°C and 12hr:12hr photoperiod. Eggs from these females were used to establish three replicate F_1 laboratory generations. Larval development, survival, fecundity, mating success, percentage pupation and wing length were measured in the F_1 FUTAZ offspring and compared with wild FUTAZ and FUMOZ mosquitoes.

Results

Wild FUTAZ laid fewer eggs (64.1; 95%CI [63.2, 65.0]) than FUMOZ females (76.1; 95%CI [73.3, 79.1]). Survival of F_1-FUTAZ larvae under laboratory conditions was low, with an egg-to-pupae conversion rate of only 5.9% compared to 27.4% in FUMOZ. The median lifespan of F_1-FUTAZ females (32 days) and males (33 days)

was lower than FUMOZ (52 and 49 for females and males respectively). The proportion of female F_1-FUTAZ inseminated under laboratory conditions (9%) was considerably lower than either FUMOZ (72%) or wild-caught FUTAZ females (92%). This resulted in almost no viable F_2-FUTAZ eggs being produced. Wild FUTAZ body sizes (as estimated from wing size) were larger than that of lab reared F_1-FUTAZ and FUMOZ.

Conclusions

This study indicates that poor larval survival, mating success, low fecundity and shorter survival under laboratory conditions all contribute to difficulties in colonizing of *An. funestus*. Future studies should focus on enhancing these aspects of *An. funestus* fitness in the laboratory, with the biggest barrier likely to be poor mating.

2.0 Background

Malaria transmission in Africa is dominated by species in the *Anopheles gambiae* and *Anopheles funestus* species complexes. Control of these vectors has been the primary driver of malaria reduction since 2000 [2,217], and requires thorough understanding of their ecology, behaviours and transmission potential [45,58,62,103,116,225,226]. Laboratory colonies of *An. gambiae sensu lato* (*s.l.*) have been an invaluable resource for research by enabling experimental studies under controlled conditions. Such colonies have facilitated the characterization of insecticide resistance [209,227,228], genetics [229,230]), immunity [231,232] and key vector demographic profiles [59,109,233]. Mosquitoes generated from laboratory colonies are also extensively used for semi-field bioassays [58,81,135].

In contrast to *An. gambiae s.l.*, *An. funestus s.l.* has proven extremely difficult to colonize and maintain under laboratory conditions. The *An. funestus* species complex group consists of at least 13 known species*: Anopheles aruni, Anopheles brucei, Anopheles confusus, Anopheles funestus sensu stricto* (*s.s.*), *Anopheles funestus*-like, *Anopheles fuscivenosus*, *Anopheles leesoni, Anopheles longipalpis* type A, *Anopheles longipalpis* type C, *Anopheles parensisis*, *Anopheles rivulorum*, *Anopheles rivulorum*-like and *Anopheles vaneedeni* [66,84–86]. These species vary in vectorial capacity [87], with only *An. funestus s.s.* thought to play a significant role in malaria transmission [88,89]. Others, such as *An. rivulorum,* have been reported as minor vectors in Kenya [234] and Tanzania [235], as has *An. vaneedeni* in South Africa [236].

Colonization of *An. funestus s.s.* has however been problematic. Only two strains have been successfully colonized from wild populations despite several attempts. Both strains were colonized at the Vector Control Reference Laboratory (VCRL) in the National Institute for Communicable Diseases, South Africa, from populations in Angola (FANG) and Mozambique (FUMOZ) [92,104]. The FUMOZ strain is also maintained at other laboratories worldwide, including in Cameroon, the UK [228], and in Tanzania (at the Ifakara Health Institute). Several attempts have been made to colonize new *An. funestus* strains [170] from wild populations, but methods used to establish FUMOZ and FANG have not

been successful elsewhere [237], including when attempted with the same wild populations where FUMOZ was originally derived (Coetzee, *pers. commun.*). This inability to repeatedly colonize and establish *An. funestus* in laboratories is responsible for the more limited understanding of the biology of this species compared to other vector species.

Several factors may account for the difficulty of colonizing *An. funestus*. Chief amongst these is eurygamy the inability to mate in cage/confined space [37,38]. Eurygamic species are difficult to colonize because they do not exhibit natural mating behaviours, such as swarming [238], under insectary conditions [37,239,240]. Many *Anopheles* mosquitoes mate naturally in aerial swarms [33,34,241] or, to a smaller extent, indoors [35]. Whilst *An. gambiae* will mate readily in the laboratory [58], wild and F_1 progeny of *An. funestus* rarely swarm inside cages. Although mating is hypothesized to be the main barrier to *An. funestus* colonization, other factors cannot be ruled out due to incomplete or absence of reporting on other aspects of their life history and fitness during attempted colonization. Therefore, it is crucial to comprehensively evaluate how all aspects of *An. funestus* life history, development and demography respond to standard methodologies for colonization to identify where modifications should be focused.

To address these knowledge gaps, the fitness and behaviour of wild *An. funestus* from Tanzania and their offspring (defined as "FUTAZ", i.e. *An. funestus* from Tanzania), were quantified during repeated colonization attempts under standard laboratory conditions. The first step to optimize the colonization process is to understand which aspects of *An. funestus* life-history and fitness are most impaired during colonization, and thus target modifications appropriately. To assess this, detailed measurements of the fitness and life-history of wild and F_1 *An. funestus* were conducted during repeated laboratory colonization attempts. Fitness measures of individuals in this nascent colony were compared to those of a stable *An. funestus* colony (FUMOZ) to identify the key barriers that hinder successful colonization of this species. The term "fitness trait" refers to measures of mating success (insemination status), fecundity (number of eggs produced), adult body size and survival (larval and adult).

Insights gained will guide future research to overcome barriers to colonizing *An. funestus*, and also increase knowledge on this important vector and its control.

2.1 Methods

2.1.1 Study area

Wild *An. funestus* adults were collected from three villages (Tulizamoyo, Ikwambi and Sululu) in Kilombero (8.1539ºS, 36.6870ºE) and Ulanga (8.3124ºS, 36.6879ºE) districts in Tanzania (Figure 2.1). These villages were selected because of they have high density populations of *An. funestus s.l*, of which >93% are known to be *An. funestus s.s.* [195]. Wild-caught females were transported to the Ifakara Health Institute and used in experiments at the Vector Biology & Control Laboratory, the "VectorSphere" (Figure 2.1).

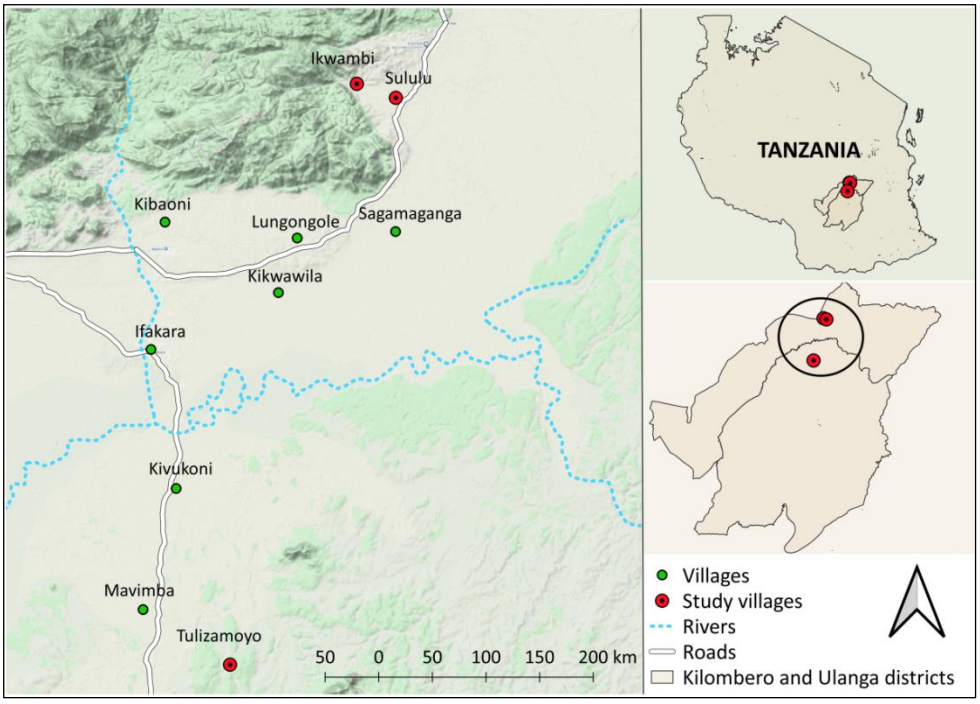

Figure 2.1: A map of study area showing the location of the villages where *Anopheles funestus* females (wild-FUTAZ) were sampled for colonization experiments.

2.1.2 Mosquito sampling

Five houses were selected for mosquito collection in each village. Mosquito collections were conducted for one week in each village in 2019 (Tulizamoyo: 17-23rd June; Ikwambi: 8-16th July and Sululu: 1-10th September). Due to collection logistics and space limitation in the VectorSphere, only one set of experiments (i.e. with mosquitoes from just one village) was done at a time. Trapping was done using CDC light traps [127,242] that were set from 6pm to 6am for five consecutive nights per village (yielding ~200-300 female *An. funestus s.l.* per week). Light traps were fitted with larger catch bags to help keep mosquitoes alive without desiccation until morning. Every morning, live female *An. funestus s.l.* were aspirated from collection bags into netted cages (30 x 30 cm), provided with 10% glucose solution and brought to the VectorSphere for blood-feeding and further rearing. Inside the VectorSphere mosquitoes were kept under standard conditions of 70-85% RH, 25-27^{o}C and a 12hr: 12hr photoperiod.

2.1.3 Laboratory maintenance and fitness measurements for FUTAZ mosquitoes

In the VectorSphere, wild female of *An. funestus s.l.* were given an initial blood meal from a chicken for a maximum of 30 minutes (from 6:30pm) inside cages covered with dark cloth. After this meal, mosquitoes were left in the cage until the next morning when their feeding success was recorded by visual observation. Those with a distended, red abdomen were considered to be fully engorged and transferred into individual oviposition cups for egg laying (Figure 2.2). Cotton pads soaked in 10% glucose solution were placed onto the top netting over the cups for additional nutrition. After three days, a small amount of water (~5ml) was put in each cup to stimulate oviposition. Cups were then inspected daily to record if and when eggs were laid, and dead mosquitoes were removed. Mosquitoes that did not lay eggs after 12 days were killed by freezing for 10 minutes and later dissected to assess insemination. The terminalia and last abdominal segment (segment IX) were cut-open in distilled water to expose the spermathecae. Slide mounts of spermathecae were inspected using a microscope at 400x magnification for presence of sperm (Figure. 2.3a&b).

F_1 eggs from wild-caught *An. funestus s.l.* were identified to species-level based on their morphological characteristics [15]. Eggs were observed under a stereomicroscope, and sub-samples of emergent adults verified by PCR [243]. All eggs morphologically confirmed as belonging to *An. funestus s.s.* were retained for use in subsequent colonization and life history experiments and defined as F_1-FUTAZ (Table 2.1). Here, all F_1-FUTAZ eggs were pooled and redistributed into a series of 5L round plastic basins (30 cm diameter, filled to 3.3 cm with tap water, replaced every two days) at approximate densities of 400-600 eggs per basin and left to hatch. There were a total of 12 replicate basins set up for each of the 3 independent colonization experiments (1 per study village). From here onwards, the term *An. funestus* refers specifically to *An. funestus s.s.*

Figure 2.2: Images of laboratory set-up for the colonization process:
a) Series of wooden cages with oviposition cups, each contains a single fully-engorged Anopheles funestus female. Individual cups were used to measure the number of eggs laid by a single mosquito after full blood meal, **b)** Technician counting the number of eggs and measuring the wing sizes of individual mosquitoes which have laid eggs, **c)** Technician aspirating mosquitoes from the rearing cage using mouth aspirator and **d)** bowl contains eggs of Anopheles funestus. All experiment was done within the VectorSphere at Ifakara Health Institute.

Larvae were fed daily with a pinch (approx. 0.36 grams) of a mixture of finely crushed dog biscuits and brewer's yeast at a ratio of 3:1 [104]. Basins were checked daily to record egg hatching and larval survival. Pupae that emerged

over three consecutive days were recorded and retained for use in F_1 adult mosquito fitness experiments. The female: male ratio of emerging F_1 adults was recorded by looking at the genital lobe shape (i.e. at the end of the pupae abdominal segments just below the paddles) and also males tend to be smaller than females [188,244]. Pupae were placed in a single cage (30 x 30 cm) and monitored until emergence (1-2 days). A total of three cages were set up for each of the three independent colonization experiments.

F_1-FUTAZ females were offered their first human blood meal five days post-emergence. Females from the FUMOZ colony whose fitness was compared to F_1-FUTAZ were also provided a human blood meal on the same day and time (see below). Females of both strains were provided with additional blood meals every five days. The rationale for blood feeding every 5 days was to estimate the survival under conditions where they had access to blood meals at a frequency similar to that expected in the wild. Studies indicate that the gonotrophic cycle length of *An. funestus* ranges between 2-5 days [31,32]; with 5 being selected here due to practical considerations. From the first blood meal onwards, cages were inspected daily to record and remove all dead mosquitoes. A total of three cages were used as replicates in each of the 3 independent colonization experiments. All dead females were dissected to assess insemination status. Random sub-samples of F_1 females were selected after the second blood meal and moved into individual oviposition cups to measure their egg production.

2.1.4 Laboratory maintenance and fitness measurements for FUMOZ mosquitoes

In July 2018, eggs from the FUMOZ *An. funestus* colony were obtained from the VCRL laboratory in South Africa and used to establish a colony within the VectorSphere at Ifakara Health Institute in Tanzania. The founder FUMOZ colony at VCRL has been maintained since 2000 [104]. At IHI, the FUMOZ colony was maintained for four generations before starting these experiments. This colony was kept under the same insectary conditions (70-85% RH, 25-27^{o}C and 12hr: 12hr photoperiod) and same feeding regime as described above for F_1-FUTAZ. In this study, the following fitness variables were measured in FUMOZ for comparison with FUTAZ: number of eggs laid per mosquito (fecundity),

proportion of eggs hatched, wing lengths, proportion of adult females inseminated, proportion of larvae survived (from 1st instar to pupation), larval development period, the proportion of pupae that emerged as adults, female: male sex ratio at pupae stage and number of days survived by adult both males and females. The definition of all fitness traits measured, and the colonies in which they were made were given in (Table 2.1).

2.1.5 Mosquito wing size measurements

The wing lengths of all female *An. funestus* (Wild FUTAZ, F_1-FUTAZ and FUMOZ) were measured and used as a proxy for their body size. One wing was removed from each mosquito and placed onto a drop of water on a microscope slide. The wing lengths were measured using the micrometre ruler under a microscope (50mm micrometre scale in 0.1mm divisions, 70mm x 20mm x 3mm) [245]. Measurements were taken from the apical notch to the auxiliary margin, excluding the wing fringe (Figure 2.3d).

Table 2.1: Descriptions of terms used and strains compared as used in this study

Variable measured	**Strains compared**	**Definition as used in this paper**
Fecundity	Wild-FUTAZ vs. FUMOZ	Number of eggs laid by a single female mosquito
Wing size	Wild-FUTAZ vs. F_1-FUTAZ vs. FUMOZ	Length (mm) of a wing from apical notch to the auxiliary margin
% eggs hatched	F_1-FUTAZ vs. FUMOZ	Number of eggs hatched as a percentage of the total number of eggs laid
% Larvae survival	F_1-FUTAZ vs. FUMOZ	Number of larvae pupating, as a percentage of all eggs produced per individual female
Larval development period	F_1-FUTAZ vs. FUMOZ	Number of days from 1st instar larvae to pupation
Sex ratio	F_1-FUTAZ vs. FUMOZ	Ration of the number of females to number of males as identified at the pupal stage
% Adult emerging	F_1-FUTAZ vs. FUMOZ	Number of adult emerged as a percentage of pupae stage
% Female inseminated	Wild-FUTAZ vs. F_1-FUTAZ vs. FUMOZ	Number of females found to be inseminated as a percentage of total dissected
Adult survival rate	F_1-FUTAZ vs. FUMOZ	Number of days survived by an adult mosquito in the laboratory

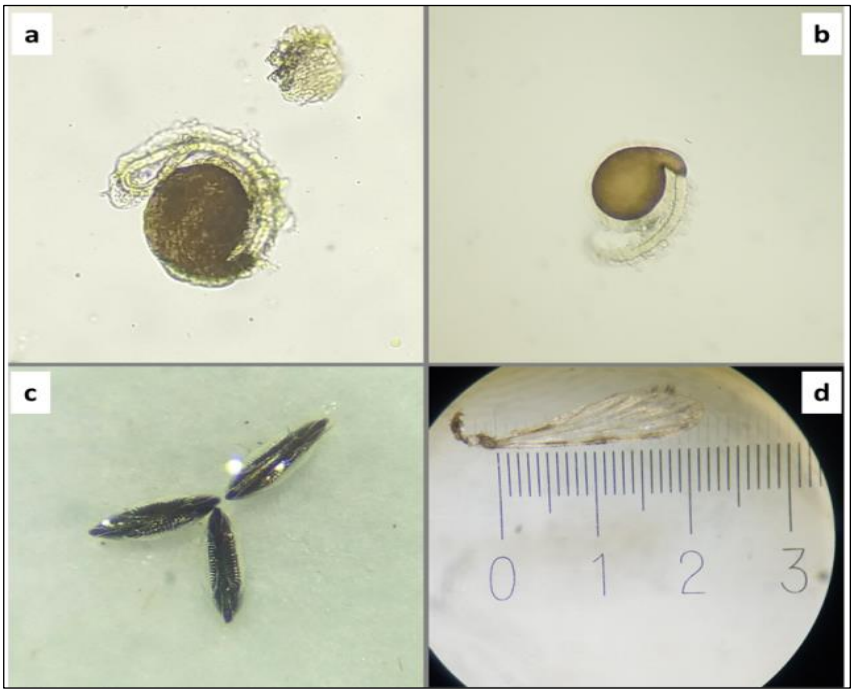

Figure 2.3: Microscopic images of different parts of *Anopheles funestus*: showing the **a)** presence of the spermatozoa as the confirmation for insemination and **b)** absence of spermatozoa suggesting non-inseminated, **c)** Egg structure of the female Anopheles funestus s.s. as seen under microscope, a quick and cheap method of species distinction within Anopheles funestus group during colonization instead of standard Polymerase Chain Reaction (PCR) and **d)** the wing measurement under microscope showing the apical notch and the auxiliary margin.

2.1.6 Statistical analysis

Data analyses were conducted using the R statistical software version 3.5.0 [246]. Mean values of these fitness traits were measured and compared between FUTAZ and FUMOZ (Table 2.1). Where possible, fitness traits (i.e. wing length and proportion inseminated) were also compared among the wild FUTAZ, F_1-FUTAZ and FUMOZ. Additional analysis was conducted to assess the relationship between female body size and fecundity (number of eggs produced) in wild FUTAZ and FUMOZ. It is known for other species that, the larger the body size the higher the number of eggs laid per female mosquitoes [247,248].

Generalized linear mixed models (GLMM) implemented in *lme4* package [249] were used to estimate mean values of fitness traits in wild, F_1-FUTAZ and FUMOZ strains. Fecundity (the number of eggs laid per mosquito) was modelled as following a Poisson distribution with wing length included in the model as a fixed

effect. Proportion data (here, emergence, insemination, larval survival and sex ratio) were modelled as binomial variates with strain used as fixed effects. Wing length and strain were also fit as fixed effects when assessing insemination rates. Wing length was modelled as a Gamma variate with an inverse link function, incorporating strain as fixed effect. In all the model fitting process, experimental replicates and villages were included as random effects. Tukey's post-hoc tests were used to assess the means differences for different fitness measurements.

Analysis of adult survival was conducted using a Cox proportional hazard model using the *survival* package [250] to assess the odds of mortality in males and females and of different strains (F_1-FUTAZ vs. FUMOZ). Here, the response variable was the death occurring on each day of observation, while strain and sex were included as fixed explanatory variables. In the analysis of F_1-FUTAZ, site of collection (village) was included as a random effect by fitting a frailty function [251,252] using a Gamma distribution. Separate analyses were performed for each strain except when the differences between strains were investigated. Log likelihood ratio tests (LRT) were used to test the significance of each variable of interest in all models. All figures were produced using *ggplot2* [253] and *survminer* [254] R packages.

2.2 Results

A total of 1,130 adult females of the wild-FUTAZ strain were collected from the three different villages of Tulizamoyo (n=332); Ikwambi (n=425); Sululu (n=373). More than two-thirds (n=804) of these successfully fed when offered a chicken blood-meal in the insectary, of which 39% (n=316) laid eggs in the insectary.

2.2.1 Mosquito wing lengths, mating status, fecundity and pupation

Anopheles funestus wing lengths varied significantly between groups (x^2=14.97, p<0.001, Figure 2.4a). A Tukey's *post-hoc* test showed that wild-collected FUTAZ were larger than lab reared F_1-FUTAZ (z=3.23, p<0.01, Figure 2.4a) and FUMOZ (z=2.52, p<0.05, Figure 2.4a). There was no difference in wing lengths between

the two laboratory-reared strains, FUMOZ and F_1-FUTAZ (z=1.43, p=0.303, Figure 2.4a).

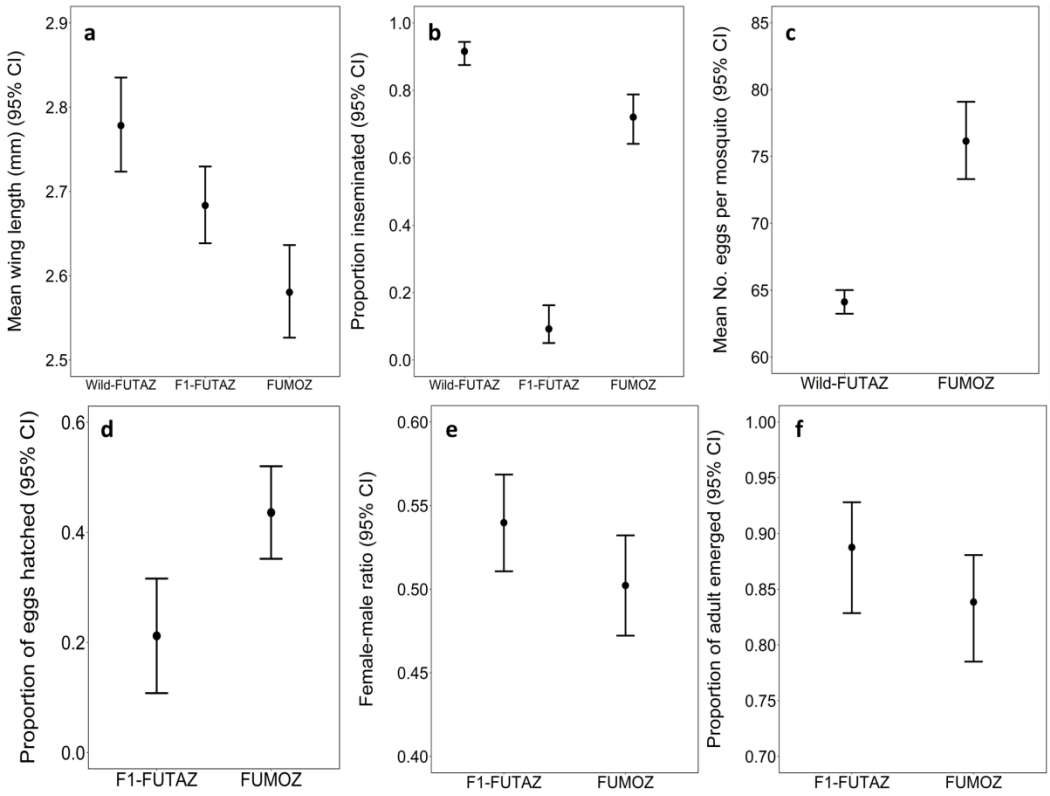

Figure 2.4: Showing *Anopheles funestus* **a**) wing sizes; **b**) female inseminated, **c**) fecundity, **d**) eggs hatched, **e**) sex ratio, **f**) adult emerged for Wild-FUTAZ, F_1-FUTAZ and FUMOZ strain. Error bars are 95%CI predicted from the GLMM.

The wing lengths of F_1-FUTAZ (x^2=10.4, p<0.01) but not FUMOZ (x^2=0.123, p=0.688) were positively associated with insemination status. Furthermore, the proportion inseminated varied significantly between strains (x^2=177.2, p<0.001, Figure 2.4b, Table 2.2), and the two generations of FUTAZ (x^2=172.3, p<0.001, Figure 2.4b, Table 2.2). Insemination was considerably lower in F_1-FUTAZ (9%) compared to wild caught FUTAZ females (92%) and FUMOZ (72%; Figure 2.4b, Table 2.2).

Table 2.2: Relative odds (OR) and means of insemination, sex ratio, pupation and adult emergence for different strains of Anopheles funestus, number in brackets are 95% confidence intervals with their respective p-values.

Variable	Strain	Mean ± 2SE	OR [95% CI]	P-value
% Insemination	Wild-FUTAZ	91.5 ± 1.30	1	
	F_1-FUTAZ	9.2 ± 4.99	0.009 [0.004, 0.020]	<0.001
	FUMOZ	72.0 ± 5.16	0.24 [0.13, 0.42]	<0.001
% Sex ratio (F/M)	F_1-FUTAZ	53 ± 4.0	1	=0.049
	FUMOZ	50 ± 2.9	0.86 [0.74, 0.99]	
% Larval survival	F_1-FUTAZ	5.3 ± 3.5	1	<0.001
	FUMOZ	27.4 ± 12.5	12.05 [4.27, 34.03]	
% Adult emergence	F_1-FUTAZ	88.8 ± 7.35	1	=0.174
	FUMOZ	81.6 ± 8.90	0.66 [0.36, 1.20]	

Fecundity varied between strains (x^2=66.54, p<0.001) with FUMOZ females producing significantly more eggs than wild-FUTAZ (Figure 2.4c, Table 2.3). FUMOZ clutch size ranged from 41-137 eggs while wild-FUTAZ clutch sizes ranged from 3-236 eggs. The proportion of eggs hatched into 1st instar larvae was 21% [95% CI; 10.8, 31.6] in F_1-FUTAZ and 44% [35.2, 52.0] in FUMOZ (Figure 2.4d). No eggs were produced by F1-FUTAZ. The impact of wing length on fecundity varied between strains (x^2=62.57, p<0.001, Figure 2.5a).

Table 2.3: Relative rate (RR) and means of fecundity for different strains of *Anopheles funestus*, number in brackets are 95% confidence intervals.

Variable	Strain	Mean ± 2SE	RR [95% CI]	P-value
Fecundity	Wild-FUTAZ	64.1 ± 5.26	1	
	FUMOZ	76.1 ± 7.61	0.84 0.81, 0.88]	<0.001

The median larval development period from 1st instar larvae to pupation was 22, IQR: 21-23 days in FUMOZ, and only 13, IQR: 11-14 days in F_1-FUTAZ (Figure 2.5b). Overall, the proportion of eggs surviving to pupation was 5.87% in F_1-FUTAZ and 27.4% in FUMOZ, reflecting significant variation between strains (x^2=11.28, p<0.001). The sex ratio (females: males) in pupae varied marginally between strains (x^2=3.89, p=0.049, Table 2.2 & Figure 2.4e), with a slightly

higher proportion of females in F_1-FUTAZ compared to FUMOZ (Table 2.3). The proportion of adults that emerged from pupae was similar in F_1-FUTAZ and FUMOZ (x^2=1.91, p=0.167, Figure 2.4f), with most adults emerging on the second day of pupation (Figure 2.5c).

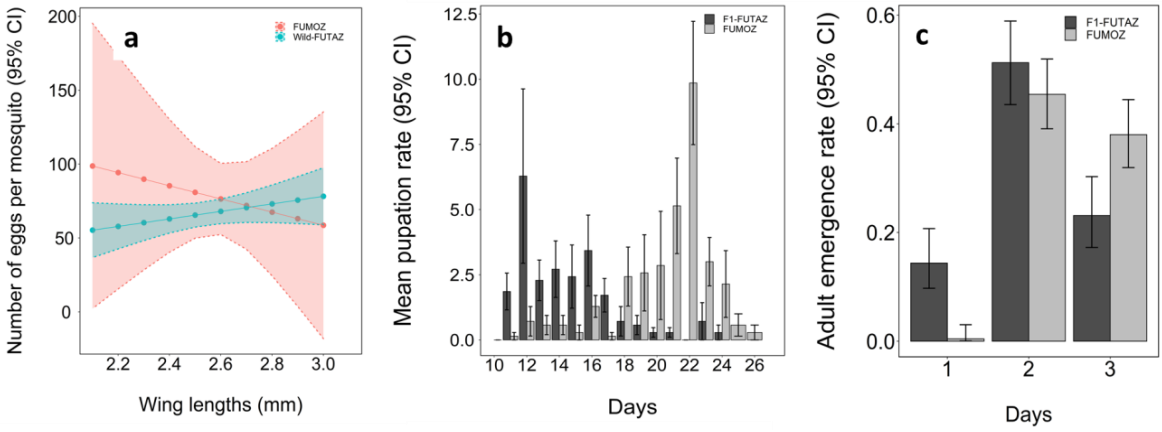

Figure 2.5: *Anopheles funestus* **a)** relationship between mosquito body sizes and number of eggs produced per *Anopheles funestus* mosquito, **b)** larvae period and **c)** adult emergence rate from pupae

2.2.2 Adult survival

The median survival of adult female F_1-FUTAZ was 32 (IQR: 26, 40) and 33 days (IQR: 27, 41) for males (Figure 2.7a, Table 2.4). In FUMOZ, the median survival for females was 52 days (IQR: 39, 56) and 49 days (IQR: 42, 56) for males (Figure 2.7b). There was no difference in the survival of males and females within either strain, F_1-FUTAZ (p=0.468, Table 2.4) and FUMOZ (p=0.752, Table 2.4). However, restricting analysis to adult females, survival was significantly lower in F_1-FUTAZ than FUMOZ (p<0.001, Table 2.3, Figure 2.7c). Likewise, adult male survival was significantly lower in F1-FUTAZ than FUMOZ (p<0.01, Table 2.4).

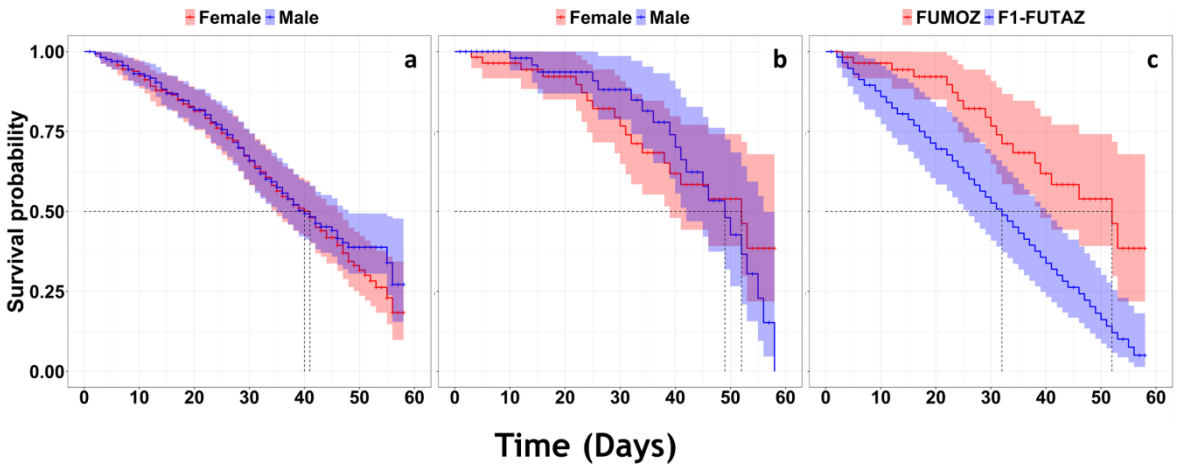

Figure 2.6: Survival curve for *Anopheles funestus* males and females: **a)** Anopheles funestus (F1-FUTAZ) and **b)** Anopheles funestus (FUMOZ) when fed after every five days, **c)** females of both F1-FUTAZ and FUMOZ. Lines represent the survival function as estimated from fitting the Cox proportion hazard model and shaded area express 95% CI. Dotted grey horizontal and vertical lines show the median survival days.

Table 2.4: Hazard Ratio and median values of the adult survival between males and females of the F_1-FUTAZ and FUMOZ, associated p-values indicate the significance difference of sex and species on the number of days survived by F_1-FUTAZ and FUMOZ strains.

Strain	Sex	Median [IQR]	HR [95% CI]	P-value
F_1-FUTAZ	Female	32 [26, 40]	1	=0.468
	Male	33 [27, 41]	0.89 [0.65, 1.24]	
FUMOZ	Female	52 [39, 56]	1	=0.752
	Male	49 [42, 56]	1.11 [0.59, 2.06]	
Female	FUMOZ	52 [39, 56]	1	<0.001
	F_1-FUTAZ	32 [26, 40]	2.63 [1.55, 4.46],	
Male	FUMOZ	49 [42, 56]	1	<0.01
	F_1-FUTAZ	33 [27, 41]	2.05 [1.22, 3.45]	

2.3 Discussion

Limited understanding of mosquito biology and ecology poses a challenge for the development of effective vector control approaches. Laboratory colonization of target species provides an opportunity to address these knowledge gaps by facilitating detailed investigation of vector biology under controlled conditions where experimental manipulation is possible. Here the fitness traits of *An. funestus* during colonization attempts from a wild population in Tanzania were characterized to identify the bottlenecks that make this species so difficult to colonize. This is the first documentation of fitness constraints during attempted colonization of this species and the first report of attempted colonization of *An. funestus* from Tanzania.

Consistent with most previous attempts, colonization of this wild *An. funestus* population proved unsuccessful with no offspring being produced from the F_1 generation. Several life history processes and demographic traits were identified as being impaired when FUTAZ were brought into the laboratory. First, the number of eggs laid by wild FUTAZ when brought in the insectary was lower than in the well-establish FUMOZ line, as were hatching rates, larval survival, mating success and adult female/male survival. F_1-FUTAZ body sizes were slightly smaller compared to their maternal generation in the wild, but did not differ with the FUMOZ strain indicating this trait is unlikely to predict colonization success. Of all these fitness traits, the primary hurdles to colonization are likely to be the extremely low mating success and larval survival of F_1 *An. funestus* in the laboratory. Until these fitness traits can be improved under laboratory conditions, the colonization of *An. funestus* is unlikely to be successful and repeatable.

Eggs laid by wild-FUTAZ *An. funestus* had a low proportion of hatching compared to those of FUMOZ. In both strains, insemination rates were lower than 50%, indicating many were unviable eggs laid by non-inseminated females. Previous studies investigating the impact of different water sources used for larval rearing in an *An. funestus* colony (FUMOZ) indicated that their egg hatching rate can exceed 70% [222,255]; confirming hatch rates in this study were uncharacteristically low. It is known that females of other *Anopheles* species can

produce unviable eggs without successful mating, or after mating with sperm-less males [256]. Therefore, poor hatching observed in the nascent strain (FUTAZ) here is likely due to the low mating success of *An. funestus* in captivity as has been previously documented in other *Anopheles* species [237], and low hatch rates. Low hatch rates could have been due to suboptimal temperatures in the laboratory relative to conditions in the field; which FUMOZ strain might have been well adapted to it but not FUTAZ [257].

The larval development period of F_1-FUTAZ (11-14 days) was similar to that reported for *An. funestus* in other laboratory settings [222,255,258], but faster than FUMOZ development period (21-23 days) observed in this study. The duration of larval development in *An. funestus* (FUTAZ and FUMOZ) observed here were considerably longer than described for *An. gambiae* complex in the laboratory [259]. For example, life table analyses of *An. gambiae* indicate a larval development period from eclosion to adult emergence of about 11 days at 27^{o}C [32,48,52,259,260]. This extended larval development period for F_1-FUTAZ results in a long estimated generation time of 30 – 33 days from eggs to first oviposition; which is higher than estimated for other African *Anopheles* species [255,261,262]. Other life table analyses performed on *An. funestus* colonies estimated a generation time of approximately 33 days in insecticide-resistant (FUMOZ) and susceptible strains (FANG) [222]. As a consequence of this extended period of larval development, egg to pupa survival was very low; approximately 6% for F_1-FUTAZ and 27% for FUMOZ. Due to this long larval development and associated high larval mortality, very large numbers of eggs would be required generate modest numbers of adults in the laboratory. Therefore, the fitness and reproductive success of these resulting adults would have to be very high to yield a further generation.

Analysis of wild FUTAZ adults and their F1 offspring indicate their fitness is reduced compared to that of a stable *An. funestus* colony (FUMOZ). Wild-FUTAZ *An. funestus* brought into the laboratory laid 16% fewer eggs than the FUMOZ colony, and the F_1 generation of FUTAZ produced no viable eggs at all. A previous study measuring the fecundity of F_1 *An. funestus* using Madagascan population reported that this species can lay and average of 56 to 108 eggs per mosquito in captivity [237], which corroborates with 65 and 76 eggs from wild-

FUTAZ and FUMOZ, respectively, from the current study. The number of eggs here is consistent to that reported in resistant and susceptible *An. funestus* strains in the laboratory, [222].

Mosquito body size is often interpreted as a proxy of their fitness [61,263,264]. Here, wild FUTAZ were somewhat larger in body size than F_1-FUTAZ and FUMOZ. Consistent with the hypothesis of body size being an indicator of fitness, wing length was positively correlated with fecundity in the wild population of *An. funestus* (FUTAZ). However, the opposite was seen in the stable FUMOZ strain where wing size was negatively associated with fecundity. Thus, at least in this one stable laboratory colony, large body size in *An. funestus* was not a good indicator of reproductive success. Caution is required in extrapolating fitness differences based on *An. funestus* body size, particularly between field and laboratory strains. Although body size was higher in wild-FUTAZ than F_1-FUTAZ, the latter strain was still bigger than the FUMOZ which had the highest fecundity.

The mating success of *An. funestus* from these populations was extremely low in the laboratory, supporting hypothesis that mating is the key bottleneck for the colonization of this species. Compared to wild-FUTAZ, insemination rates in F_1-FUTAZ were extremely low (9.2% vs. 72%) and insufficient to establish a further F_2-FUTAZ generation. This poor mating success is likely due eurygamy, the inability of some *Anopheles* species including *An. funestus* to initiate natural swarming behaviour in flight [265,266]. These findings match those of other studies documenting mating as the major obstacle for successful colonization of *An. funestus* [170,237,267]. To overcome this problem, techniques such as forced mating and exposing mosquitoes during sunset to induce swarming have been applied [268,269]. Other studies have experimented with the use of large cages to stimulate natural mating for *Anopheles,* and simulate sunset which may be crucial cue for mating [270,271]. However so far these methods have had little or no success over multiple attempts [104,170]. In the current study, no F_2-FUTAZ offspring were generated because none of the F_1-FUTAZ laid viable eggs. Further research on how to induce mating behaviour in *An. funestus*, particularly using more realistic semi-field systems, would be of great value. Such studies

must focus on both females and males, to determine if males are unwilling to initiate swarming behaviour or not fit enough to do so.

Analysis of adult mosquito survival indicated that the nascent Tanzania colony (F_1-FUTAZ) had a reduced lifespan compared to stable *An. funestus* colony (FUMOZ). However adult survival in both cases was relatively high (32 median days for FUTAZ and 52 days for FUMOZ); with both strains living well beyond the minimum period required to produce eggs and transmit malaria. Another laboratory study conducted on FUMOZ where adult life span ranged from 39 to 64 days [222]; again much higher than F_1-FUTAZ here. The shorter life span of FUTAZ relative to FUMOZ may be a result of the stress from the change of environment, or lack of adaption to laboratory conditions. Nevertheless, this F_1-FUTAZ survived much longer compared to another competent vectors of malaria transmission, *Anopheles arabiensis* and *An. gambiae s.s.* in laboratory conditions [81]. Previous experiments on parity shows that the median survival of *An. funestus* in the wild is much shorter, ranging from 7-10 days [41]. Thus, poor adult survival relative to the wild cannot explain the failure of colonization here.

A potential limitation of my study is that the unfed *An. funestus* females were used to seed laboratory colonies, requiring me to blood feed them artificially (on chicken blood) in the laboratory to acquire eggs for the next generation. Thus, the mating status and age of the wild females used for colonization were uncertain and likely variable. An alternative would have been to collect only visibly blood fed females during field collections (these individuals would likely had fed on humans if caught inside houses), and used their eggs to generate the F1 generation. This was considered, but given the much lower abundance of blood fed *An. funestus* inside houses compared to the numbers of unfed females that can be obtained in CDC light traps; the latter approach was chosen to ensure sufficiently large samples were obtained for colonization experiments. These wild mosquitoes could not be provided with a human blood meal given their malaria infection status was unknown and they were not adapted to membrane feeding, thus chicken blood was provided. This variation in host blood source could have generated some differences in fitness between strains. However, this is unlikely given that previous studies indicate that human and

chicken blood meals generate similar egg production in other African malaria vector species under laboratory conditions (*An. arabiensis* and *An. gambiae* [78,81]). The F_1 population, upon which the main fitness indicators were assessed, was fed on human blood. Further investigation is required to confirm whether *An. funestus* fitness is impacted by the type of host blood meals provided and whether there is an optimal diet for laboratory maintenance.

2.4 Conclusions

Laboratory colonies remain fundamental for research on the biology and control of mosquito vectors by providing a stable and standardized source of mosquitoes for experimental studies. This study provides additional evidence of the intractability of *An. funestus* to colonization. By quantifying a comprehensive range of fitness traits during unsuccessful attempts, this study generates insights into the most important barriers to colonization. Of the range of traits investigated, the primary barrier to colonization was identified as low mating success, compounded further by the slow development and poor survival of the small numbers of larvae produced. Additionally, both the fecundity and adult survival of *An. funestus* offspring from wild parents were reduced under laboratory conditions, but these impacts may have been relatively minor compared to the consequences of poor mating success and poor larval survival. This combination of fitness deficits presents a major challenge for successful colonization and mass rearing of *An. funestus*. To overcome this, future research should focus on enhancing the efficiency these life-cycle processes under insectary conditions. Additionally, the demographic rates estimated from wild and F_1 FUTAZ will provide useful baseline information for understanding and modelling *An. funestus* population dynamics in general, and guiding further attempts to its colonization.

3.0 Chapter 3: A statistical calibration tool for methods used to sample outdoor-biting mosquitoes

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Abstract

Background

Methods for sampling outdoor-biting mosquitoes are urgently needed to improve surveillance of vector-borne diseases. Such tools could potentially replace the human landing catch (HLC), which, despite being the most direct option for measuring human exposure, raises significant ethical and logistical concerns. Several alternatives are under development but detailed evaluation still requires common frameworks for calibration relative to the HLC. The aim of this study was therefore to develop and validate a framework for predicting HLC-derived outdoor exposure rates from different exposure-free alternatives for African malaria (*An. arabiensis* and *An. funestus*) and filiariasis (*Culex* spp.) vectors.

Methods

I obtained mosquito abundance data (*Anopheles arabiensis, Anopheles funestus* and *Culex sp.*) from a year-long Tanzanian study comparing six outdoor traps and the HLC. The outdoor traps were: Suna Trap (SUN), BG Sentinel (BGS), M-Trap (MTR), M-Trap + CDC (MTR-C), Ifakara Tent Trap-C (ITT-C) and Mosquito Magnet Trap (MMX). Four generalised linear models were developed within a Bayesian framework to investigate associations between traps and the HLC; considering intra- and inter-specific density dependence. The best model was used to create a calibration tool for predicting HLC-equivalents of each trap for each vector group.

Results

For *An. arabiensis*, SUN catches had the strongest correlation with HLC (R^2=19.4), followed by BGS (R^2=17.2), MTRC (R^2=13.1). The least correlated was MMX (R^2=2.5). For *An. funestus*, BGS had the strongest correlation with the HLC (R^2=53.4), followed by MTRC (R^2=37.4), MTR (R^2=37.4). The least favoured was SUN (R^2=14.5). For *Culex* mosquitoes, the traps most highly correlated with the HLC were MTR (R^2=45.4) and MTRC (R^2=44.2). In general, the association between the HLC and alternative trapping methods was the best described through a simple linear relationship. Only BGS in all the three species exhibit a non-linear relationship which is modified by intra and interspecies mosquito density. An interactive *Shiny App* calibration tool was developed for this and similar applications.

Conclusion

I successfully developed a calibration tool to assess the performance of outdoor-traps relative to this HLC to provide a framework for assessing human exposure. The performance of candidate traps relative to the HLC varied between mosquito taxa, thus there was no single optimal method. Though all the traps underestimated HLC catches (proxy for human-biting rates), it was possible to mathematically define their representativeness against HLC. Further studies should focus identifying traps that have the greatest consistency and representativeness relative to the HLC, as opposed to simply finding traps that catch the most mosquitoes.

3.0 Background

Malaria control primarily relies on the use of Insecticide Treated Nets (ITNs) and Indoor Residual Spraying (IRS) [123,182,183,272]. LLINs and IRS provide protection by targeting mosquitoes that are host seeking or resting indoors. Wide-scale use of these tools has yielded significant progress but there are still challenges such as insecticide resistance [176,212,214,273] and outdoor-biting [77,89,90]. Drivers of outdoor-biting may include human behaviours [274–276], plasticity in mosquito behaviours (e.g. shifting from feeding indoors to outdoors) [16,79,103,212] and the effects of some indoor interventions [181,277]. Sampling mosquito populations is a core component of malaria surveillance activities [278], and it's aims include determining when and where people are most at risk. For best results, this surveillance should consistently capture the key drivers of biting risk indoors and outdoors. Unfortunately, representative sampling of mosquito vectors remains a challenge; particularly in outdoor settings.

The main entomological indicators assessed during vector surveillance include the Human Biting Rate (HBR) [103,279], sporozoite infection prevalence in mosquitoes [280,281], the Entomological Inoculation Rate (EIR) [88,279], time of exposure and proportion of exposure prevented by ITNs [108,109,124,282]. The HBR is a fundamental variable for estimating the transmission of malaria and other mosquito-borne diseases [65]. As defined in the Ross MacDonald model, the HBR is required for estimation of the reproductive rate (R_0) of malaria. Both the HBR and sporozoite prevalence are required for estimation of EIR [65]; calculated as the number of infectious bites a person would be expected to receive in a given location over a given time period. The HBR and EIR are frequently used to estimate the impact of vector control interventions by highlighting how much they reduce exposure [76,93,182,279].

The Human Landing Catch (HLC) has been the gold standard for direct measurement of human exposure and other key entomological variables. However, this method has several limitations and ethical constraints [118,119,283–285] due to its requirement that human volunteers expose parts of their body (usually lower legs) to mosquitoes [129,282,286]. Due to this combination of ethical concerns and practical limitations, it is widely

recognized that alternative exposure-free methods for measuring the HBR are needed [121,122,282,286,287]. Alternatives such as CDC light traps are already widely used for sampling host-seeking mosquitoes indoors [114], but are unsuitable in outdoor settings. The urgency to identify suitable methods for measuring exposure outdoors is therefore greater [123,125,282,288], especially due to the growing recognition of the importance of outdoor exposure to residual transmission [77,93,274].

To date, a number of alternative exposure-free methods have been independently developed and tested in different settings in Africa (e.g. [122–124,282,286,288–291]). Some methods provide a good representation of vector species composition and their biting activities, but underestimate density (e.g. [123,287,288](. Others catch more mosquitoes than the HLC and thus overestimate typical human exposure (e.g. [122,292]). Finally there are some traps that are easy to implement, but which provide biased estimates of outdoor exposure by disproportionately sampling endophilic rather than exophilic species (e.g. [293]). These strengths and weaknesses suggest that different traps are optimal for different surveillance applications. Unfortunately, there are no standardized calibration methods to allow estimation of HLC-equivalent exposure from different outdoor sampling methods. Development of a standardized and validated calibration framework for such prediction would enable comparison of results from different studies and methods. Such a calibration tool would need to reflect the potential non-linear relationship between trap counts and HLC values; meaning that no single conversion ‘value’ between methods may apply across the full range of mosquito densities or species.

Several studies have indicated that trap performance relative to the HLC is density dependent [114,294]. However, density dependence is often considered in terms of “intraspecific” density (e.g. the baseline density of the target vector species [282,294]) but not the density of all mosquitoes, target vectors or not, that are attracted to the trap. However, the mechanisms that could give rise to intra-specific density dependence in trap performance could also generate dependence with the overall densities of all mosquitoes attracted to the trap; including other species not of interest. While such interspecific dependence on the wider mosquito community is plausible, this has not been

formally evaluated in trap evaluation studies.

The overall aim of this study was to provide an extensive comparison of six exposure-free traps for 3 vectors (*An. arabiensis, An. funestus* and *Culex* sp. which is the common mosquito group and a vector of filiariasis in Tanzania). Specifically, I aimed to 1) identify valid and safe alternatives to the HLC for outdoor mosquito' sampling that are exposure-free and do not rely on human participants, 2) assess of contribution of intra and interspecific mosquito density dependence to trap performance, and 3) develop an interactive calibration tool (in the form of a Shiny App) through which the number of a given species caught in an HLC can be predicted from catches made by alternative traps.

3.1 Methods

3.1.1 Study area and vector species

Prior to the start of my PhD., a team from the Ifakara Health Institute carried out a mosquito trapping study in six adjacent villages in the Ulanga and Kilombero districts of south-eastern Tanzania, namely: Kivukoni (8.2135°S, 36.6879°E), Minepa (8.2710°S, 36.6771°E), Mavimba (8.3124°S, 36.6771°E), Milola (8.3306°S, 36.6727°E), Igumbiro (8.3511°S, 36.6725°E) and Lupiro (8.385° S, 36.670°E). Data were collected over 12 months between 2015 and 2016. The valley has relatively high mosquito abundance which peaks at the end of the rainy season. The common vectors of malaria transmission are *Anopheles arabiensis* and *Anopheles funestus* [88,103,295]. Mosquitoes in the *Culex* genera are also highly abundant, with some species being potential vectors for arboviruses found in the study area [120,296].

3.1.2 Data collection

I was not involved in the design of this study or the field data collection, but the data were made available to me for analysis in my PhD. Mosquito sampling was carried out in both the wet and dry seasons, using six different traps for sampling outdoor-biting mosquitoes around human dwellings. The traps were: Mosquito Magnet trap (MMX) [297], BG-Sentinel trap (BGS) [298], Suna trap (SUN)

[123], Ifakara Tent Trap-C (ITT-C) [291], M-Trap (MTR) [299], M-Trap fitted with CDC Light trap (MTRC) (this study) and the Human Landing Catches (HLC) [123]. Most of these traps have been extensively described elsewhere except for the MRT fitted with a CDC light trap (MTR), which was adapted from the original exposure-free M-trap designed by Mwangungulu *et al* [299]. In this current study, the original MRT was divided into two compartments made of UV-resistant shade netting: one in which a human volunteer sat to attract mosquitoes and the other section in which mosquito are entered [299]. A CDC light trap was suspended inside the other section of the trap to attract more mosquitos to the light source.

The traps were located at least 100m apart. Initial trap allocation was random, but their positions were switched over successive sampling nights in a Latin square design. This way each trap was used in each position once over a seven night cycle. After completion of each cycle, the study team moved to the next village so that one round of sampling in all six villages was completed over 42 trap-nights. Six rounds of data collection were completed spanning the wettest and the driest periods of the year (252 trap nights between April 2015 and April 2016). Mosquito sampling was done overnight from 6pm to 6am. The collected mosquitoes were morphologically sorted by taxa. A subsample of *An. gambiae* s.l. (n=1,405, 26% of total) were analysed by PCR [71] to assess sibling species composition within the complex.

3.1.3 Model fitting

The main goal of analyses was to create a calibration tool to evaluate outdoor mosquito traps and to validate the tool by comparing the performance of candidate trapping methods relative to HLC, the “gold standard”. In particular, I wanted to test the shape of the association curve linking the numbers of mosquitoes collected by each trap type with those collected by the HLC. First, I pooled all the hourly collections into a single collection cup per trap per night. Then, for each of the focus mosquito groups (*Culex* genera, *An. arabiensis* and *An. funestus* s.l), I modelled nightly HLC catches as a function of the catching rate of each alternative trap.

Four linear models were developed within a Bayesian model fitting framework to allow me to test for linear and non-linear associations through increasing the levels of complexity. The Bayesian approach allowed specific constraints on parameters based on biological plausibility, in the form of priors and uncertainty when converting the counts from alternative traps into HLC equivalent values in the form of full posteriors.

For any given trap and mosquito group, I defined the response variable (N_i) as the number of female mosquitoes on every i^{th} sampling night. Preliminary investigation of data using Poisson likelihood showed over-dispersion for all the three mosquito groups. Final models did not account for other environmental covariates at specific trap locations (e.g. temperature, humidity). Initial analysis suggests that, environmental covariates did not improve model fitting. I accounted for the over-dispersion by using a negative binomial likelihood model formulated as a Gamma-Poisson mixture distribution [300]:

$$N_i \sim Poisson(\boldsymbol{\theta_i}\, \boldsymbol{\lambda}_i)$$

with

$$\boldsymbol{\theta_i} \sim Gamma(\tau, \tau)$$

Where the Poisson rate $\boldsymbol{\lambda_i}$ is defined by the shape of the relationship between N_i and the number of mosquitoes collected with the alternative trap (n_i, Table 3.1) and theta θ_i was drawn from a gamma distribution with parameter tau τ as rate and shape of 1.

Since the algebraic form of this relationship is not known, I made three assumptions with specified mathematical definitions, as follows: 1) that the relationship must start at the origin (i.e. when HLC catches zero mosquitoes, the other traps will, on average also collect zero mosquitoes), 2) that the relationship is positive (i.e., no negative relationships between trap catches), and 3) that any given trap could potentially suffer from a density effect (i.e., the slope of the relationship is not constant and it can change according to the abundance of mosquitoes, either just of the same mosquito group or of all mosquitoes).

To define $\boldsymbol{\lambda_i}$, I therefore formulated four possible scenarios to describe the relationship between HLCs and other trapping methods as summarized in Table 3.1 and Figure 3.1. In Model 1, I considered a simple linear relationship between N_i and n_i (Table 3.1, Figure 3.1A)[1]. In Model 2 I tested if the efficiency of the alternative trap was dependent on the density of the focal mosquito (e.g. "intra-specific" density dependence) by adding a quadratic term ${n_i}^2$ (Table 3.1, Figure 3.1B). In Model 3 I tested if the captures of a given group by a given trap were dependent on the abundance of the other taxonomic groups (e.g. "inter-specific" density dependence) by adding, an interaction term between n_i and the number of all the females from other mosquito groups collected with the same trap (m_i) (Table 3.1, Figure 3.1C). Model 4 was similar to Model 3, but I considered all the other K_i taxonomic groups separately. Therefore it included all the pair wise interaction terms between n_i and the number of females of each k^{th} mosquito group (s_{k_i}) (Table 3.1, Figure 3.1D). My analysis mainly focused on three mosquito groups, but I collected a higher number of species hence $K > 3$ (Table 3.2).

Table 3.1: Description of models used to investigate the relationships between female mosquito catches by human landing catch and the alternative traps.

Model	Structure
Model 1	$\log(\boldsymbol{\lambda}_i) = \beta_1 n_i$
Model 2	$\log(\boldsymbol{\lambda}_i) = \beta_1 n_i + \beta_2 {n_i}^2$
Model 3	$\log(\boldsymbol{\lambda}_i) = \beta_1 n_i + \beta_2 n_i m_i$
Model 4	$\log(\boldsymbol{\lambda}_i) = \beta_1 n_i + \sum_{k=1}^{K} \beta_k n_i s_{k_i}$

The analysis was performed in the statistical environment R version 4.0 [246], with Bayesian model fitting to the data done using the program JAGS [301] interfaced within R via the package *rjags* [302]. For parameters β_1, β_2 and β_k I used a gamma prior (shape = 0.1, rate = 0.1). The prior for β_1 was chosen to ensure a positive relationship between n_i and N_i and a positive effect of the

[1] Note that this relationship is not linear. See Chapter 7 (Addendum to chapter 3) for a corrected version of this analysis including model formulas as described in Table 3.1.

quadratic and the interaction terms for β_2 and β_k. To achieve convergence, the models were run for up to $3\,x\,10^4$ iterations. Means of posterior distributions with corresponding credible intervals were obtained for each model coefficient β. I compared different models by their Deviance Information Criteria (DIC) and the goodness of fit of each model using pseudo R^2 values. Models with the lowest DIC were selected as best.

3.1.4 Interactive calibration tool

I designed a lookup table (Table 3.3) containing means of posterior predictions for different combinations of mosquito taxa, trap types and models. This allowed me to predict the expected number of a given mosquito taxa from an HLC (with credible intervals) based on the number caught in the alternative traps. I also developed an interactive online tool, in the form of an *R Shiny* App [303] to facilitate these evaluations. This tool provides users with an interactive graphical user interface (GUI) to select the number of captured mosquitoes for a group of interest by trap type, and to explore the predicted number of mosquitoes caught in an HLC by method.

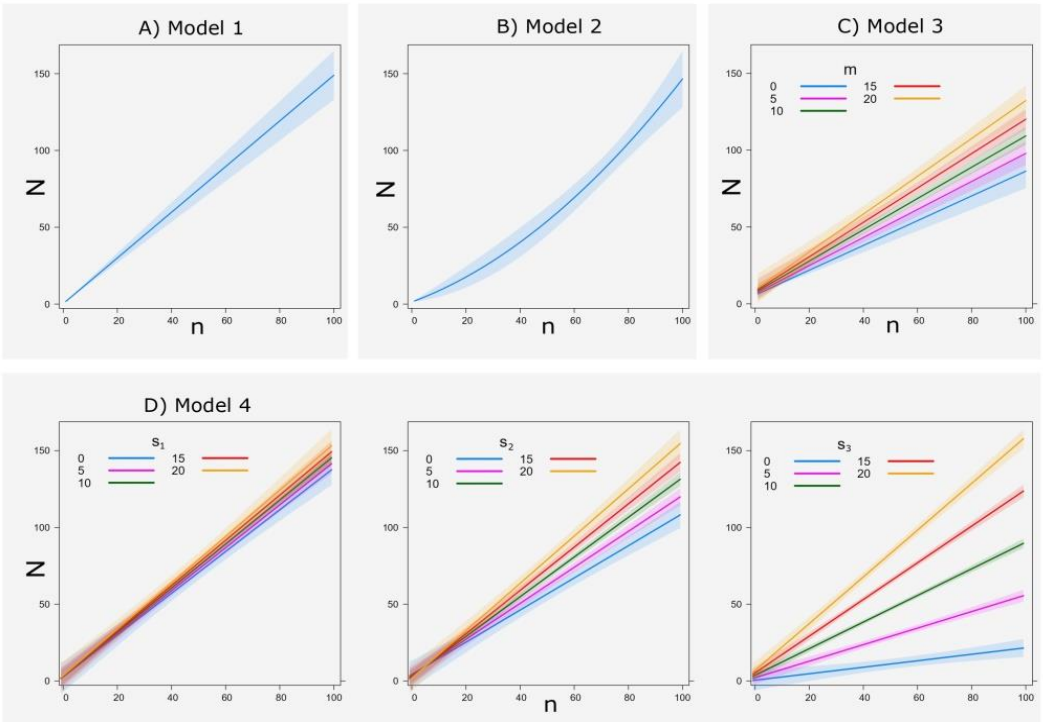

Figure 3.1: Illustration of models used to investigate the relationship between number of female mosquitoes collected with human landing catch and six alternative traps.
N: Number of female mosquitoes collected with human landing catch; n: number of female mosquitoes collected with a given alternative trap; m: pooled female

mosquitoes of all the other species collected with the same alternative trap of n; s_1, s_2 and s_3: number of female mosquitoes of each of the other K species, where K refers to all the species collected I developed the focus one (here $K = 3$). Model 1 considers a simple linear relationship with n (A). Model 2 considers a quadratic term n^2 (B). Model 3 includes an interaction term between *n* and the number of all the females of the other species collected with the same trap (m) (C). Model 4 considers all the pairwise interaction between *n* and s_1, $s_2$and s_3 (D).

3.2 Results

The statistical correlations between HLCs and other trapping methods for each of the three mosquito groups are summarized in Table 3.2. The fit of models varied between trap types and mosquito group, with correlations with the HLC (R^2 values) ranging from 0.8% - 53.4% (Table 3.2). The strength and nature of associations (Models 1-4) varied considerably between mosquito groups and traps; thus no one single model was best in all cases. I provide an example of a prediction table (Table 3.3) which describes how mosquito abundance in a HLC can be estimated from catches made by the alternative traps (using model 1, with intervals grouped by 10). Other models (model 2-4) outputs/predictions can be easily retrieved from the *Shiny* App tool. Environmental covariates (temperature and humidity) were dropped during the initial model fitting process as they were not improving the goodness of fit of the model (Model 1-4).

Table 3.2: Summary of all other mosquitoes collected for each trap type including the Human Landing Catches (HLC)

Species	HLC	SUN	BGS	ITT-C	MMX	MTRC	MTR
Anopheles arabiensis	5282	1187	531	615	370	2125	1454
Anopheles funestus	226	66	40	76	37	115	115
Anopheles coustani	670	1216	207	3	444	564	314
Anopheles pharoensis	101	906	82	0	69	103	76
Anopheles squamosus	56	196	49	1	99	145	127
Anopheles wellcomi	16	3	3	0	9	72	25
Anopheles ziemani	204	1368	183	0	269	340	156
Culex sp.	7191	3666	3709	4970	1018	7710	6645
Mansonia sp.	2101	4527	1001	111	1961	2314	1273
Aedes aegypti	20	2	5	2	30	7	5
Coquellitidia	240	84	117	14	56	127	229

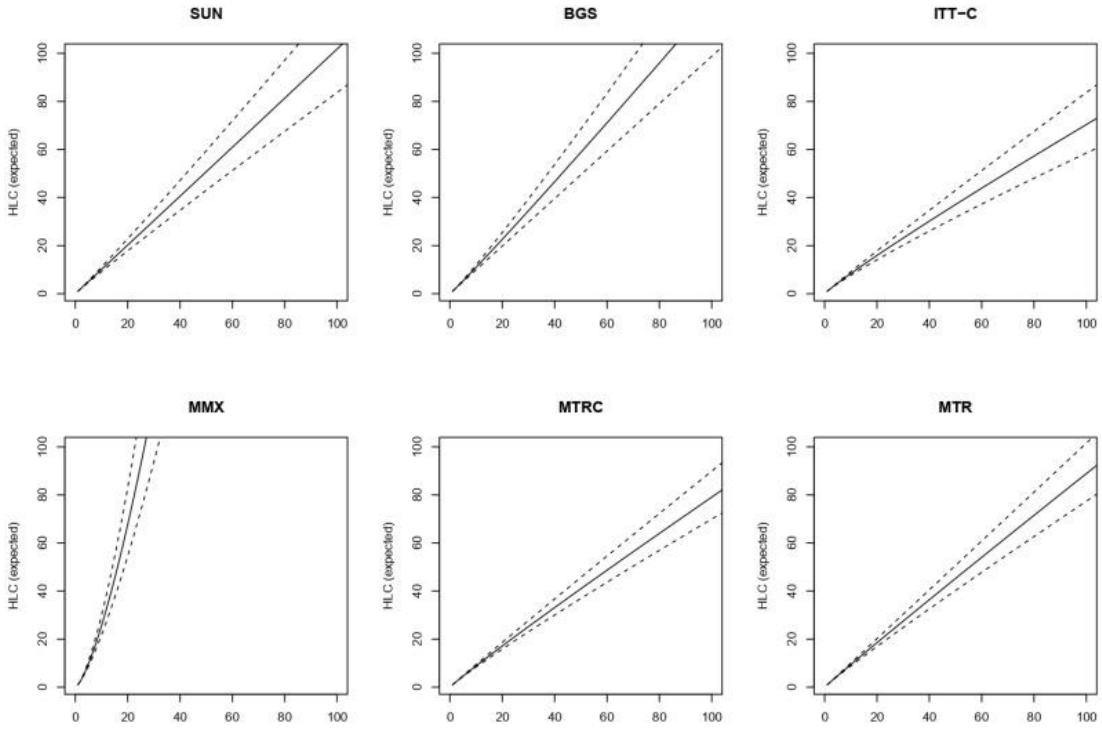

Figure 3.2: Expected number of female *Culex spp.* mosquitoes collected with HLC (y-axis), given the number of females collected with alternative traps (x-axis). Continuous line is the prediction of a Gamma-Poisson model assuming a linear relationship; dashed lines are 95% credible intervals.

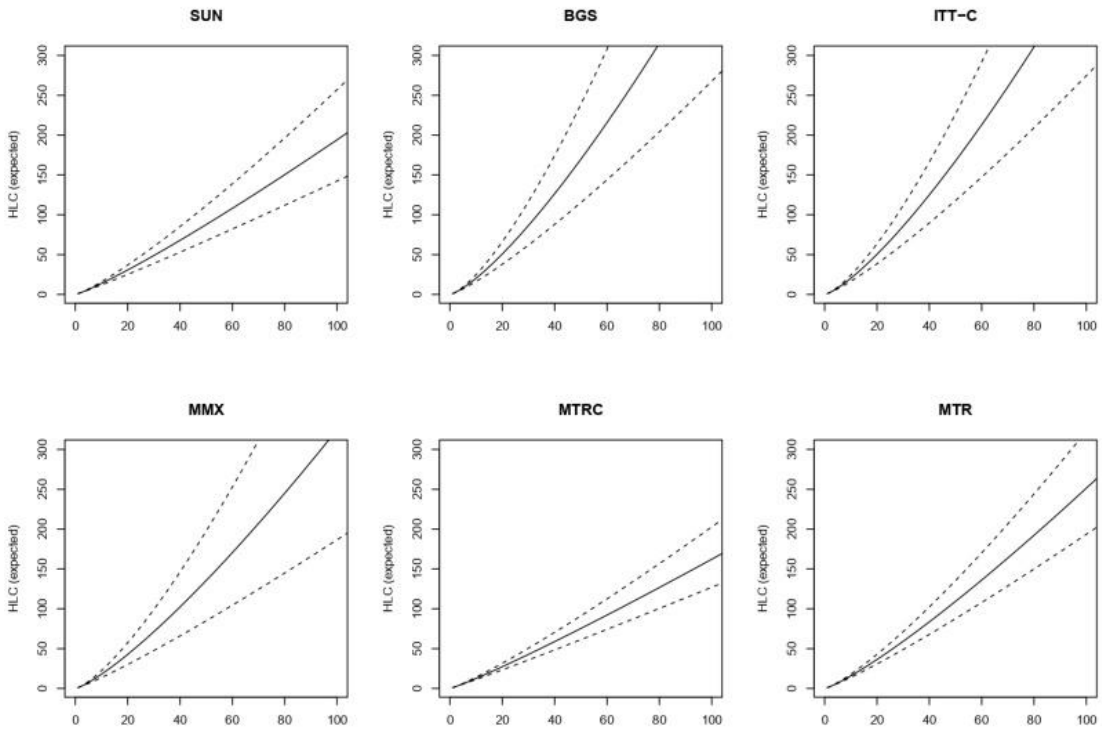

Figure 3.3: Expected number of female *Anopheles arabiensis* mosquitoes collected with HLC (y-axis), given the number of females collected with alternative traps (x-axis). Continuous line is the prediction of a Gamma-Poisson model assuming a linear relationship; dashed lines are 95% credible intervals.

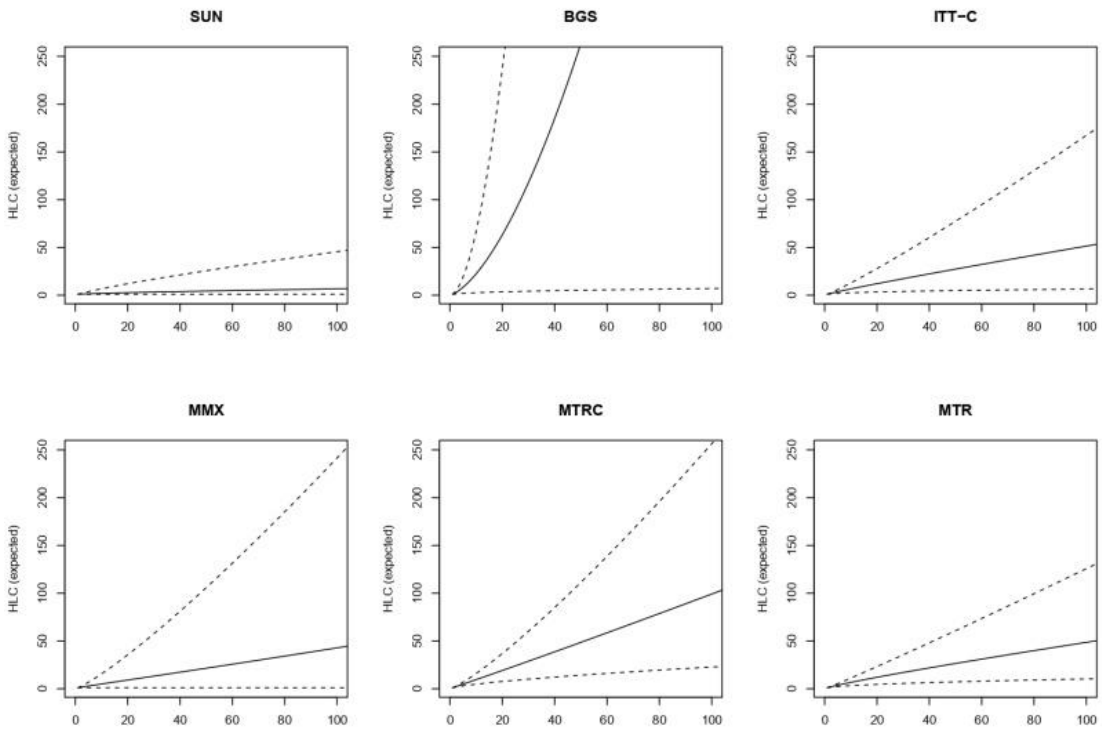

Figure 3.4: Expected number of female *Anopheles funestus* mosquitoes collected with HLC (y-axis), given the number of females collected with alternative traps (x-axis). Continuous line is the prediction of a Gamma-Poisson model assuming a linear relationship; dashed lines are 95% credible intervals.

3.2.1 *Anopheles arabiensis*

In most of the models, trap catches of *An. arabiensis* were only weakly correlated with HLC counts (Table. 3.2b). SUN was the only alternative trap where the correlation with the HLC was consistently 17% or more (R^2>17). For this trap, the relationship with HLC catches was best described by Model 4 (R^2=19.4, Table 3.2b), which incorporates both intra- and interspecific density dependence. The DIC values however did not vary much between models of differing complexity (ΔDIC = 1.32, Table 3.2b). Overall, SUN consistently underestimated HLC catches (for example 100 mosquitoes collected with SUN corresponded to 194 [CIs: 142-257]; Table 3.3b, Figure 3.3).

Table 3.3: Summary (R^2 and DIC values) of models used to investigate the relationship between the numbers of female mosquitoes collected with human landing catch (HLC) and the six alternative outdoor traps. See Table 1 for description of models.

Species and Model	SUN R^2	SUN DIC	SUN ΔDIC	BGS R^2	BGS DIC	BGS ΔDIC	ITT-C R^2	ITT-C DIC	ITT-C ΔDIC	MMX R^2	MMX DIC	MMX ΔDIC	MTRC R^2	MTRC DIC	MTRC ΔDIC	MTR R^2	MTR DIC	MTR ΔDIC
a) *Culex*																		
Model 1	27.3	1026.6	2.36	25.1	1038.3	0.00	19.0	769.3	5.07	12.4	841.0	12.50	44.7	1236.5	7.95	44.2	1174.7	6.00
Model 2	27.1	1025.3	1.05	25.1	1045.2	6.88	19.2	770.2	5.88	12.8	838.7	10.27	44.8	1236.1	7.62	44.2	1177.1	8.42
Model 3	27.2	1027.5	3.25	24.9	1040.6	2.32	19.4	769.1	4.78	12.8	828.5	0.00	44.7	1228.5	0.00	44.2	1168.7	0.00
Model 4	28.1	1024.3	0.00	25.5	1044.5	6.23	21.9	764.3	0.00	15.9	835.5	7.03	45.4	1235.8	7.29	43.8	1172.5	3.77
b)*An. arabiensis*																		
Model 1	17.4	780.3	0.00	10.8	549.6	0.61	9.3	534.7	6.37	0.8	383.9	3.51	13.1	991.6	4.43	11.3	1077.6	12.95
Model 2	17.5	780.3	0.03	10.8	549.0	0.00	9.4	528.3	0.00	0.4	382.8	2.46	10.0	1000.8	13.68	11.3	1073.0	8.37
Model 3	17.4	782.5	2.21	10.7	550.9	1.89	10.4	534.5	6.17	1.4	385.8	5.42	13.1	987.1	0.00	11.3	1070.5	5.84
Model 4	19.4	781.6	1.32	17.2	551.2	2.19	11.8	530.6	2.30	2.5	380.3	0.00	12.7	990.5	3.39	11.1	1064.6	0.00
c) *An. funestus*																		
Model 1	14.6	76.5	2.79	46.6	52.9	0.00	33.9	111.5	0.00	31.8	40.9	0.62	32.6	151.1	1.43	36.8	120.0	0.00
Model 2	14.5	77.0	3.30	50.2	53.7	0.78	33.6	112.5	0.97	30.6	40.3	0.00	32.4	149.6	0.00	36.6	120.6	0.56
Model 3	14.5	76.0	2.29	52.6	53.4	0.50	34.3	112.4	0.87	30.6	40.7	0.35	33.4	151.2	1.53	36.3	122.5	2.45
Model 4	16.2	73.7	0.00	53.4	53.8	0.87	34.3	112.2	0.69	30.0	40.4	0.09	37.4	152.8	3.13	37.4	120.7	0.65

SUN: Suna trap, BGS: BG-Sentinel trap, ITT-C: Ifakara Tent Trap version C, MMX: Mosquito Magnet trap, MTRC: M-trap combined with CDC Light, MTR: M-trap;

BGS was the only trap where R^2 values substantially increased with model complexity. Here, the most complex model (Model 4), which incorporated intra- and interspecific density dependence had an R^2 value of 17.2% (Table 3.2b). For all other trap types, correlations with the HLC were best explained by the simplest linear relationship (Model 1). Collections from BGS also underestimated the number of mosquitoes caught by HLC, and to a larger extent than the SUN (e.g. 100 mosquitoes caught by BGS is equivalent to 423 [CIs: 268-629] by HLC; Table 3.3b, Figure 3.3).

The MMX trap had the poorest correlation and was least representative of the HLC (R^2 values: 0.8 - 2.5%); particularly at low densities where it often failed to capture any individuals. This trap therefore also significantly underestimated the catches relative to HLC (for example 100 catches of MMX is equivalent to 325 [CIs: 187-504]; Table 3.3b, Figure 3.3).

3.2.2 *Anopheles funestus*

There were no major differences between the alternative models when describing associations between HLC and the other traps for collecting *An. funestus*. Thus on the basis of parsimony, I concluded that the simple linear model (Model 1) was sufficient to describe these relationships. BGS was the most highly correlated with the HLC (R^2 ranged from 46.6% to 53.4%) in *An. funestus*. The highest R^2 value was from the most complex model (Model 4). However, similar to *An. arabiensis,* the BGS underestimated the number of *An. funestus* caught by HLC (Table 3.3c). On the other hand, MTR, MTRC, ITT-C and MMX traps were only moderately correlated with HLC (R^2 values ranging from 30.0 to 37.4%), and SUN was the worst performing trap for this species (Table 3.2c).

In general, predictions obtained with all *An. funestus* trap models (Models 1-4, for all trap types) were characterized by very large credible intervals (Figure 3.4), meaning that there was insufficient precision to define a useful calibration factor. This large uncertainty amount HLC equivalent of trap catches was particularly pronounced at higher *An. funestus* densities.

Table 3.4: Predicted values for estimating the expected mosquito catches by human landing catch and alternative traps, according to the linear model (Model 1). Numbers in the first column refer to the mosquitoes collected with a given trap. To obtain the estimate of the equivalent number that one would collect with HLC, refer to the column corresponding to the trap itself. Numbers in brackets are (95% credible intervals).

		Expected HLC					
	Collected	SUN	BGS	ITT-C	MMX	MTRC	MTR
a) *Culex spp.*	10	10 (9-11)	11 (10-12)	8 (8-9)	25 (22-30)	9 (8-9)	9 (9-10)
	20	20 (18-23)	23 (20-25)	16 (14-18)	68 (54-83)	17 (16-19)	19 (17-20)
	30	30 (26-35)	35 (30-39)	23 (20-26)	120 (94-150)	25 (23-28)	27 (25-30)
	40	41 (35-47)	47 (40-54)	30 (26-35)	179 (137-230)	33 (30-37)	36 (33-40)
	50	51 (43-59)	59 (49-68)	37 (32-43)	246 (185-320)	41 (37-46)	45 (40-51)
	60	61 (51-72)	71 (59-83)	44 (37-51)	318 (236-418)	49 (44-55)	54 (48-61)
	70	71 (59-84)	83 (69-98)	51 (43-59)	395 (290-525)	56 (50-63)	63 (55-71)
	80	81 (68-97)	96 (79-114)	57 (48-68)	476 (347-639)	64 (57-72)	72 (63-81)
	90	92 (76-110)	108 (89-129)	64 (53-76)	562 (406-760)	72 (63-81)	80 (70-91)
	100	102 (84-123)	121 (99-145)	70 (59-84)	652 (467-888)	79 (70-90)	89 (77-102)
b) *An. arabiensis*	10	14 (12-16)	20 (16-25)	20 (17-24)	18 (14-22)	13 (11-14)	16 (14-18)
	20	31 (25-37)	51 (38-66)	50 (39-64)	43 (30-57)	27 (23-32)	36 (31-43)
	30	49 (39-60)	87 (62-117)	86 (63-112)	71 (48-99)	43 (36-51)	59 (49-71)
	40	68 (53-85)	126 (88-175)	125 (90-166)	102 (66-146)	59 (48-70)	84 (68-102)
	50	88 (67-111)	170 (115-239)	168 (118-227)	135 (85-198)	75 (61-91)	109 (88-135)

		Expected HLC					
	Collected	SUN	BGS	ITT-C	MMX	MTRC	MTR
b) *An. arabiensis*	60	108 (82-139)	216 (144-308)	213 (147-292)	170 (105-253)	92 (74-112)	136 (108-170)
	70	129 (97-167)	264 (174-382)	261 (178-361)	207 (125-311)	109 (87-134)	164 (129-207)
	80	150 (112-196)	315 (204-460)	311 (209-435)	245 (145-373)	127 (100-157)	192 (150-244)
	90	172 (127-226)	368 (235-543)	363 (241-512)	284 (166-437)	144 (114-179)	222 (172-283)
	100	194 (142-257)	423 (268-629)	417 (275-592)	325 (187-504)	162 (127-203)	251 (194-323)
c) *An. funestus*	10	2 (1-7)	22 (3-67)	7 (3-13)	5 (1-16)	10 (5-16)	7 (3-11)
	20	3 (1-12)	63 (4-237)	12 (3-28)	9 (1-35)	19 (8-37)	12 (5-23)
	30	3 (1-17)	118 (4-497)	17 (4-44)	13 (1-58)	29 (10-60)	17 (6-36)
	40	4 (1-21)	185 (5-840)	22 (4-60)	17 (1-81)	39 (12-85)	22 (6-48)
	50	4 (1-26)	263 (5-1262)	27 (5-77)	21 (1-106)	49 (14-111)	26 (7-61)
	60	5 (1-30)	352 (6-1760)	32 (5-95)	26 (1-131)	59 (16-139)	31 (8-74)
	70	5 (1-34)	452 (6-2332)	37 (6-113)	30 (1-158)	69 (18-167)	35 (9-86)
	80	6 (1-38)	561 (6-2975)	42 (6-131)	34 (1-185)	79 (20-196)	40 (9-99)
	90	6 (1-42)	679 (7-3689)	47 (6-149)	38 (1-213)	89 (21-226)	44 (10-113)
	100	7 (1-46)	807 (7-4471)	51 (6-167)	43 (1-242)	99 (23-256)	49 (10-126)

In that sense, the trap that resulted in (relatively) narrower prediction was the MTR, where for 100 mosquitoes collected, the model would estimate 49 HLC-equivalent, with CIs ranging between 10 and 126 (Table 3.3c).

3.2.3 *Culex* species

Overall, there were moderately higher correlations between the alternative traps and HLC for *Culex* catches than for the *Anopheles* groups (Table 3.2). There was however no major differences between the tested models (based on ΔDIC estimates), thus the simplest linear model was adopted based simply on being the thriftiest. Full details of all models are presented in Table 3.2a. Catches from MTRC and MTR traps had the highest correlations with HLCs (R^2 values ranging between 44.2% and 45.4%). On the other hand, MMX and ITT-C were the worst performing traps, and significantly underestimated the HLC catches (Table 3.2a, Figure 3.2).

3.2.4 Interactive calibration tool

To support detailed assessment and comparison of these and any future trap types for outdoor-sampling, I developed an interactive calibration tool incorporating the key parameters as identified in the analysis above. This tool is designed with simple user interfaces to simplify model inputs and outputs. For example, reporting full conversion tables for the Model 3 and 4, which include density dependence, would be challenging since the associated interaction terms would require every possible combination of mosquito group, trap types and catch range. To get estimates according to these models, readers can use the interactive online tool, which is available as an *R Shiny* App. The coefficients of these models will be updated regularly as additional data are gathered. This tool may be expanded to cover additional geographic regions and mosquito species not currently captures. The tool is hosted by an online server of the "Boyd Orr Centre for Population and Ecosystem Health" (University of Glasgow), and it is freely available at https://boydorr.gla.ac.uk/lucanelli/trapcalibration/.

3.3 Discussion

Despite the growing importance of outdoor-biting mosquitoes and their role in malaria transmission in different settings, there are limited methods for sampling outdoors. Human Landing Catches remain common and are sometimes considered as the gold standard, but there are multiple ethical, cost and logistical concerns limiting their application [112,117]. Multiple alternative tools have therefore been tested as potential HLC replacements in different settings (e.g. [114,115,121,124,287,291,293]). While most efforts have focused on finding an alternative that catches as many mosquitoes as HLC, it is now recognized that what matters more is how representative the catches from any specific trap are relative to HLC catches. It means therefore that efforts to improve surveillance methods should include not just new traps, but also a statistical tool for assessing their representativeness.

In this study, I therefore developed and validated a statistical framework for predicting credible intervals of HLC-derived exposure rates based on catches from multiple exposure-free alternatives. I have provided extensive comparison and corrections factors for the different trapping methods, as well as evidence for the most representative alternative to the HLC. Furthermore, I have translated the results of my modelling approach into an easy-to-use interactive calibration tool, which generates the expected means and credible intervals of nightly human biting rates (using HLC as a proxy) based on inputs of other trap catches.

Of several trapping methods that have been proposed for outdoor mosquito sampling of malaria vectors; only a few have been calibrated relative to the HLC (e.g. [114]); and even fewer in outdoor setting (e.g. [282,288]). These traps provide disparate levels of efficacy relative to HLC, but rely primarily on two mutually inclusive principles; i) substituting human subjects by human odours and a carbon dioxide source (e.g. [182,289]); or ii) designing traps that protect human volunteers from bites with physical barriers (e.g. [124,287,288,299]). Many studies have assessed the correlations between mosquito abundance as estimated from the HLC and an alternative (e.g. [122,123,242,286–288,291,294,304], but only a few provide the relevant quantitative estimates of "accuracy" (i.e. how close the estimates are to HLC) and precision (i.e. how

variable the estimates are) [122,286–288,291]. Furthermore, to my knowledge none have provided an explicit calibration tool to facilitate rapid predictions of mosquito counts from an alternative trap into an HLC-equivalent. Such a calibration tool would need to reflect the potential non-linear relationship between trap counts and HLC values; meaning that no single conversion "value" between methods may apply across the full range of mosquito densities. This hypothesis is backed-up by a multi-country study which evaluated the limitations of CDC light traps for estimating exposure to African malaria vectors indoors after observing non-linearity in its association with HLC [114].

In general, the overall measure for goodness of fit (R^2) for models predicting HLC counts was highest in *An. funestus*, followed by *Culex* species and *An. arabiensis*. Despite the higher value of R^2 in *An. funestus*, the wider credible intervals were probably due to the much small sample sizes of this species (total mosquito caught with HLC: *An. funestus* = 226, *An. arabiensis* = 5282, *Culex* = 7191); although there could also have been affected by other ecological features that were not directly captured with this study (e.g. other environmental conditions apart from humidity and temperature). During the model fitting exercise, temperature and humidity were excluded. The proportion of *An. funestus* in the study area compared to other species such as *An. arabiensis* and *Culex* has been historically low [88,89,103] though this species carries a significant amount of infection compared to other commonly known malaria vectors [88]. Study shows that they do bite outdoors in appreciable numbers but hard to relate trap counts from other methods to HLC equivalents for this species because of very high variability in data.

The performance of some alternative traps in comparison to the HLC has been shown to be density dependent in several investigations (e.g. [114,294]). However, such density dependent impacts are usually only considered in terms of "intra-specific" dependencies, such as the baseline density of the target vector species [282,294], overlooking the larger mosquito community. However, the same mechanisms that cause intra-specific density dependence in trap performance may also cause dependence on the overall densities of all mosquito species lured to the trap, including species that aren't of public health importance. While such reliance on the wider mosquito community is plausible, it has yet to be tested in trap evaluation studies. This study and the calibration

tool that I developed therefore also included a robust assessment of how density dependence may play a role. Model 3 and 4 included these variables and will allow users to incorporate these as covariates when predicting outdoor-biting rates in their settings of interest.

Overall, this study found little evidence that the relative performance of the trapping methods investigated here is modified by the density of the target mosquito taxa or other members of the mosquito community. Models that incorporated intra or inter specific density dependence in trap performance did not yield any substantial improvements over those assuming simple linear relationship between mosquito counts in the HLC and alternative method. This indicates that neither intra nor interspecific density dependence has a large impact on the relative efficiency of the alternative traps tested here. This was generally the case with exception of BGS and SUN trap which shows signs of both intra and interspecific density dependencies. Given the wide range of trap catches, the calibration tool I developed here allows users to incorporate such density dependence effects (both within and between species) and to examine if these are applicable in their settings. Previously, (intraspecific) density dependence has been detected in the performance of some trapping methods [288,291,292]. However, evidence of density dependence in trap performance can be variable even for the same trapping method. For example, studies investigating the performance of the Mosquito Electrocuting Trap relative to the HLC have detected density dependence in some cases [114,124], but not others [288].

One limitation of this study is that while the HLC is broadly considered the gold standard for collecting host seeking mosquitoes both indoors and outdoors, I only focused on traps for outdoor sampling. Although I compared a large number of trap types commonly used in Africa settings, other traps may perform differently and potentially better than some of the candidates investigated here [124,287,288]. Additional studies including other alternative traps for indoor and outdoor use would be of further value; with the calibration tool developed here providing a useful framework for their evaluation and comparison. Based on the results presented here, I recommend that for whatever trap used, the users should generate credible estimates of what the human biting rates (as estimated from HLC) could be. Due to the potential variation in trap performance between

different ecological settings and mosquito species, I do not yet recommend any one specific trap as the best replacement for the HLC. Instead, I recommend that users consider and define the statistical relationships between a prospective trap and the HLC when planning surveillance and interpreting results. The interactive conversion tool I have developed here can be used for that purpose and is now available online as a *Shiny App* interface.

3.4 Conclusion

Methods for sampling outdoor-biting mosquitoes are urgently needed to improve surveillance of vector-borne diseases. Even if an alternative trap does not catch as many mosquitoes as the HLC, it is desirable to define the statistical relationship between them so that credible ranges of actual biting risk can be predicted in units of HLC equivalents. In this study, I successfully evaluated six different outdoor traps and developed a calibration tool to assess their performance relative to the HLC. This tool was validated using data from year-round field collections and enabled a framework for predicting HLC-derived exposure rates representative of individual human biting risk. The tool incorporates multiple models, including two that allow assessment of effects of both inter and intra-specific density dependence of the performance of candidate traps. In the specific field trials from which data was obtained here, density-dependence between and within mosquito species influenced the performance of only one trap, BGS, but not any others. An interactive *Shiny App* calibration tool was developed for this and similar applications. I conclude that this calibration approach provides a valuable framework for assessing human exposure from different outdoor trapping methods. As the performance of candidate traps relative to the HLC varied between mosquito taxa, there was no single optimum. While all the candidate traps underestimated HLC catches, and thus human-biting rates. Further studies of trapping methods and associated evaluation criteria should focus on consistency and representativeness as opposed to simply finding traps that catches as many mosquitoes as HLC.

4.0 Chapter 4: Using Bayesian state-space models to understand the population dynamics of the dominant malaria vector, *Anopheles funestus* in rural Tanzania

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Abstract

Background

It is often assumed that the population dynamics of the malaria vector *Anopheles funestus*, its role in malaria transmission and the way it responds to interventions are similar to the more extensively characterized *Anopheles gambiae*. However, *An. funestus* has several unique ecological features that could generate distinct transmission dynamics and responsiveness to interventions. The objectives of this work were to develop a model which will: 1) reconstruct the population dynamics, survival, and fecundity of wild *An. funestus* populations in southern Tanzania, 2) quantify impacts of density dependence on the dynamics, and 3) assess seasonal fluctuations in *An. funestus* demography. Through quantifying the population dynamics of *An. funestus*, this model will enable analysis of how their stability and response to interventions may differ from that of *An. gambiae sensu lato*.

Methods

A Bayesian State Space Model (SSM) based on mosquito life history was fitted to time series data on the abundance of female *An. funestus sensu stricto* collected over 2 years in southern Tanzania. Prior values of fitness and demography were incorporated from empirical data on larval development, adult survival and fecundity from laboratory-reared first generation progeny of wild caught *An. funestus*. The model was structured to allow larval and adult fitness traits to vary seasonally in response to environmental covariates (i.e. temperature and rainfall), and for density dependency in larvae. The effects of density dependence and seasonality were measured through counterfactual examination of model fit with or without these covariates.

Results

The model accurately reconstructed the seasonal population dynamics of *An. funestus* and generated biologically-plausible values of their survival, larval development and fecundity in the wild. This model suggests that daily *An. funestus* survival and fecundity values annual were highly variable across the year, but did not show consistent seasonal trends in association with rainfall or temperature. While the model fit was somewhat improved by inclusion of density dependence, this was a relatively minor effect and suggests that this process is not as important for *An. funestus* as it is for *An. gambiae* populations.

Conclusion

The model's ability to accurately reconstruct the dynamics and demography of *An. funestus* could potentially be useful in simulating the response of these populations to vector control techniques deployed separately or in combination. The observed and simulated dynamics also suggests that *An. funestus* could be playing a role in year-round malaria transmission.

4.1 Background

Anopheles funestus is one of the major malaria vectors in Africa and is widely distributed across the continent [66,68]. With the exception of *Anopheles gambiae sensu stricto* (*s.s.*), the species appears to have higher vectorial capacity than other members of the *Anopheles gambiae* complex [77,88,89,104,305,306]. *Anopheles funestus* makes a higher contribution to transmission than *An. gambiae sensu lato* (*s.l.*) in numerous parts in sub-Saharan Africa [88,109,204,207,307]; particularly in settings where *An. gambiae* abundance has plummeted due to either effective indoor-based vector control interventions [76,185] or environmental change. It is hypothesized that *An. funestus* persistence despite the recent scale-up of insecticide–treated nets may have been facilitated by their earlier development of strong physiological resistance [91].

Anopheles funestus is typically grouped with *An. gambiae s.l.* when modelling transmission and formulating policies for malaria vector control [9,198,308]. The lack of explicit consideration of *An. funestus* ecology and transmission potential may be partially due to this species having been relatively neglected compared to *An. gambiae*. Comparatively the ecology of *An. funestus s.l.* is less well understood, and it is much more difficult to maintain under insectary or semi-field conditions ([24], Chapter 2). However, this species has several unique ecological features, such as its different larval habitat and dry season persistence [95], that could give rise to distinct population dynamics and differentiate its response to core and supplementary interventions. For example, *An. funestus* prefers larger aquatic habitats that are semi-permanent or permanent throughout the year, and contain clear water with some emergent vegetation [95]. This differs from *An. gambiae s.s.* which generally prefer small temporary habitats, such as puddles, ditches or animal hoof prints [54,95], or *An. arabiensis*, which can breed extensively in rice fields and other sunlit open pools [66]. The use of more permanent larval habitats means that *An. funestus* has greater persistence through the driest periods of the year compared to *An. gambiae* [103], whose habitats evaporate quickly in the absence of rainfall [54,309]. This ecological feature means that the seasonal phenology of *An. funestus* and its response to aquatic microclimate differs from *An. gambiae*

[54,96,103]; and could thus generate differential response to seasonally-targeted interventions, such as Indoor Residual Spraying (IRS) and larviciding.

Differential use of aquatic habitats may also impact the relative importance of key intrinsic drivers of mosquito population dynamics such as density dependence. Density dependence in malaria vectors occurs during larval development as a product of competition for space and nutritional resources [59,310]. In space-limited habitats, high larval densities can influence larval development rates and survival, but also subsequent adult fitness traits such as body size, survival, fecundity and mating success [53,57,61,311]. While there is evidence that density dependence is an important driver of *An. gambiae* population dynamics [59], the relative importance of this process for *An. funestus* is unknown. Given that larval crowding and competition are less likely within the larger habitats preferred by *An. funestus,* density dependence is hypothesised to be less pronounced for this vector species. Quantifying the strength of density dependence is important to inform the ease with which vector populations can be suppressed and how quickly they can recover [57,310,312].

Models of vector population dynamics and their response to interventions must be parameterised by reliable estimates of their demography and fitness. For vectors in the *An. gambiae* complex, such estimates are often acquired from insectary and semi-field studies (e.g. [78,81,135,176,225,313]) as well as field studies. Similar data has been difficult to obtain for *An. funestus* because of its poorly understood ecology and the difficulties of creating laboratory colonies; which so far has been achieved on only two occasions [24,207,222]. State-space models (SSM) provide an alternative approach to indirectly estimate these parameters by fitting a population dynamics model to observed time series data [159,175]. These models are widely used in other fields of ecology and conservation biology to investigate the population dynamics of other animals [159,180] and guide management decisions [180]. However, these models have so far had limited update in medical entomology. Given data on population fluctuations are available, these models can infer and estimate plausible demographic rates that could generate the observed dynamics [179].

SSMs are time-series models that distinguish between two stochastic components, namely, the process (i.e. biological) which captures sequential dependencies between population components (e.g. eggs, larvae, pupae), and an observation component which captures and corrects for biases and imprecisions in the data-collection process. Prior knowledge of model parameters is used to bolster the information content of the time series data with existing expert or laboratory data and uncertainty in estimates. Population projections are then quantified on the basis of posterior probability distributions for parameters and population states. SSMs have recently been used to elucidate the dynamics and impacts of interventions on malaria vectors in laboratory and semi field populations [135,176], but have not yet been applied to estimate *An. funestus* vector demographics in the wild. Here, an innovative SSM application was developed to describe the dynamics of wild *An. funestus* populations in Tanzania, and use it to assess extrinsic (environmental) and intrinsic (density dependent) drivers of their fitness and demography for the first time. Empirical data from laboratory experiments on *An. funestus* colonization (Chapter 2, [24]) were incorporated together with published literature and the wild population data to develop an SSM. Time series field data collected in 2015 [88] and 2018 in southern Tanzania, and corresponding environmental information were used to validate the model. Specific aims were: 1) to accurately reconstruct the population dynamics, survival and fecundity of wild *An. funestus* populations in southern Tanzania, 2) assess the impact of density dependence on the dynamics, and 3) to identify and quantify seasonal variations in *An. funestus* abundance and demography.

4.2 Methods

4.2.1 Time series data on wild *An. funestus* populations

Indoor densities of female *An. funestus s.l.* adults were recorded over 12 months of entomological surveys conducted in three villages (Tulizamoyo, Ikwambi and Sululu) in Kilombero (8.1539ºS, 36.6870ºE) and Ulanga (8.3124ºS, 36.6879ºE) districts, south-eastern Tanzania from June 2018 to May 2019 (Figure 4.1). The villages were selected because of the high abundance of *An. funestus s.l.* within which *An. funestus s.s.* is the dominant sibling species (93%) [1]. Annual rainfall

was 1200mm-1800mm, and temperature, 20 ^{o}C-32 ^{o}C. CDC Light traps [129] were used to sample host-seeking mosquitoes from 6pm to 6am for 5 days per week, 4 weeks a month for 12 months in 10-15 houses per village (repeated collection from the same households). The houses were randomly selected and consent obtained from household heads. The mosquitoes were sorted by taxa and sex, and females further classified as unfed, blood-fed or gravid. Daily climatic data (rainfall and temperature) were obtained from a weather station, approximately ~20km from the farthest village.

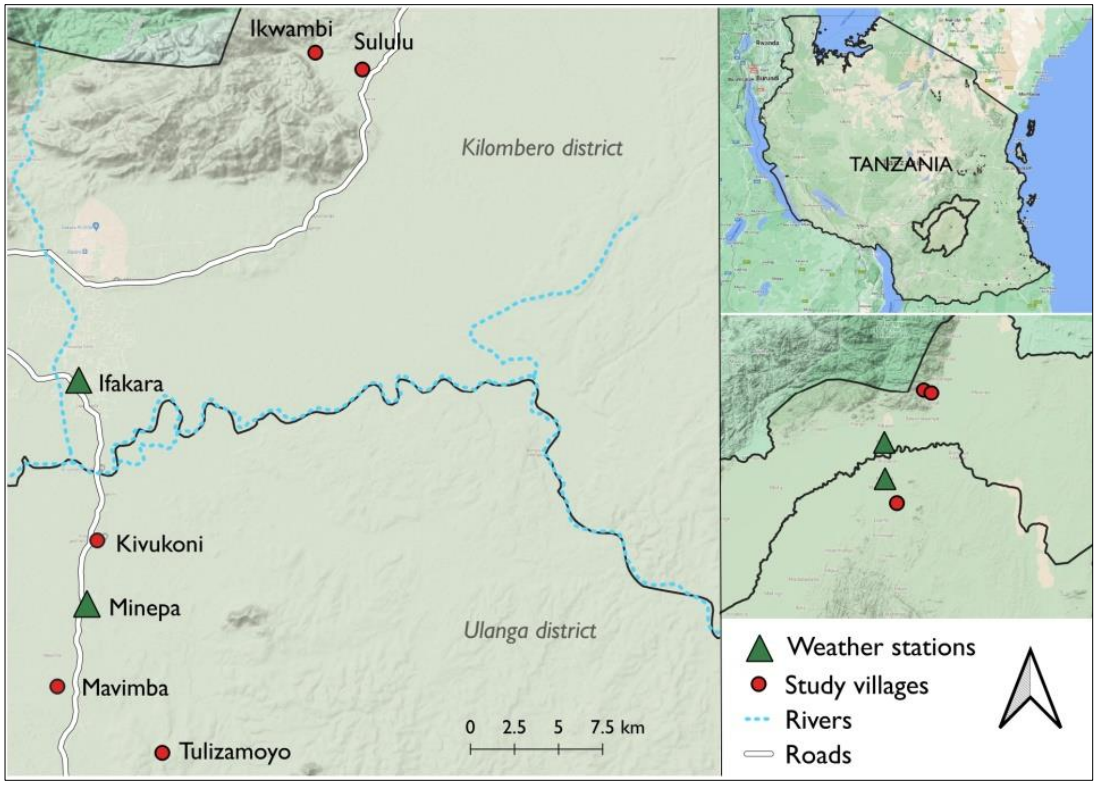


Figure 4.1: A map depicting the locations of various study villages where mosquito sampling was carried out in 2015–2016 and 2018–2019.

To complement this, additional data on *An. funestus* were extracted from a 2015 dataset from three other villages in Ulanga district (Mavimba, Minepa and Kivukoni) (Figure 4.1) [88]. These data were collected five days per week for a period of 12 months. This data allowed me to fit the model simultaneously to multiple time series so that it could learn hierarchically from *An. funestus* trajectories enfolding in different years and locations. This additional data has previously been described elsewhere and used to demonstrate the epidemiological dominance of *An. funestus*, which now contributes >85% of all

malaria infections in the region [88]. Here, time series data collected in 2015 was aggregated and considered as "*population 1*" and collection in 2018/2019 was considered as "*population 2*"

4.2.2 Prior information on life-history and gonotrophic cycle stages

Female *An. funestus* adults collected from the same three villages in 2018 were maintained in insectary conditions for one generation to estimate baseline fitness traits as already described in Chapter 2 and Ngowo *et al.* [24]. Data collected from this 1st-generation laboratory progeny included: a) proportion of eggs that hatched into larvae, larvae that transitioned to pupae, and of pupae that emerged into adults, b) the length of the transition periods (days) between life stages :(i) eggs to 1st instar larvae, (ii) 1st instar larvae to pupae, and (iii) pupae to unfed adult female (one day post emergence), c) transition period of adult females between three different stages of their gonotrophic cycle, i.e. unfed, blood-fed and gravid.

The gonotrophic cycle starts with 'unfed' females who transition to 'blood-fed' after obtaining a blood meal. In the wild, the first gonotrophic cycle usually starts after unfed females have mated [31]; which is assumed to happen soon after emergence. In insectary experiments, females had access to males immediately on emergence. As the blood meal is digested, blood-fed females transition into the 'gravid' state during which eggs develop. Gravid females then oviposit their eggs into aquatic habitats and return to the 'unfed' stage with the cycle begins again (Figure 4.2). In the wild, the rate of transition between these gonotrophic stages is governed by both intrinsic and extrinsic environmental conditions including the availability of blood-meals and oviposition sites [157]. In the insectary, the first blood-meal (arm-feeding) was offered 5 days post emergence to ensure individuals had sufficient time for mating.

Per capita fecundity was defined as the number of eggs laid per fully bloodfed adult female. The rate of surviving between life-history stages or gonotrophic stages were calculated as the inverse of the number of days required to transit from one stage to the next:

$$s_m = (j_m)^{1/r} \qquad (1)$$

Here, m is the life cycle stage, j is proportion survived as the percentage of the total from preceding life stage, r is the average number of days it took to transit from one life stage to another, and s is the daily rate of survival within the stage (Table 4.1).

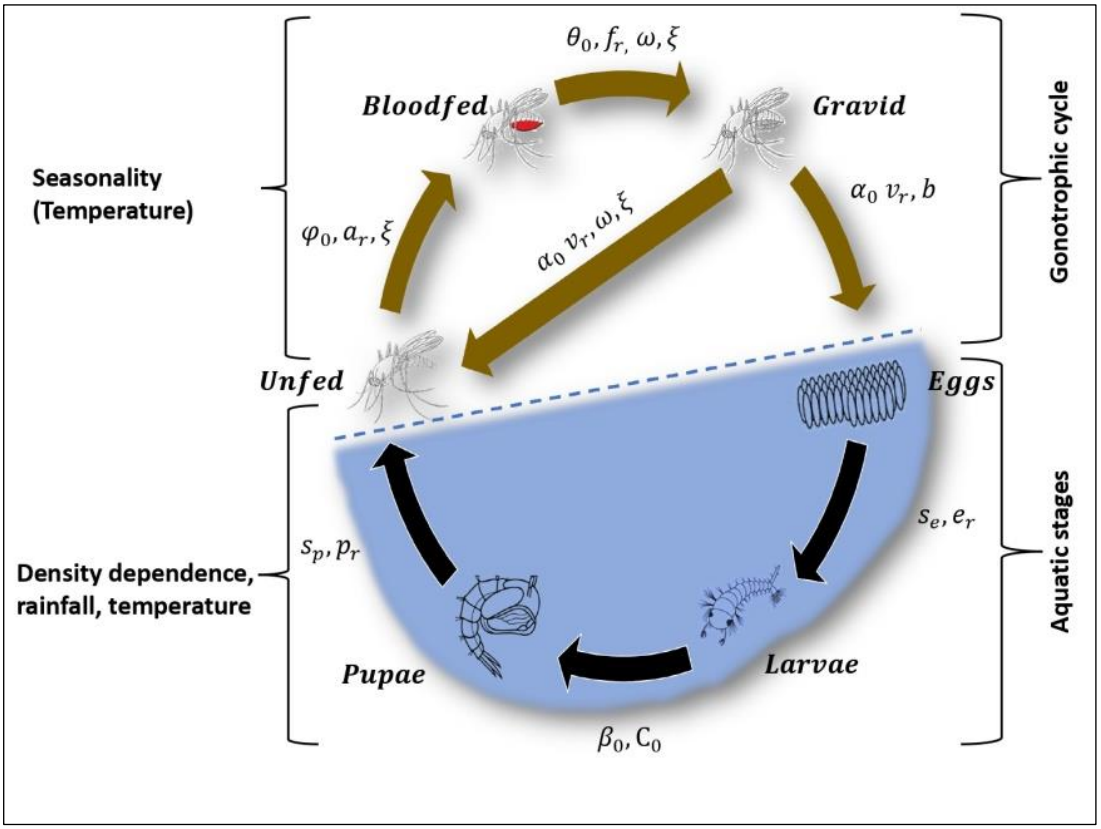


Figure 4.2: Schematic representation of the state-space population model showing different life stages compartment (circles) and flows (arrows) of Anopheles funestus. Abundance data were only available for unfed, blood-fed and gravid stages. The model assumes that once a gravid mosquito has laid eggs, they return to the unfed stage. The annotations are described in Table 4.1. The model incorporates six life stages (eggs, larvae, pupae, unfed, bloodfed and gravid) of An. funestus.

4.2.3 Biological process components of the Bayesian SSMs

Daily survival: The daily survival of larval stages was assumed to be the same for all instar stages. Adult survival was assumed to be the same for unfed, blood-fed and gravid females. Survival probabilities (s_l, s_a, s_f, s_v) were linked to their covariates through a *logit* transformation of linear predictors (here, subscripts l, a, f, v refer to larvae, unfed, blood-fed and gravid, respectively). Pupal (S_p) and egg (S_e) survival probabilities were considered to be independent of any

climatic and density-dependent covariates, and were treated as a binomial distribution, with baseline rates assigned priors as described in eq. 16-17.

A range of covariates hypothesized to be associated with the demography of *An. funestus* were incorporated to allow baseline larval and adult survival to vary with environmental conditions. Rainfall (current and 1 week-lagged) and temperature were incorporated into the larval survival model. Rainfall regulates the availability and permanence of aquatic habitats, thus influencing both survival and carrying capacity of larval habitats [62]. Density dependence was incorporated into the model of larval survival [59] to assess whether this could improve the fit of the adult population dynamics model. Additionally, the speed of larval development was modelled as a function of temperature based on its known importance [25,26]. The daily survival of larvae was thus defined as a function of daily rainfall (current and lagged), daily temperature and density dependence. The daily survival rates (lowercase $s_l(t)$) of larvae were estimated through a logit transformation of linear predictors (uppercase $S_l(t)$).

$$s_l(t) = \frac{\exp\left(S_l(t)\right)}{1 + \exp\left(S_l(t)\right)} \qquad (2)$$

Specifically, $S_l(t)$ is written as a function of both intrinsic and extrinsic drivers:

$$S_l(t) = \beta_0 - \beta_1 R_{(t-1)} - \beta_2 D_{(t-1)} \left(1 - \frac{\beta_3 Q_{(t-1)}}{\max(Q)}\right) + \beta_4 T_{(t-1)} - \beta_5 T^2_{(t-1)} + \varepsilon_{l,t} \qquad (3)$$

$$\varepsilon_l \sim Normal\left(0,\ \sigma_{l,t}\right) \qquad (4)$$

Here, β_0 is the baseline daily larval survival on the linear scale. A survival probability prior was assigned under zero rainfall and average temperature (i.e. 27°C) and then calculated the intercept of β_0 to reflect this prior information. When there is no effect of any environmental covariates prior takes values between 0.80-1, Table 4.1. The coefficient β_1 quantifies the effect of current rainfall (R); with the envisioned scenario being that higher R (i.e. flooding) tends to wash away larvae hence reducing the baseline survival [314]. This β_1 was defined by an informative gamma prior with shape = 5.382 and rate of 46.4

(Table 4.2) which permits anything from no rain effects to 100% mortality. The coefficient β_2 quantifies the effect of larval density at time t on larval survival. A monotonic negative relationship was assumed based on the biologically-plausible hypothesis that larval survival is reduced at high larval density because of resource competition and intraspecific cannibalism [53,63]. This coefficient β_2 was defined by an informative gamma prior with shape of 0.5 and rate of 1 (Table 4.2), which allows the impact of density to range from no effect to complete annihilation.

The term inside brackets in Eq. (3) represents the fact that density dependence needs to be modulated by the availability of larval habitat. The availability of suitable aquatic habitats for oviposition will increase with rainfall; thus potentially reducing the crowding of larvae into the remaining habitats that persist during the dry season. This hypothesis has been supported for *An. gambiae s.l.*, where their seasonal population dynamics can be explained by models incorporating a rainfall-dependent carrying capacity [59]. Here, the coefficient β_3 was a proportion that captures the potential interaction between larval habitat availability (defined as the cumulative rainfall (Q) over the past week) and larval density (D). When rain in the recent week has been the maximum observed (i.e. $Q = \max(Q)$), then $(1 - \frac{\beta_3 Q_{(t-1)}}{\max(Q)})$ would be the smallest amount of density dependency experienced by *An. funestus*. The prior distribution for β_3 was defined by an upward-biased beta prior with mean 0.9 and variance of 0.01 allowing β_3 to have positive impact on larvae survival.

Additional covariates were incorporated to assess the role of temperature on larval survival (via the coefficients β_4, β_5). The parameter β_4 captures the potentially positive effects of temperature on daily larval survival, which were defined by an uninformative gamma prior with mean of 1 and variance of 0.1 considering 27°C as the optimal temperature (ρ) for maximum survival [315]. This prior allows temperature to vary from having no impact, to high positive impact on larval survival. Alternatively, the relationship between larval survival and temperature may be characterized by survival being reduced at low or very high temperature, and peaking in the middle [47]. The coefficient β_5 was incorporated to capture this potential curvilinear relationship but was

dependent on β_4, to ensure that the optimum temperature was fixed at 27°C, ($\beta_5 = \frac{\beta_4}{2\rho}$). The prior of β_4 allowed extreme temperatures values away from the optimum to range from having no effect to generating 100% mortality. The parameter ε_l is capturing the unexplained stochasticity associated with larval survival. This error term was defined by a normal prior with mean of 0 and a precision σ_l from a gamma distribution with both shape and rate of 10.

The linear predictors for survival of unfed, blood-fed, gravid (uppercase $S_a(t), S_f(t), S_v(t)$) and daily probabilities of survival (lowercase $s_a(t)$, $s_f(t)$, $s_v(t)$) were structured similarly to eq 3. The daily survival probabilities of adult stages were thus defined as the functions of daily temperature; such that an increase in temperature would result in an increase in the survival of all three adults stages and reduction in survival when temperature become lethal [103,316]. The biological relationship between adult survival and temperature was assumed to be curvilinear [103,316,317].

$$S_a(t) = \varphi_0 + \varphi_1 T_{(t-1)} - \varphi_2 T_{(t-1)}^2 + \varepsilon_{a,t} \quad (5)$$

$$S_f(t) = \theta_0 + \theta_1 T_{(t-1)} - \theta_2 T_{(t-1)}^2 + \varepsilon_{f,t} \quad (6)$$

$$S_v(t) = \alpha_0 + \alpha_1 T_{(t-1)} - \alpha_2 T_{(t-1)}^2 + \varepsilon_{v,t} \quad (7)$$

$$\varepsilon_{t*} \sim Normal\ (0,\ \sigma_{t*}) \quad (8)$$

Here, φ_0, θ_0, and α_0 refer to the baseline survival of unfed, blood-fed and gravid females respectively on a linear scale, under fixed temperature conditions of 27 ± 2°C (insectary standard under which *An. funestus* have maximum survival [47,317]), and assumes no blood meal limitation. The positive impact of temperature on all three life stages was represented by the coefficients φ_1, θ_1 and α_1 with an uninformative gamma prior with mean 12.5 and variance of 6.25. The coefficients φ_2, θ_2 and α_2 correspond to the curvilinear effect of temperature on the survival of all three adult stages with their priors derived from the ratio between the linear coefficient and twice

optimum temperature. This formulation ensured that the optimum temperature is fixed at (27°C). The stochastic terms ε_a, ε_f, ε_v capture unexplained variation associated with survival during the distinct gonotrophic stages. These error terms (ε_*) were defined by normal priors with mean of 0 and a precision σ^* from a gamma distribution with both shape and rate of 10 for unfed, blood-fed and gravid females.

Development between stages: The daily development probability from one life stage to the next was defined as the reciprocal of the development time (days) between the stages (assuming that all development times take longer than a day). An increase in temperature was assumed to reduce the development period of larvae [26,316,318,319].

$$l(t) = \frac{\exp\big(L(t)\big)}{1 + \exp\big(L(t)\big)} \qquad (9)$$

Specifically, $L(t)$ is written as the function of temperature covariates:

$$L(t) = C_0 + C_1 T_{(t-1)} \qquad (10)$$

Here C_0 is the baseline daily development period on a linear scale defined by an informative beta prior with range defined in eq 16-17 (Table 4.1). The coefficient C_1 explains the positive effect of temperature on larval development period, with its prior values derived from an uninformative gamma prior with mean 0.001 and standard deviation 0.001.

The development time for other life history stages (eggs and pupae) and the time between gonotrophic stages were assumed to be independent of temperature and other environmental covariates. The numbers of individuals ($\mathcal{K}_m$) graduating from one stage to the next each day were modelled as a binomial process Eq 11.

$$\mathcal{K}_m(t) \sim Binomial(r_m, \mathcal{W}_{m-1}(t)) \qquad (11)$$

Here r_m is a development rate as defined in eq 11 for m stage, with assigned informative prior values as described through a generic prior in eq 16-17. Parameter $\mathcal{W}_{m-1}(t)$ refers to the number surviving the preceding life stage.

Fecundity: The number of eggs laid at each time step was drawn from a Poisson distribution whose rate was the product of per-capita fecundity (number of eggs laid by blood-fed *An. funestus* under insectary conditions (b_0), a penalized rate for the egg survival (s_e), the number of gravid mosquitoes ($V_{(t-1)}$) and ratio of females-males (assumed to be 0.5) as assessed at the pupae stage [24].

$$b_t = \exp(b_0 + \varepsilon_{b,t}) \quad (12)$$

$$B(t) \sim Poisson\left(0.5 b_t s_e V_{(t-1)}\right) \quad (13)$$

The error term ε_b was defined as a normal process with mean of 0 and a precision from a vague gamma prior with both shape and rate of 10.

4.2.4 Observation-derived components of the Bayesian SSMs

Observations of the abundance of adults (unfed, bloodfed, gravid) A at time t were modelled as a normal distribution with varying daily means $\bar{a}$ determined by the biological model and a precision τ representing observation error. A fixed coefficient of variation (ξ) for the daily observation process was assumed and assigned an uninformative prior with values between 0.1-0.9 (Table 4.1). The CDC light trap typically samples mosquitoes from populations of unknown size, for which the daily catch rates are difficult to quantify independently. A parameter ϑ was therefore incorporated both into the precision τ and daily varying means to account for an observed weekly periodicity in adult abundance, which was otherwise hard to interpret. The weekly periodicity observed here was a consistent reduction in the catches between the first day of weekly collection and the last day. This parameter was allowed to vary both by day of the week j and between the two populations k (2015 and 2018-19 datasets). The ϑ values were derived from a logit function $\exp(\rho)/(1+\exp(\rho))$, with ρ defined from the uninformative normal prior with mean and standard deviation of 0 and

10 respectively. Therefore precision τ can be written as $\frac{1}{(\xi_t \bar{a}_t \vartheta_{jk})^2}$ for all the adult stages. Thereafter, the observation abundance was estimated as follows:

$$A(t) \sim Normal\left(\bar{a}_t \vartheta_{jk}, \quad \frac{1}{(\xi_t \bar{a}_t \vartheta_{jk})^2}\right) \tag{14}$$

Trap bias: The trapping method (CDC Light traps) primarily targets unfed host-seeking mosquitoes [129]. Blood fed and gravid mosquitoes are assumed to no longer host-seek, and represent a small proportion (~0.5-3%) of the females caught in CDC traps [88,103]). To account for these biases in sampling, a new parameter of "trap-biasness" ω was added in the observation model for both precision τ and varying daily means $\bar{a}_\eta$. The prior values for ω were estimated from independent studies from the same locations [88,195], and ranged from 0.05-0.15 (Table 4.1), with variations between the two life stages η. Therefore, the observation model for blood-fed and gravid (A_η) was rewritten by modifying eq 14 as follows

$$A_\eta(t) \sim Normal\left(\bar{a}_{\eta t} \vartheta_{jk} \omega_\eta, \quad \frac{1}{(\xi_t \bar{a}_{\eta t} \vartheta_{jk} \omega_\eta)^2}\right) \tag{15}$$

Prior distributions: Since this model contains a large number of parameters, use of un-bounded informative priors restricted model convergence and mixing. I therefore opted for bounded and rescaled beta distributions of the informative priors [320]:

$$Y \sim Beta\,(5, 5) \tag{16}$$

$$X = X_{min} + Y(X_{max} - X_{min}) \tag{17}$$

Here Y is a dummy variable that takes values in the interval [0, 1] with mean of 0.5 and standard deviation of 0.15, selected to provide low likelihood at the extremes of its range, 0 and 1. The values of X_{min} and X_{max} define the range of the parameter of interest as dictated by the prior information. Since the information on priors was provided in form of mean (μ) and standard deviation

(σ), these rescaling values were defined as $X_{min}, X_{max} = \mu \pm 2\sigma$. Survival, development period, trap-biasness, variability in daily catches and fecundity parameters were all assigned priors according to Eq. 17.

4.2.5 Model selection, model fitting and outputs

Model fitting was done using the R statistical software version 4.0.5 [246]. Population models were fitted using a Markov Chain and Monte Carlo sampling (MCMC) algorithm via the JAGS software [301] interfaced to R via the *runjags* package [302] (code provided in Appendix 4). To achieve convergence, the model with 6 chains was run in parallel for 10^5 samples with a burn-in of 10^5, keeping every 10th iteration for memory-saving reasons. Convergence was assessed by visual investigation of the trace plots, prior-posterior distribution using the *coda* package [321], effective sample sizes and the Gelman Rubin diagnostic [322]. Model comparisons were done using the deviance information criterion (DIC) [323], and the ones with the lowest DIC selected as the most preferred. The predicted and observed densities of *An. funestus* adult females were plotted to evaluate consistent prediction biases visually. Posterior means and 95% credible intervals for the key survival parameters, development period, density dependence, environmental covariates (temperature and rainfall) and fecundity were also reported to reveal different dynamical aspects of the system.

4.3 Results

4.3.1 Population trajectories and seasonal trends

A Bayesian state-space model was used to describe the dynamics of wild populations of *An. funestus*. The full results, including summaries of posterior means for all the fitness and demographic parameters are reported in Table 4.1. The most parsimonious model (model-7, Table 4.3) included density dependence, and temperature and rainfall (current and lagged) impacts on larval survival, and the effect of temperature on the larval development period. The only covariate that was not retained in the "model-7" was temperature impacts on adult survival. This model satisfactorily reconstructed the population

dynamics of *An. funestus* in the study villages, with all environmental covariate relaxation applied based on DIC selection. Population trajectories were estimated for all six *An. funestus* life history and gonotrophic stages after accounting for potential impacts of environmental covariates and density dependence (Figure 4.3).

These trajectories reflect the annual trend in abundance spanning in periods from low or no rainfall to high rainfall. All trajectories show a relatively high abundance of *An. funestus* right after the rainy season (May-June), followed by reduced but sustained abundance during the dry period (August-October) for all the life stages. After accounting for observation biases during sampling, the observed abundance of unfed, gravid and blood-fed groups largely falls within the credible intervals of the predicted values (Figure 4.4).

4.3.2 Survival and fecundity

Estimated *An. funestus* larval survival trajectories demonstrate substantial mean variability during the two seasons, with no clear pattern of seasonality (Figures 4.5a and 4.5b, Table 4.1) due large credible intervals. Similarly, the survival trajectories of the adult stages (all gonotrophic states) were variable throughout the year, with daily survival rate ranging from 0.2 to 1.0 and not consistently differing between wet and dry seasons (Figures 4.5c to 4.5h, Table 4.1). Per capita fecundity was estimated to be between 75 and 81 eggs per female *An. funestus* (Table 4.1). While the abundance of this species fluctuated seasonally, per capita fecundity remained consistent throughout the year (Figures 4.5k and 4.5l).

Temperature was an important predictor of larval survival with a curvilinear relationship (ΔDIC = 138, Table 4.3, Figure 4.6b). Temperature also had a positive monotonic relationship on the larval development rate (ΔDIC = 336, Table 4.3, Figure 4.6a); with the larval development period estimated to last about 16 days on average.

Table 4.1: Priors as used in the state-space population models of *Anopheles funestus* and the estimated posteriors mean and 95% credible intervals. Day (d^{-1}) was considered as a unit of time in this modelling development.

Parameter		**Prior distribution**				**Posterior distribution**	
Notation	**Description**	**Type**	**Source**	**Mean**	**95-percentiles**	**Mean**	**95-percentiles**
s_e	Eggs daily survival rate	Beta	This study	0.794	[0.619, 1]	0.789	[0.776, 0.804]
λ	Eggs development rate (d^{-1})	Beta	This study	0.5	[0.4, 0.6]	0.499	[0.485, 0.514]
β_0	Baseline larval daily survival	Beta	This study	0.923	[0.801, 1]	0.950	[0.943, 0.956]
C_0	Baseline larval development rate (d^{-1})	Beta	This study	0.063	[0.055, 0.071]	0.063	[0.062, 0.064]
s_p	Pupae daily survival rate	Beta	This study	0.941	[0.874, 1]	0.944	[0.930, 0.950]
p_r	Pupae development rate (d^{-1})	Beta	This study	0.522	[0.253, 0.792]	0.525	[0.506, 0.546]
φ_0	Baseline unfed daily survival	Beta	This study	0.935	[0.877, 0.992]	0.937	[0.933, 0.941]
a_r	Unfed development rate (d^{-1})	Beta	This study	0.20	[0.19, 0.21]	0.200	[0.198, 0.201]
θ_0	Baseline blood-fed daily survival	Beta	This study	0.807	[0.654, 0.961]	0.810	[0.799, 0.820]
f_r	Blood-fed development rate (d^{-1})	Beta	This study	0.25	[0.05, 0.45]	0.269	[0.256, 0.280]
α_0	Baseline gravid daily survival	Beta	This study	0.904	[0.848, 0.961]	0.903	[0.899, 0.907]
v_r	Gravid development rate (d^{-1})	Beta	This study	0.333	[0.133, 0.533]	0.311	[0.297, 0.324]
b_0	No. eggs/female (Per capita fecundity)	Beta	This study	80	[60, 100]	78	[75, 81]
ξ	Coefficient of variability	Beta	Uninformative prior	0.5	[0.1, 0.9]	0.79	[0.810, 0.825]

Parameter		Prior distribution				Posterior distribution	
Notation	**Description**	**Type**	**Source**	**Mean**	**95-percentiles**	**Mean**	**95-percentiles**
$\omega_{\eta=f}$	Coefficient of "Trap biasness" for the blood-fed	Beta	Msugupakyula et al. [195] and Kaindoa et al. [88]	0.1	[0.05, 0.15]	0.122	[0.117, 0.127]
$\omega_{\eta=v}$	Coefficient of "Trap biasness" for the gravid			0.505*ω_f	[0.025,0.076]	0.062	[0.059, 0.064]

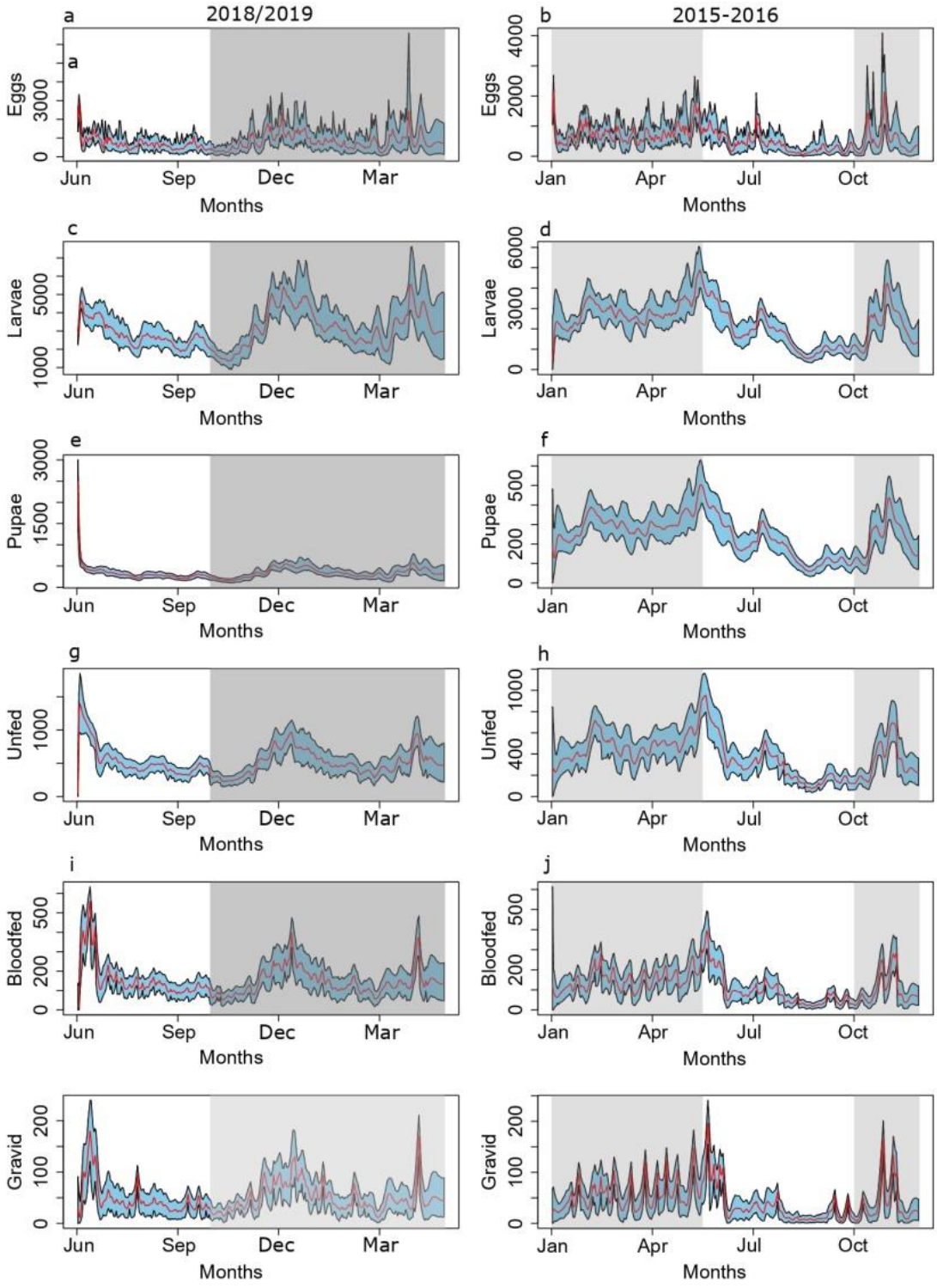


Figure 4.3: Reconstruction of the abundance trajectories for all the six life-stages. The red line indicates the mean posterior values and the respective 95% confidence intervals are shown in "sky-blue". Left column (a,c,e,g,i,k) is data collected from June 2018 to May 2019 and right column (b,d,f,h,j,l) is data collected from Jan-Dec 2015. The grey area indicates the period with rainfall.

Additionally, daily rainfall (flooding) was estimated to be an important driver for of dynamics of *An. funestus* by reducing larval survival (ΔDIC = 5605, Table 4.3, Figure 4.6c) with a negative monotonic relationship.

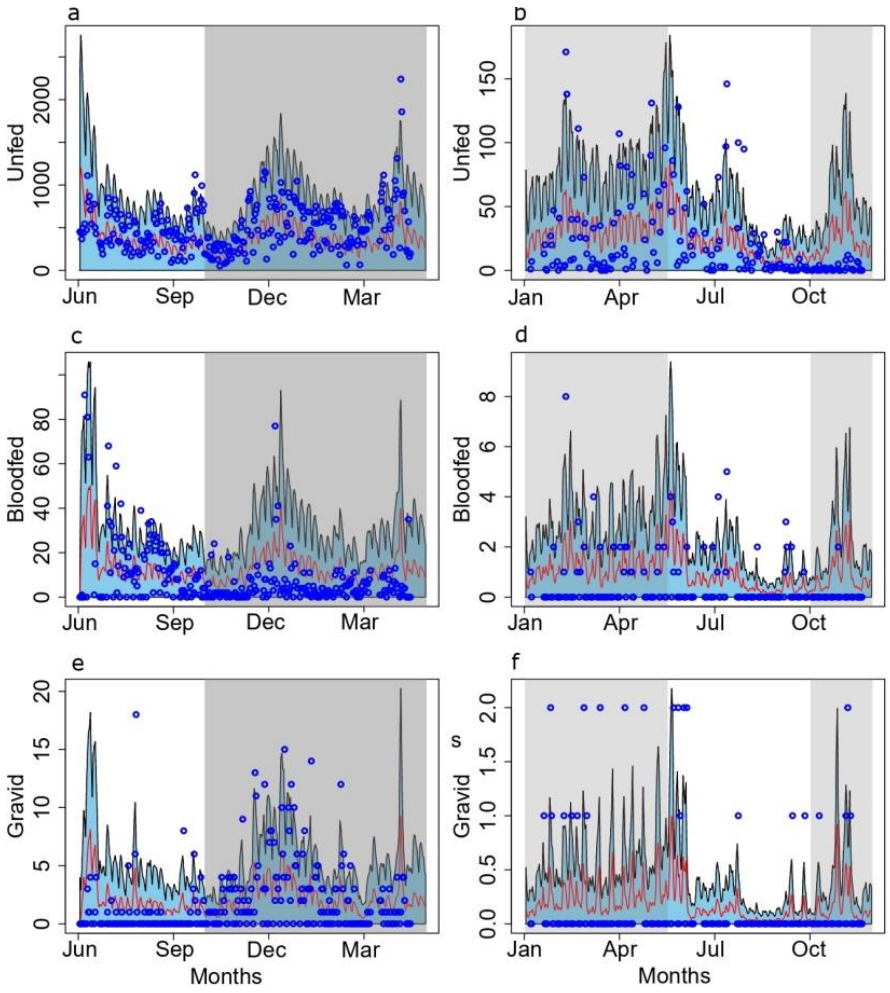


Figure 4.4: Observed vs. model estimated values for the three adult stages with data collected using CDC light trap both in June 2018 –May 2019 (left column- a,c,d) and Jan-Dec 2015 (right column- b,d,e). Red lines are the model estimated trajectories with "sky-blue" showing their 95% credible intervals. The blue circles are the observed values from the Light trap catches. Grey areas are the periods with rainfalls episodes.

4.3.3 Effects of density dependence

Density dependence was the only intrinsic feature incorporated in this dynamic model of *An. funestus*. The model was able to converge efficiently without crashing when density dependency was removed, suggesting this process plays a detectable but relatively minor role population regulation when compared with extrinsic factors (ΔDIC = 222, Table 4.3, Figure 4.6e). To verify this, a simulation

was run and discovered that the estimated density dependence was actually quite low (this was detailed described in the next Chapter 5). The model fitting process also suggested an interaction parameter (β_3) between larvae density (D) and one week cumulative rainfall before sampling day (Q) contributes to *An. funestus* dynamics by positively increasing larval survival (ΔDIC = 38, Table 4.3, Figure 4.6d).

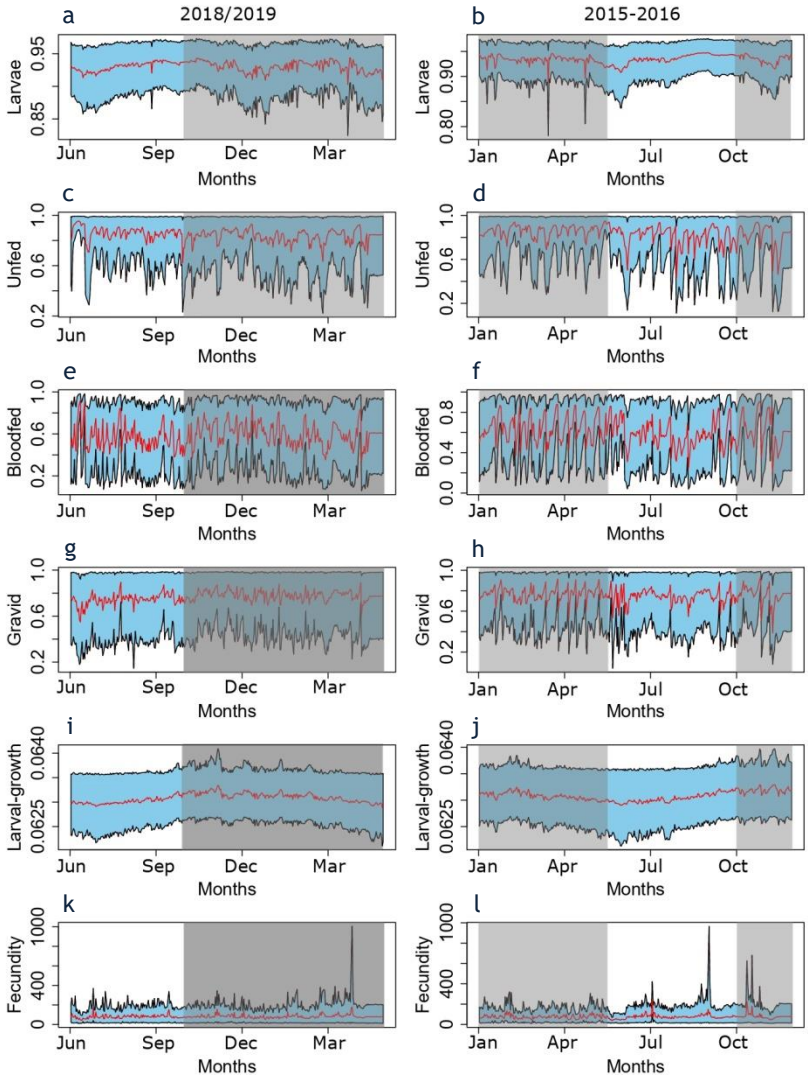


Figure 4.5: Reconstruction of the survival trajectories for all the four stages (larvae, unfed, bloodfed, and gravid) which were affected by the environmental covariates. The two bottom rows show the larval development period and fecundity trends. Left column (a,c,e,g,i,k) is trajectories from June 2018 to May 2019 and right column (b,d,f,h,j,l) is from Jan-Dec 2015. Grey area is the period with rainfall. Y-axis shows the survival rates of different life stages and the bottom row (k&l) shows per-capita fecundity

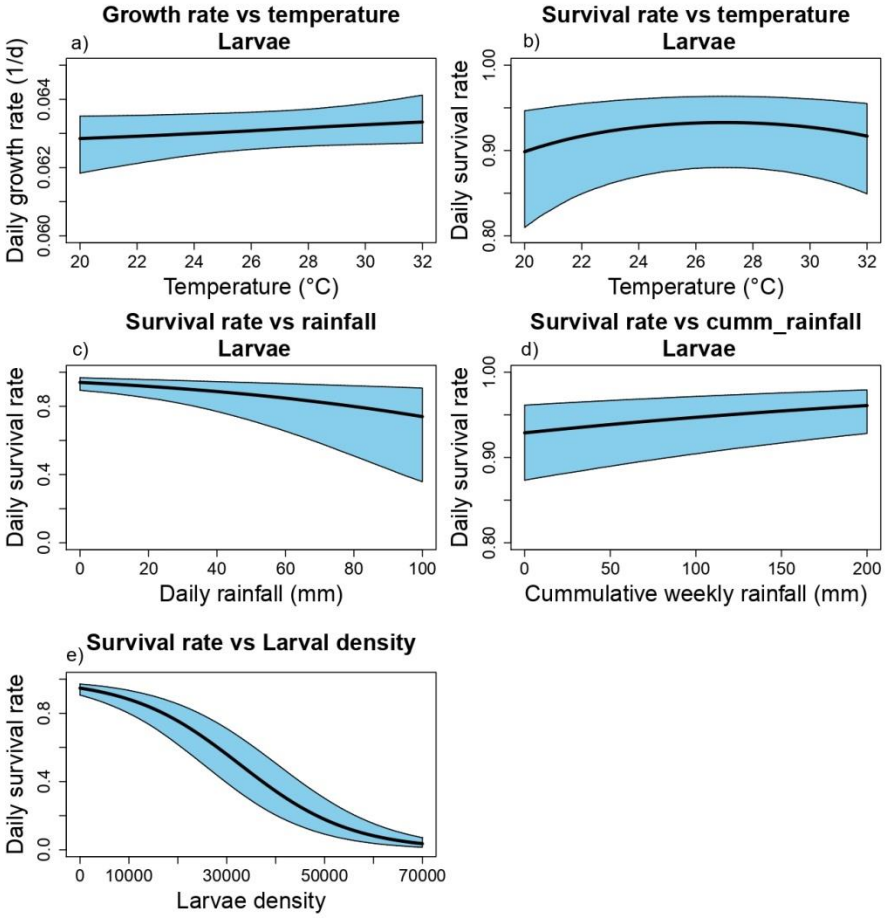


Figure 4.6: Relationship between environmental covariates and fitness parameters as estimated from the SSMs of population dynamic of *Anopheles funestus*. The predictions were produced from a posterior value.

Table 4.2: Priors for the intrinsic and extrinsic drivers of the population dynamic as used in the state-space model of *Anopheles funestus* and the estimated posteriors mean and 95% credible intervals.

Parameter		Prior distribution				Posterior distribution	
Notation	**Description**	**Type**	**Source**	**Mean**	**sd**	**Mean**	**95-percentiles**
β_1	Linear coefficient for rainfall on larvae survival rate	Gamma	Uninformative prior	0.1	0.05	0.01681	[0.00604, 0.0308]
β_2	Density dependent coefficient for larvae on larvae survival rate	Gamma	Uninformative prior	0.5	0.7	1.0283e-4	[1.0e-4, 1.1205e-4]
β_3	Coefficient of interaction between larvae and rainfall on larvae survival	Beta	Upward-Biased prior	0.9	0.1	0.9601	[0.80810, 0.99999]
β_4	Linear coefficient for temperature on larvae survival rate	Gamma	Uninformative prior	1	0.316	0.487	[0.203, 0.806]
β_5	Quadratic coefficient for temperature on larvae survival rate		a function of β_4	$\beta_5 = \frac{\beta_4}{2 * \rho}$		-0.00902	[-0.01492,-0.00376]
C_1	Linear coefficient for temperature on larvae development rate	Gamma	Uninformative prior	0.001	0.001	5.362e-4	[2.51e-8, 2.311e-3]
φ_1	Linear coefficient for temperature on unfed, survival rate	Gamma	Uninformative prior	1	0.316	0.074	[0.068, 0.081]
φ_2	Quadratic coefficient for temperature on unfed survival rate	Gamma	a function of φ_1	$\varphi_2 = \frac{\varphi_1}{2 * \rho}$		-1.378e-3	[-1.50e-3, -1.26e-3]

Parameter		Prior distribution				Posterior distribution	
Notation	**Description**	**Type**	**Source**	**Mean**	**sd**	**Mean**	**95-percentiles**
θ_1	Linear coefficient for temperature on bloodfed survival rate	Gamma	Uninformative prior	1	0.316	0.074	[0.068, 0.081]
θ_2	Quadratic coefficient for temperature on bloodfed survival rate	Gamma	a function of θ_1	$\theta_2 = \frac{\theta_1}{2 * \rho}$		-1.378e-3	[-1.50e-3, -1.26e-3]
α_1	Linear coefficient for temperature on gravid survival rate	Gamma	Uninformative prior	1	0.316	0.074	[0.068, 0.081]
α_2	Quadratic coefficient for temperature on gravid survival rate	Gamma	a function of α_1	$\alpha_2 = \frac{\alpha_1}{2 * \rho}$		-1.378e-3	[-1.50e-3, -1.26e-3]

Table 4.3: Model selection: Description of all models fitted with and without environmental covariates and their corresponding delta-Deviance Information Criterion ΔDIC

Model	Removed covariate(s)	Fitness measure	Penalized Deviance (pD)/DIC	ΔpD/DIC
Model 1-Full	None		35500	25339
Model 2	Temperature	Larval survival	10555	394
Model 3	Rainfall	Larval survival	10277	116
Model 4	One week cummulative rainfall*density dependency	Larval survival	11886	1725
Model 5	Density dependency	Larval survival	11011	850
Model 6	Temperature	Larval development period	10873	712
Model 7^	Temperature	Adult survival	10161	0
Model 8	Model 7 – Temperature	Larval survival	10299	138
Model 9	Model 7 – Rainfall	Larval survival	15766	5605
Model 10	Model 7 - 1 week rainfall:density dependency	Larval survival	10199	38
Model 11	Model 7 - Density dependency	Larval survival	10383	222
Model 12	Model 7 - Temperature	Larval development	10497	336

^ The best model (lowest DIC/Penalized Deviance) value-model-7 followed by model-10. **Model 8-12** consists of model-7 minus one more environmental covariate. **Model 4** involved the removal of the interaction term.

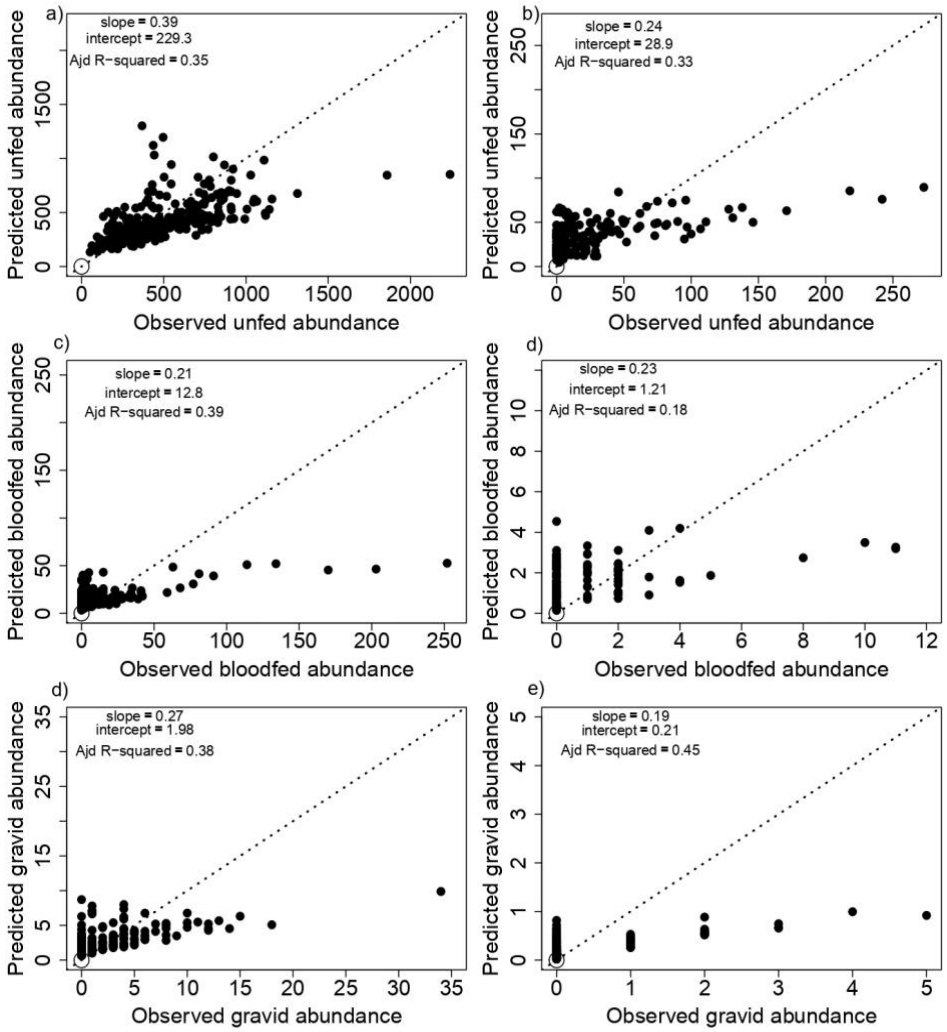


Figure 4.7: Goodness of fit: Observed versus predicted unfed, bloodfed and gravid densities across all populations. Adjusted R-squared, intercept and slope values are from a linear model of the predicted against observed values. Dotted lines correspond to 1:1 line. Left column (a,c,d) is data collected from June 2018 to May 2019 and right column (b,d,e) is data from Jan-Dec 2015.

4.4 Discussion

A state-space model (SSM) was developed and fit to field and laboratory data to accurately reconstruct the population dynamics of wild population of *An. funestus* from Tanzania. The SSM inferred the trajectories of multiple life-cycle and gonotrophic stages of wild *An. funestus* females. This allowed the reconstruction of the observed trajectories of larvae and adult females for wild *An. funestus* in Tanzania for the first time. This analysis indicated that the dynamics of *An. funestus* were best explained in a model that included density dependency, temperature (curvilinear relationship), daily rainfall (negative monotonic relationship), an interaction between larvae density and one week cumulative rainfall before sampling on larval survival. Temperature effect on the larval development rate (positive monotonic relationship) was also used to describe the model. In contrast, model fit was not improved by incorporating temperature dependency into adult survival (all gonotrophic stages). *Anopheles funestus* abundance vary seasonally between wet (highest abundance) and dry periods (lowest abundance); but demographics rates (i.e. survivals, fecundity and development period) did not show a clear seasonal pattern after accounting for the impact of environmental covariates and density dependence. These results are very useful for generating hypotheses about the nature and relative magnitude of drivers of *An. funestus* population dynamics in the wild. This model can be extended to include a component on malaria dynamics in humans; or to compare the efficacy and effectiveness of different interventions in combination or singly. This would allow more sophisticated evaluation of the suitability of *An. funestus*-specific interventions; including prediction of the potential combined effect of strategies that act at different life-cycle stages and/or target different demographic processes (e.g. survival versus fecundity).

Extrinsic covariates such as rainfall and temperature were all hypothesised to be the main drivers for the dynamics of this vector species. This study supports the hypothesis that rainfall is a significant driver of the population dynamics of wild *An. funestus*. Overall, the abundance of all life stages was relatively higher in rainy compared to dry periods of the year as previously documented [79,103,223,324]. Rainfall covariates were directly included in the larval survival model since it is the only stage on which rainfall was hypothesized to have a

significant impact. Daily larval survival as estimated by the SSM showed high variability both within seasons and across the year. There was support for a monotonic association between rainfall and larval survival; characterized as reduction in larval survival during periods of heavy rainfall. In previous studies, *Anopheles funestus* abundance has been shown to be positively associated with the cumulative lagged rainfall [103]. Here one week cumulative rainfall was included in the model to account for its effect on larval survival. In this work, an interaction between one week cumulative lagged rainfall and larval density shows an increase in the larval survival. Similar to other vectors of malaria transmission such as *An. gambiae*, rainfall has always been considered as the main factor regulating the dynamics [27,62,103,325]. In contrast to *An. gambiae* abundance which peaks much earlier in the rainy season, *An. funestus* on the other hand peaks at the very end of the rainy season.

The SSM also provided support for the hypothesis that temperature is an important driver of *An. funestus* dynamics; although the nature of temperature effects was complex and variable between life history stages. For example, temperature was associated with both larval survival and development, but not adult survival or fecundity. Furthermore the estimated impacts of temperature on larval ecology were complex; with the SSM suggesting a curvilinear relationship with survival but a positive monotonic impact with the larval development rate. These findings validate the prior studies that demonstrated that temperature had a curvilinear influence on *Anopheles* larval survival, with a rise in temperature above/below the optimum lowering survival [26,48,60]. The larval development period of *An. gambiae* is temperature dependent [48,318]; thus this model incorporated a positive monotonic effect such that development period is shorter when temperature is high and just below maximum threshold for larval development [23,48]. In this work, indeed temperature has been shown to speed up the development rate of the larvae to pupation. In the final model the effect of temperature on the survival of adult females (all gonotrophic stages) was not found to be an important across the range of values observed, and was thus removed during the model selection process.

Little is known about the effect of density dependence on *An. funestus* due to its ecology and reliance on the large semi-permanent and permanent breeding

habitats [54,95]. However, density dependence is already well-known to be an important driver for dynamic of other malaria vectors like *An. gambiae* [53,57,59,61,312,324,326] and other non-malaria vectors like *Aedes aegypti* [327,328]. Here the SSM fit better when density dependence of larval survival was included, however the relative magnitude of this process was quite small and thus likely to have minor impact on the overall dynamics of *An. funestus* populations. These findings suggest that *An. funestus* populations are likely to be regulated more by extrinsic than intrinsic processes; and corroborate the original hypothesis that density dependence may have a weaker regulatory role in this species than on those that use smaller, less permanent habitats (e.g. *An. gambiae s.s.*). The larger and more permanent habitat used by *An. funestus* (e.g. [95,329]) can likely sustain higher resources and thus reduce competition. This is among of the first report documenting the role of density dependence on the dynamics of the wild populations of *An. funestus*. A recent laboratory study on the effect of larval density on the life history traits of *An. funestus* shows that increasing larval density lengthens the larval development period [330]. Now that colonies are becoming more feasible, more thorough investigation on the role of density dependence in the dynamic of *An. funestus* is prerequisite.

In addition to highlighting potential drivers of *An. funestus* populations, the SSM here generated plausible estimates of key demographic and life-history processes in the wild. This model estimated that *An. funestus* larvae takes an average of 15.6-16.1 days to grow from first instar larvae to pupae; which is relatively long compared to the other major vectors in the *An. gambiae* complex (9-11 days [60,318]). This apparently longer development period of *An. funestus* may be a product of their adaptation to more permanent, year-round breeding habitats that are unlikely to dry up; thus reducing selection for rapid development. The SSM estimated that the daily survival rate of wild *An. funestus* larvae could be as high as 0.95, compared to the 0.83 [0.80, 0.86] mean daily survival rate of other African malaria vectors like *An. gambiae* [60,174]. This also matches observations from insectary experiments conducted by myself (Chapter 2 [24]) and others [100,222] in which *An. funestus* larvae have higher survival than *An. gambiae*. Given the apparently higher larval survival in *An. funestus* than in *An. gambiae*, these findings suggest that more lethal

intervention may be required to control *An. funestus* both at larvae and adult stages.

The impact of any vector control largely depends on the ecology of the specific vector species. Differences in ecology between *An. gambiae* and *An. funestus* are likely to affect the relative impact of interventions. For instance, *An. gambiae* prefer breeding in small and temporary habitats which dry up quickly when there is no rainfall; in contrast to the larger and more permanent aquatic habitats of *An. funestus*. This makes *An. funestus* habitats more likely to be "few, fixed, and findable" and thus suitable for targeting for larviciding [95]; particularly during the dry season. However treating large habitats such as rivers or bigger ponds could also pose logistical challenges. The persistence of *An. funestus* throughout the year even during the driest periods suggest this vector is less seasonal compared to *An. gambiae s.l.*, which experiences much more dramatic “boom and bust” dynamics in relation to seasonal rains [88,103,223]. This model suggests that *An. funestus* is likely responsible for year-round malaria transmission, while other vector species that rely on temporary aquatic habitats only be a substantial source of transmission during the rainy season.

Models of vector population dynamics can provide a useful guide for the selection of optimal vector control strategies; particular through enabling more focal investigation of the benefits of seasonal or spatial targeting and use of combined versus single interventions. Despite its complexity, this population dynamics model provides a useful framework for investigation of the stability of *An. funestus* populations. With additional data, this model can be further refined to include additional modifications related to vector ecology and behaviour that may impact intervention (e.g. host choice and its impacts on fitness, predation during larval or adult phase and spatial components). Such further elucidation may increase the predictive accuracy of this SSM (Figure 4.7) in specific contexts, but even the more general framework developed here has flexibility to introduce stage-specific mortality effects expected from different types of vector control [135,176,308]. For example, this framework could be used to model the impact of combined interventions including those that target adult females (insecticide-treated nets (ITNs), IRS) and larviciding; and assessment of how mortality varies with different coverage [135,308]. It can also

be used to investigate the possible response of vector population to climate change anticipated in Tanzania and other African countries. An important limitation of this study is lack of knowledge on what percentage of the *An. funestus* mosquito population is sampled by the trap, which is important for understanding the relative magnitude of demographic stochasticity in modelled dynamics. This highlights the need to explicitly incorporate this source of uncertainty into vector and transmission dynamics; including the need for further calibration and standardization of the efficiency and biases associated with particular mosquito trapping methods (e.g. Chapter 3).

4.5 Conclusions

This study used Bayesian State Space Models (SSM) parameterized with empirical data to quantify key demographic and fitness processes underpinning the population dynamics of *An. funestus* in Tanzania. This is the first use of SSM to understand the population dynamic of the wild vector of residual malaria transmission, *An. funestus* in Tanzania. The model identified that both environmental covariates (i.e. rainfall and temperature) and density dependence influence *An. funestus* and can contribute to observed patterns of seasonality; although the magnitude of density dependent effects was much smaller than that of environmental factors. The ability of this model to accurately reconstruct the seasonal dynamics and demography of *An. funestus* indicates its value for simulating the response of these populations to vectors control measures applied either individually or in combination. Finally, this model also highlights the clear importance of accounting for regional and daily observation biases when modelling mosquito population dynamics.

5.0 Chapter 5: Density dependence and demography alone cannot explain explosive dynamics in *Anopheles funestus*

Abstract

Background: Population dynamics models are regularly used to understand the ecology of vectors of malaria transmission and how these vectors respond to varying environmental conditions. Recently, I developed the first State-Space Model to describe the population dynamics of the *Anopheles funestus* malaria vector in southern Tanzania (Chapter 4). While this model was able to adequately reconstruct the observed dynamics of these populations and detect associations with climatic variables, there was uncertainty about its generalizability and ability to predict missing data. This was mainly driven by the following shortcomings 1) the detection of weak relationship between demographic parameters and environmental covariates, 2) the difficulty with fitting the model simultaneously to two different populations (Chapter 4) and 3) the model had not been tested under conditions of prediction (i.e. by removing data and validating). Here, I addressed three issues through model fitting and simulation exercises. First, I examined whether inferences about the relative importance of intrinsic and extrinsic population drivers derived from the hierarchical model were representative of individual populations. Second, by investigating the ability of the model to capture key features of population rises and declines, I assessed the potential existence of additional, unexplained drivers of dynamics. Third, I examined the ability of the model to predict *An. funestus* population dynamics outside of the seasons on which it was parameterized. By quantifying the prediction shortfalls of this model, it is possible to derive insights into *An. funestus* population dynamics and the potential existence of regulatory forces beyond known demographic and density dependent processes.

Methods: A Bayesian State Space Model (SSM) based on *An. funestus* life history was refitted to time series data from one of the two female populations on which the original hierarchical SSM model was developed (Chapter 4). To address these shortcomings I tried to see how much of the data could be accounted for

as signals and not noise by i) creating a focused model fit to one of the two populations, ii) increasing the flexibility of the density dependence formulation in the model, and iii) testing the predictive performance of the model under data removal condition. Here, the ability of the model to predict dynamics over different seasons was evaluated by removing a segment (i.e., 25%, 50%) of the time series data used in fitting and observing the impact on reconstructed trajectories.

Results: In general, the dynamics of the local *An. funestus* population were much better described by the single population SSM model than the hierarchical model generated from two populations. The ability of flexible forms of density dependence to account for observed dynamics remains minimal, suggesting the presence of additional factors regulating the dynamics. Model fitting to a single population revealed a stronger association between demographic processes and environmental drivers. The model was able to reconstruct missing time series data as long as some data from both wet and dry seasons was included in initial fitting.

Conclusion: This single population model's ability to explain the response of demographic processes to environmental covariates indicates its value in simulating the response of *An. funestus* populations to vector control. However, need to specify the model to single-village level before strong environmental links were revealed indicates that these relationships may be spatially non-stationary. The difficulty of capturing sustained troughs and explosive peaks purely via flexible density dependent modules indicates the need for additional currently missing biological processes (i.e., aestivation or diapause) in the life-history of the mosquitoes. The overarching importance of seasonality in capturing environmental and intrinsic population regulation was underlined by data-removal experiments which showed dramatic loss of predictive power when the model did not have access to information from both the wet and dry seasons.

5.1 Background

Models have been widely used to understand the dynamics of populations and how they interact with their surroundings [331,332]. In the context of infectious diseases, mathematical models have served as powerful, explanatory and predictive tools for identifying the intrinsic and extrinsic determinants of mosquito vector populations [43,44] and forecasting the knock-on effects for transmission in the face of global climate change [9,43,333–338]. The size of the mosquito population, which is highly dependent on the availability of aquatic larval habitats, is one of the predictors of malaria transmission [339]. Consequently, extensive effort has been devoted to developing models that can predict the abundance and seasonal dynamics of mosquito vector populations [161,166,324], and other life-history and demographic rates of direct relevance to transmission (e.g. adult survival [24,40,133,161]).

Vector-borne diseases are known to be highly sensitive to environmental conditions [47,62,103,340]. A range of biotic and abiotic variables are strongly associated with the distribution and transmission intensity of vector-borne diseases [150,167,206,339], and drive the abundance, seasonal dynamics and demography of mosquito population [166,341–343]. Most models of mosquito dynamics are parameterized using mean values of demographic rates or environmental conditions from a limited number of well characterized laboratory or wild populations [150,279,344]. Use of such mean values likely dampen out heterogeneities under diverse environmental conditions, and thus may give rise to predictions that are poorly representative of specific scenarios or ecological settings of interest. While models using mean or non-random parameter values still hold great value for analysis of general population trends and responses, they may not be appropriate for providing more targeted, setting-specific information. While limitations with model generalizability are generally recognized [345]; the magnitude of these problems are rarely quantified. Formal assessment of the generalizability of model predictions on drivers of population dynamics in other areas or time periods from which model parameters were derived is a useful first step to assess their applicability.

A desired requirement of mosquito population models is the ability to predict dynamics across a full range of seasonal variation. In the case of malaria in Africa, mosquito vector populations undergo substantial seasonal variation in response to changes to micro-climatic factors such as temperature in and rainfall [79,153,346]. Seasonal changes in the environmental conditions that impact vector populations have a significant impact on malaria transmission; with incidence peaking during wet warm months when mosquito densities may be more than 100-fold higher than dry periods [88,99,166,347]. Another desirable feature of a mosquito population model is ability to predict the relative contribution of extrinsic and intrinsic factors to dynamics, such that inferences can be extrapolated to different populations beyond which the model was parameterized on. For example, density dependence is often an important intrinsic driver of population growth [22,59,61,326], and has been implicated in the population dynamics of the African malaria vector *An. gambiae* s.l. [326,348]. Here, density dependence arises through resource competition during larval development which impacts larval and adult survival, and hence overall abundance [46–49]. However, little is known about whether the relative importance of this process varies between populations; and how that influences the generalizability of inferences arising from models of one mosquito population to another.

Recently, Ngowo *et al* developed the first hierarchical state-space population dynamics model of *An. funestus*; a major vector of residual malaria transmission in the southern Tanzania (Chapter 4 [153]). This detailed biological model encapsulated the full life cycle of mosquitoes and was fit to data from two time-series data (referred here and in Chapter 4 as two populations) in southern Tanzania. Data from different populations were used to ensure that spurious features of the data from one population would be eliminated. This hierarchical model was used to assess the relative importance of extrinsic (temperature and rainfall) and intrinsic (density dependence) to population dynamics (Chapter 4). In this hierarchical model I have specified known facets of *An. funestus* life-history into a detailed population model incorporating the based on laboratory and published literature into parameter priors. I also allowed a mixture between process stochasticity and observation error and fitted the model to multiple time series of integrated population data while recognising latent variables. Given

these steps, any residual disagreement of the model with the data is informative about what aspect of the model is specific to each population (for example, the strength of seasonal patterns and the absolute abundances sampled) and what aspect of the model is insufficient (for example the density dependence).

The rationale for re-fitting the hierarchical model to a single population was to explore the limitations of the observation model. In doing so, emphasis was on whether there was only a weak relationship detected between demography and population regulation, which would indicate that the observation model was assigning much of the fluctuations to noise and not signals. I also assessed how hard it was to fit the model simultaneously to two different populations, in terms of whether the observation models required for that exercise required many differences between the subpopulations. Finally the model was tested under conditions of prediction by removing portions of the time series dataset during fitting and validating.

These model fitting and simulating analysis exercises allowed me to address the following aims: First, I examined whether inferences about the relative importance of intrinsic and extrinsic population drivers derived from the hierarchical model were representative of an individual population. Second, by investigating the ability of the model to capture key features of population rises and declines, I assessed the potential existence of additional, unexplained drivers of dynamics. Third, I examined the ability of the model to predict *An. funestus* population dynamics outside of the seasons on which it was parameterized. In combination, this will enhance understanding of *An. funestus* population dynamics through investigation of the strengths and limitations to current model generalizability. This can facilitate development of a more robust population dynamics model that could be used to guide selection of optimal packages of vector control interventions for *An. funestus* in specific settings.

5.2 Methods

5.2.1 Population dynamic model

A hierarchical State-Space population model (SSM) of *An. funestus* dynamics was developed by Ngowo et al (Chapter 4, [153]) to investigate extrinsic and intrinsic

drivers of populations in southern Tanzania, and to assess observational uncertainty. This model was developed in the JAGS [301] platform using a State-Space approach in a Bayesian framework. In brief, the model used daily time series data on the abundance of adult *An. funestus* females collected in 2015 [88] and 2018/19 from two populations respectively for model fitting and validation. Prior information on maximum survival (adult and larval) and larval development rates were derived from a mixture of laboratory experiment performed on F_1 *An. funestus* in an insectary [24] and other published literature [88,195]. This model was structured to consider four mosquito life history stages (eggs, larvae, pupae, adult female), with the adult stage being further subdivided into the physiological stages of unfed, blood-fed and gravid.

This population model also incorporated microclimatic data on temperature and rainfall on a daily average scale. Rainfall and temperature were prioritized as the primary environmental covariates in the model given their strong, known impacts on mosquito populations. Further explanation and justification of the choice of these covariates is given in Chapter 4 (methods section).

In the current work, I re-fitted the model with the same prior information to one of the two time series data (i.e. 2018/2019 time series data). To address the first aim, the joint posterior parameter values generated from this single population model were used to plot the responses of demographic values across the full range of observed environmental covariates to assess and investigate, a) the relationship estimated between rainfall and larval survival rate, and b) the relationship between temperature and larval and adult survival. Here, I examined whether the associations estimated from the single population response were consistent with those inferred by the hierarchical model (Chapter 4). To address the second aim, I increased the flexibility of the density dependence formulation in the model. This process allowed observation of volatility in the system and any factors which can be causing population decline/increase beyond density dependence and demographic parameters. To address the third aim, I tested the model predictive performance under data removal conditions. Specifically, I refitted the current single population model under two scenarios: the first being using 75% of the time series data to predict the remaining 25%. This 75% cut across both the wet and dry seasons, such that the model could learn from observed dynamics during both periods of the

year. In the second scenario, 50% of the data the time series data were used to predict the remaining 50%. The latter did not allow the model to learn from both seasons, as a large part of the wet season dataset was removed.

5.2.2 Model fitting and diagnosis

The state-space model with all environmental covariates was fit using a Markov Chain-Monte Carlo (MCMC) algorithm via the JAGS software [301] interfaced to R via the *runjags* package [302]. A model with 3 chains was run in parallel for 10^5 samples and a burn-in of 10^5, thinning every 10^{th} iteration. Two single population dynamic models were refitted with missing time series data (i.e. 25% and 50%) and their prediction capability was assessed visually by looking at the proportion of curve within the credible intervals. Convergence was assessed by visual inspection of the trace plots and prior-posterior distribution using the *coda* package [321], effective sample size and the Gelman Rubin diagnostics [322]. Posterior means and 95% credible intervals for the environmental covariates and density dependence were reported. The differences between posterior means were estimated using a Bayesian t-test [349] implemented using *BayesFactor* Package [350]

5.3 Results

5.3.1 Generalizability of inferences from a single population and hierarchical model

By re-fitting the hierarchical model of *An. funestus* dynamics (Chapter 4) to a single population, I assessed generalizability of inferences about the role of environmental covariates and density dependence. The full summaries of the posterior means for all the environmental covariates evaluated in the single population model are reported in Table 5.1. The model reconstructed the dynamics of the single *An. funestus* population very well (Figure 5.1). Population trajectories (abundances, survivals) were reconstructed for each of the six life stages together with fecundity (Figure 5.1) after consideration of the impact of environmental covariates. Temporal values for larval development periods were reconstructed, and showed no clear variation between the dry and wet season

(Figure 5.2). There was no noticeable contrast between the seasonal trend in *An. funestus* abundance as estimated in the hierarchical model and single population model (Figure 5.1 and Figure 4.3). The population trajectories produced by both models predicted a clear seasonal pattern characterized by sustained low abundance of *An. funestus* throughout the dry season, which rose to a peak within several weeks after the onset of rains (Figure 5.1).

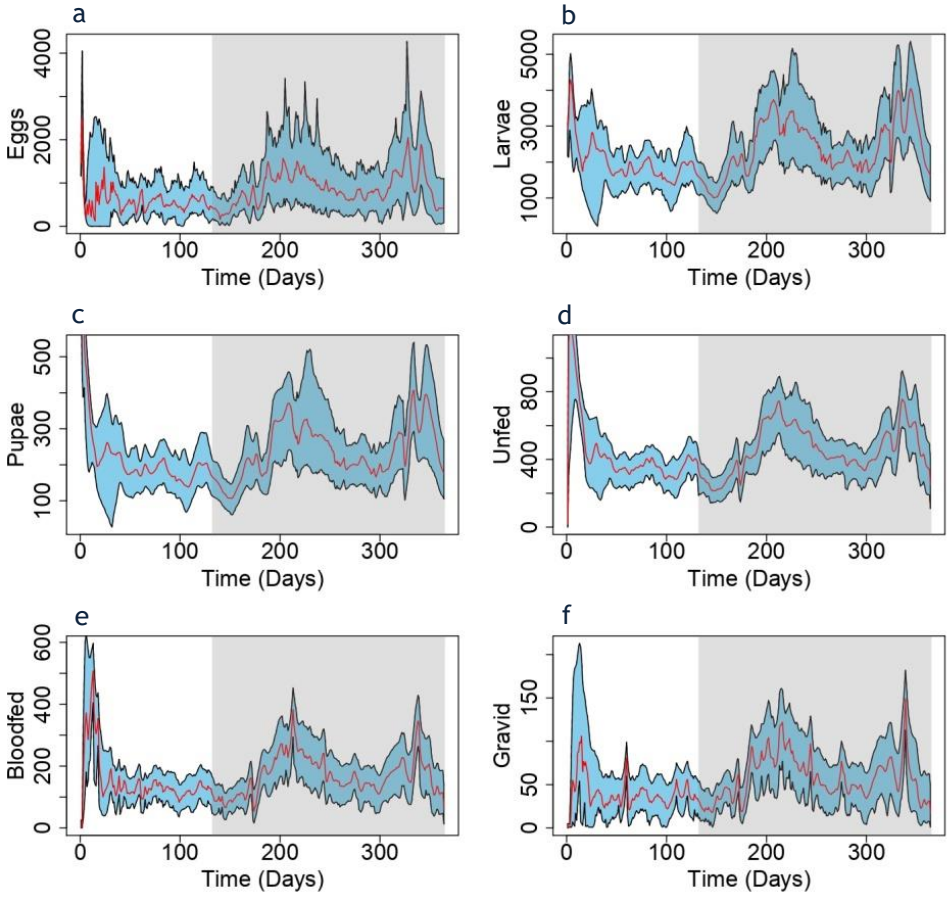


Figure 5.1: Reconstruction of the abundance trajectories of the six mosquito life-stages; a) eggs, b) larvae, c) pupae, d) unfed, e) bloodfed and f) gravid from the SSM fitted to a single population. The red line indicates the mean posterior values and the respective 95% credible intervals are shown in "sky-blue". The grey-shaded region indicates periods with rainfall. Time step "0" refers to the start of data collection June 2018 and ending May 2019.

In the single population model, demographic parameters such as adult survival (all gonotrophic stages) were highly sensitive to temperature; with values dropping notably outside the optimum value (27°C) (Figure 5.3). In contrast, adult survival was estimated to be less temperature-dependent in the

hierarchical model, and hence removed from the final model during model selection.

To highlight these differences, in the hierarchical model (before model selection), the daily survival of adults (all stages) was estimated to vary no more than 2% across a range from 20°C – 32°C. In contrast, the single population model estimated a 4.5-fold difference in adult daily survival across the range from 20°C – 32 °C (Figure 5.3); with a low of 0.21 at 20°C; compared to 0.937 at the 27°C optimal.

Table 5.1: Posterior means for the intrinsic and extrinsic drivers of the population dynamic as estimated by the state space model in [153] and the refitted single population model for *Anopheles funestus* and their respective means and 95% credible intervals.

Parameter		Multiple population		Single population	
Notation[¥]	Description	Mean	95-percentiles	Mean	95-percentiles
β_1	Linear coefficient for rainfall on larvae survival	0.01681	[0.00604, 0.0308]	0.07634*	[0.04172, 0.10681]
β_2	Density dependent coefficient for larvae on larvae survival	0.0001028	[1.0e-4, 1.1205e-4]	0.000123	[1.0e-4 4.990e-4]
β_3	Coefficient of interaction between larvae and rainfall on larvae survival	0.9601	[0.80810, 0.99999]	0.9468	[0.7309, 1.00]
β_4	Linear coefficient for temperature on larvae survival	0.487	[0.203, 0.806]	0.3157	[0.11703, 0.5941]
β_5	Quadratic coefficient for temperature on larvae survival	-0.00902	[-0.01492, -0.00376]	-0.00585	[-0.0110, -0.00217]
C_1	Linear coefficient for temperature on larvae development period	5.362e-4	[2.51e-8, 2.311e-3]	4.9724e-4	[5.097e-8, 2.16e-3]
φ_1	Linear coefficient for temperature on unfed, survival	0.074	[0.068, 0.081]	4.1033*	[1.8274, 5.0408]
φ_2	Quadratic coefficient for temperature on unfed survival	-0.00138	[-0.0015, -0.00126]	-0.07598*	[-0.0933, -0.0338]
θ_1	Linear coefficient for temperature on bloodfed survival	0.074	[0.068, 0.081]	4.1033*	[1.8274, 5.0408]
θ_2	Quadratic coefficient for temperature on bloodfed survival	-0.00138	[-0.0015, -0.00126]	-0.07598*	[-0.0933, -0.0338]
α_1	Linear coefficient for temperature on gravid survival	0.074	[0.068, 0.081]	4.1033*	[1.8274, 5.0408]
α_2	Quadratic coefficient for temperature on gravid survival	-0.00138	[-0.0015, -0.00126]	-0.07598*	[-0.0933, -0.0338]

* Coefficients of environmental covariates that differ between the two types of model structure highlighted in orange (Bayesian t-test was used)

[¥] Notations' meaning and the modelling equations should be referred to in the previous chapter (Chapter 4).

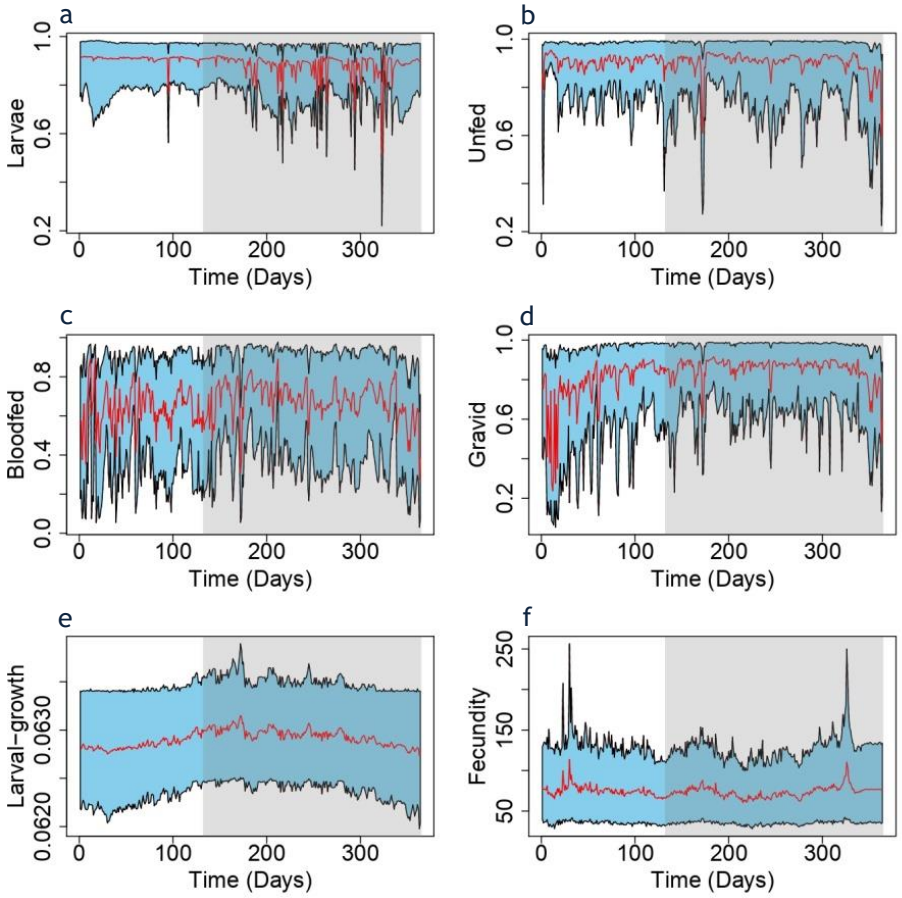


Figure 5.2: The survival trajectories of *An. funestus* from a wild population in Tanzania as estimated by the SSM model for the four life stages; a) larvae, b) unfed, c) blood-fed and d) gravid mosquitoes. The bottom row shows; e) larval development rate and f) fecundity trends over time. The red line indicates the mean posterior values and the respective 95% credible intervals are shown in "sky-blue". The grey shaded region indicates periods with rainfall. Time step "0" refers to the start of data collection June 2018 and ending May 2019.

Larval survival was not strongly associated with temperature or cumulative weekly rainfall in either the single or hierarchical model (Figure 5.3). However, larval survival was estimated to be strongly negatively associated with daily rainfall in the single population but not in the hierarchical model. In the single population model, daily larval survival was estimated to be less than 5% when daily rainfall reaches >50mm (Figure 5.3); compared to ~ 85% in the hierarchical model (Figure 4.6).

The importance of microclimatic variables was emphasized in this single-population model, indicating that there were stark differences between the two populations that the hierarchical parameters were not enough to capture; hence a muted response was estimated (Figure 5.3). Much of the goodness of fit of the single population model was due to the ability of the observation part of the model to assign variation in the data to noise, rather than signal.

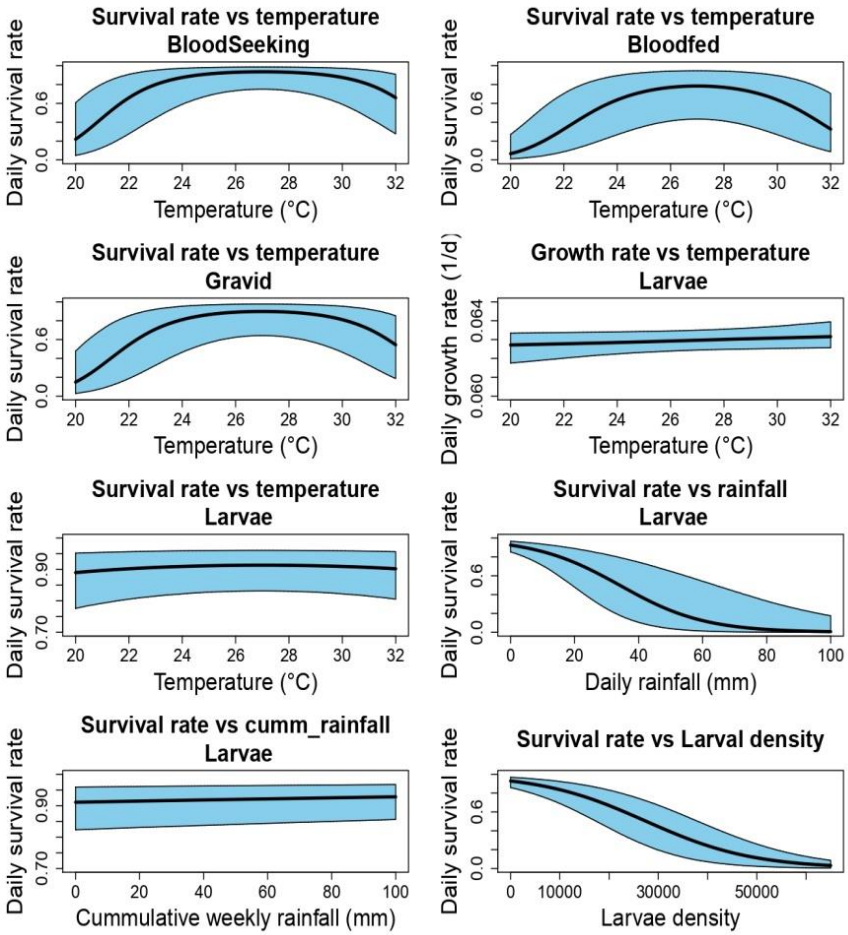


Figure 5.3: The influence of environmental covariates on the demographic parameters as estimated from the single population dynamics model of wild *An. funestus*.

5.3.2 Unexplained drivers of population dynamics

By constraining the density dependence parameter, it was possible to explore hidden mechanism which might contributes to population growth beyond density dependence and demographic parameters. In both models (single vs. hierarchical), the density dependence parameters were being reduced to low values which

suggests that observation uncertainty during data collection might be creating additional instability in the system. Also, there was evidence of hidden processes accelerating population growth that the model was only able to account for by diminishing density dependence. Both models (Figure 5.1 and Figure 4.3) show a slight increase in the abundance of the adult (unfed, bloodfed, gravid) mosquitos several weeks after the first rain event after a long period of dryness. The single population model suggests that density dependence was unable to explain the persistently low values and the rapid explosive increases simultaneously (Table 5.1). This is similar to the findings of the original hierarchical model, with no difference in the density dependence values from the original hierarchical model even after constraining the prior values for the density dependence parameters.

5.3.3 Ability of the model to predict missing seasonal data

Here, I assessed the ability of the single population model to predict a portion of missing seasonal data through visual inspection of the credible intervals of the population abundance trajectories. This indicated that the model with constrained density dependence can reliably reconstruct up to 25% of missing time series data (spanning wet and dry season) with sensible credible intervals (Figure 5.4). However, when the proportion of missing data was increased from 25% to 50% (incorporating largely dry season); the model lost its prediction capability as evidenced by increasing levels of uncertainty around predicted abundance trajectories (Figure 5.5). The high level of uncertainty in the 50% part of the missing data was mainly driven by the low value of the density dependence parameter which was unable to keep the population from exploding.

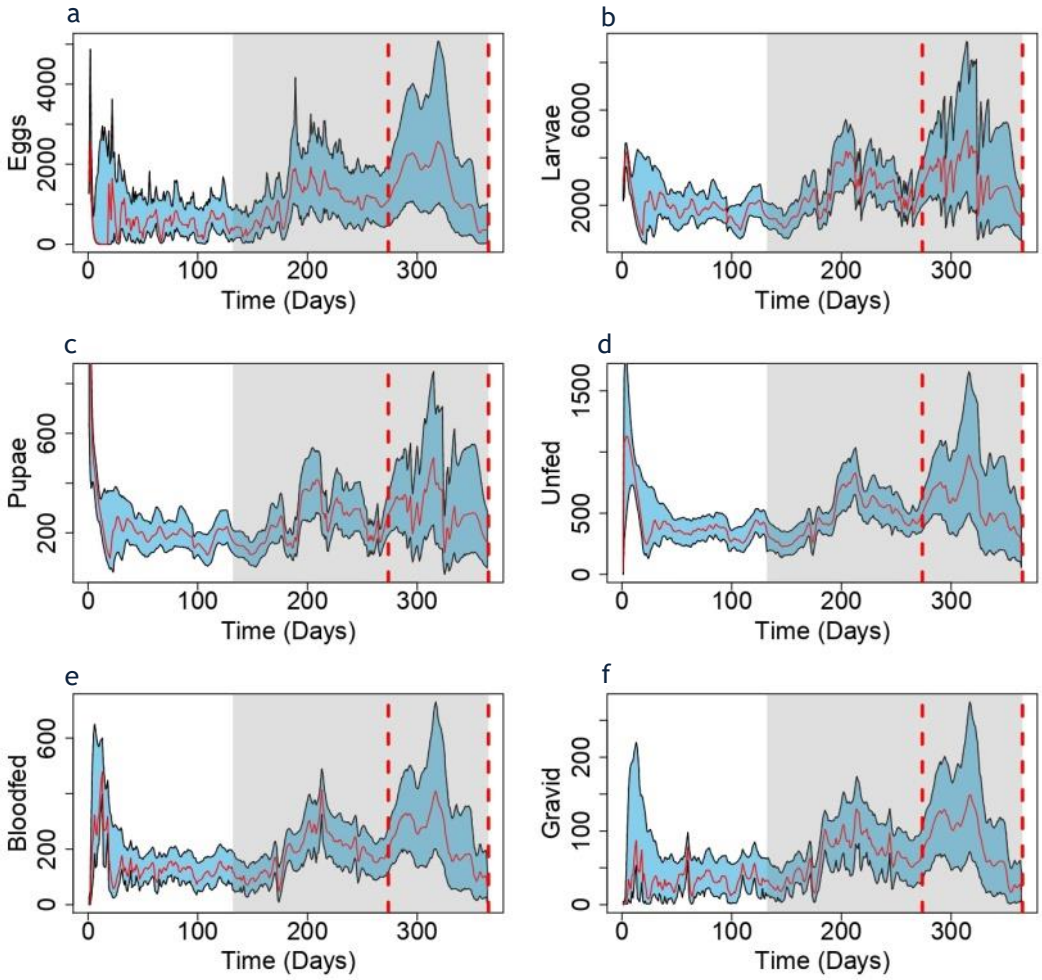


Figure 5.4: Abundance trajectories showing the prediction of the 25% missing data at the end. The vertical red dotted lines corresponds to missing data (25%) which were removed during model fitting and allow the model to predict. The red line indicates the mean posterior values and the respective 95% credible intervals are shown in “sky-blue”. The grey shaded region indicates periods with rainfall. Time step “0” refers to the start of data collection June 2018 and ending May 2019.

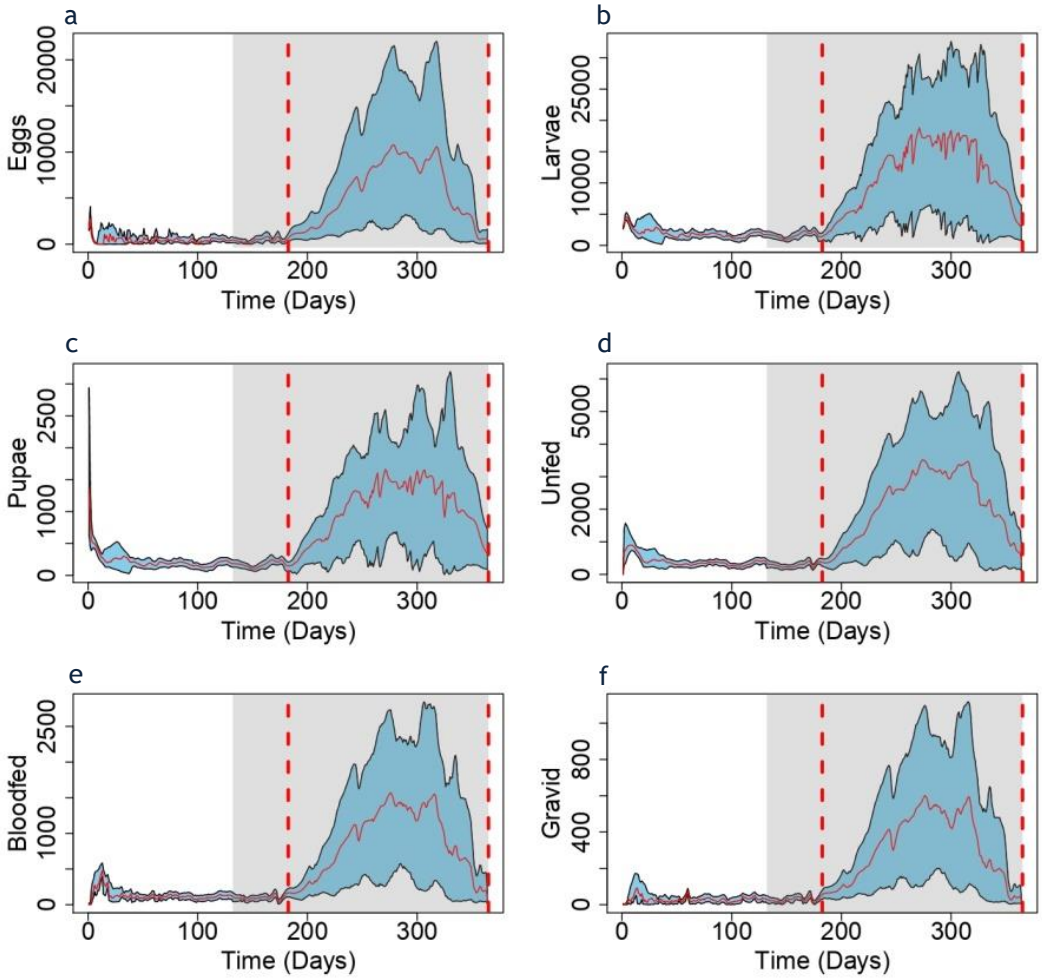


Figure 5.5: Abundance trajectories showing the prediction of the 50% missing data at the end. The vertical red dotted lines corresponds to missing data (50%) which were removed during model fitting and allow the model to predict. The red line indicates the mean posterior values and the respective 95% credible intervals are shown in "sky-blue". The grey shaded region indicates period with rainfall. Time step "0" refers to the start of data collection June 2018 and ending May 2019.

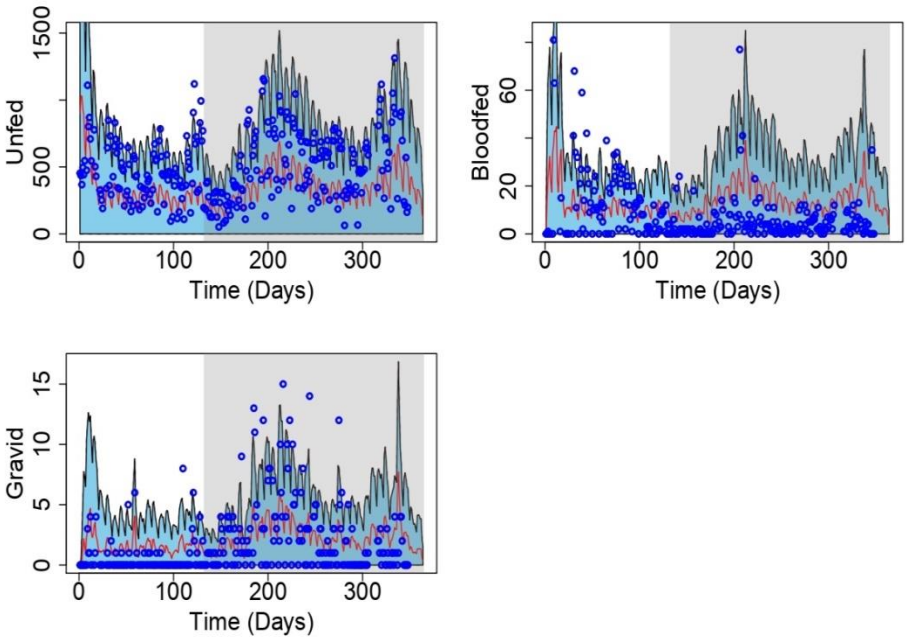


Figure 5.6: Observed vs. model estimated values for the three adult stages with data collected using CDC light trap both in June 2018 –May 2019. Red lines are the model estimated trajectories with "sky-blue" showing their 95% credible intervals. The blue circles are the observed values from the Light trap catches. Grey areas are the periods with rainfalls episodes. Time step "0" refers to the start of data collection June 2018 and ending May 2019.

5.4 Discussion

I re-examined the previously developed SSM model for the population dynamics of the wild population of *An. funestus* (Chapter 4, [153]) by fitting it to a single population. This analysis suggests that the reconstructed seasonal population trajectories of *An. funestus* were broadly similar in main features in hierarchical and single population model. In both cases, *An. funestus* were predicted to remain stable at low densities across the dry season, and start to rise several weeks after the first rains, with a peak occurring at the start of the dry season. The contribution of density dependence remained minimal in the single population model, but was sufficient to be retained by the model. I had to constrain the density dependency parameter in all the models. This requirement to constrain density dependence in the model suggests the existence of observation uncertainty during data collection which creates additional volatility in the system, and/or the

presence of a missing regulatory process that was accelerating population growth; which the model was only able to account for by diminishing density dependence.

In contrast to findings obtained for the Hierarchical model, *An. funestus* demographic parameters in the single population model were more responsive to environmental covariates. Demographic effects of covariates were much stronger in the single population model compared to hierarchical model. The hierarchical model assumed that parameters describing the response of demographic variables to micro-climatic conditions were the same in all populations. Releasing the model to fit to only one population resulted in a shift from muted to strong responses of environmental covariates. This implies that the response of *An. funestus* demography to environmental conditions may be diverse and vary between populations. For example, larval survival varied by 78% and 85% respectively along the range of variation in temperature and rainfall covariates considered in simulations. The SSMs are great in modelling population dynamics models but can be used with caution as may underestimate the role of environmental drivers when fitted over multiple populations.

This model was able to predict seasonal abundance trends relatively well when 25% of data were missing, but struggled when this was increased to 50%. The single population model was thus quite robust to missing data as long as the data included in fitting contained some representation from the dry and wet seasons. This indicates that the model can predict only if it learns from both wet and dry seasons. This model can be expanded to include other aspects of human malaria dynamics, or it can be used to simulate the efficacy and effectiveness of various interventions individually or in combination. This would allow more sophisticated evaluation of *An. funestus* specific intervention in different target areas.

In general, most of the population dynamics models previously developed for African malaria vectors have not accounted for biological processes beyond traditionally life history processes of births and death. Other processes such as aestivation, diapause or mosquito migration (e.g. [351,352]) could lead to changes in vector population abundance beyond what can be attributed to these life history processes. For example, aestivation is a process through which mosquitoes may become temporarily ‘dormant” by concealing and restricting their flight activities

until the onset of first rain [151,353,354]. In the current *An. funestus* population dynamic model, I speculate that such additional factors could play an important role in population regulation beyond the current minimal role of density dependence. This hypothesis is based on the observation that in the absence of explicit incorporation of processes like aestivation/diapause into this model, the density dependency parameter was penalised by having its value reduced to account for the missing factors.

A successful mosquito dynamics model should be able to satisfactorily capture key features of population trajectories as a result of known life history and development processes. For example, in a closed population, changes in adult mosquito abundance are expected to follow those of larval abundance, with a lag period characteristic of typical larval development periods. If unexplained rises and declines are evident, this may suggest additional biological processes are missing from the model; including deviation from the assumption of a closed population. For example, some studies have shown that seasonal aestivation and migration of adult mosquitoes has a strong influence on the overall dynamics of *An. arabiensis* [351,354]. This was detected through a signature in a population model that showed vector densities rose far quicker at the start of the wet season than could be explained by the time required for egg-larval-adult transition [351]. Study on non Anopheline species shows that, *Aedes aegypti* species eggs can survive the dry soil for more than two months [355–357] contrary to *Anopheles* eggs which survive less than 15 days [358]. With the dry period of more than two months in most of African countries which are malaria endemic, the only possible survival mechanism for adult mosquitoes is through aestivation [45]. Similar unexpected changes in population size beyond what can be explained by reproduction and mortality could be due to pulses in dispersal into or out of populations [100,359]. Additionally, it is possible for habitat composition to affect one demographic rate in one place but a different one in another. For example, the two time series data used in the hierarchical model contain differed in mean population abundance suggesting that abundance varies with geographical location. A life history-based model that fails to capture key feature of population dynamics can serve a useful purpose in highlighting the existence of additional regulatory factors. With additional data on aestivation, mosquito migration or

diapause this model can be further extended to incorporate all these environmentally driven aspects of the mosquito ecology. This may further increase the predictive accuracy of this SSM and generalizability in the assessment of stage-specific impact expected from different vector control measures.

5.5 Conclusion:

Using a novel parsimonious framework, the effect of rainfall, temperature, and density-dependence was investigated on more geographically restricted population of *An. funestus* s.l. (considered as 'one population') from southern Tanzania. The single population framework developed here could allow for a more robust analysis and evaluation of various vector control interventions, as well as their integration into malaria transmission models, in the future. This model's ability to explain the response of demographic parameters to environmental covariates indicates its value in simulating the response of this *An. funestus* population to vector control measures used individually or in combination. In this regard, the developed paradigm is a significant step forward; however it also identifies critical research gaps beyond demographic processes (i.e. aestivation or diapause) that must be addressed if we are to gain a better understanding of the ecology and population dynamics of this vector species. Furthermore, future models should include spatial elements to capture the diversity and presence of any source of heterogeneity in multiple populations.

6.0 Chapter 6: General Discussion

6.1 Overview of the main findings

The overall goal of this thesis was to contribute to a general foundation of improving knowledge on the ecology and surveillance of *Anopheles funestus*, a major vector of residual malaria transmission in many settings in Africa. It is hoped that information gained through this work will aid the campaign against malaria by improving vector control strategies in Tanzania and other settings where *An. funestus* is the major source of transmission. Malaria transmission remains high in Tanzania; hence this work will contribute important knowledge on how to exploit the ecology of *An. funestus* to further progress towards elimination. This general discussion will provide a brief summary of key findings from each of my specific objectives, discuss relevant challenges and limitations of this study, and highlight key implications for vector control and malaria elimination.

6.1.1 Fitness characteristics and colonization barriers

Extensive investigation and characterization of the biology of any malaria vector requires a stable laboratory colony. The lack of colonies from locally representative *An. funestus* populations creates a gap in terms of how to tackle transmission mediated by this vector in Tanzania. For example, assessment and monitoring of insecticide-based interventions requires carrying out bioassays on mosquitoes. Semi-field systems are often been used as the first step to study the behavior and response to intervention for any malaria vectors before the field trials. Most phase 1 intervention trials on *An. gambiae* were conducted in controlled laboratory settings, enabled by the existence of stable insectary colonies [196,360]. However, similar evaluations for *An. funestus* have been limited by the difficulty of establishing stable colonies under lab or semi-field settings. Consequently although *An. funestus* is a major vector in Tanzania, it has not been possible to evaluate the efficacy of different vector control intervention or trapping methods for local populations of this species under standardized (lab or semi-field) conditions.

Here, I evaluated key barriers associated with the colonization of this malaria vector species in the laboratory environment. I attempted to colonize this species by collecting wild mosquitos (F_0) from three villages in the rural part of south-eastern Tanzania and rearing them in the laboratory. This showed that the fitness characteristics (i.e. mating, fecundity) of F_1 offspring in the laboratory were impaired relative to that of a stable *An. funestus* colony (FUMOZ). Mating was the biggest barrier to establishing further filial generations; with <10% of F_1 females were able to mate in the laboratory whereas 92% of wild F_0 were mated. This resulted in very few viable eggs being produced despite the relatively large number of F_1 females, which were insufficient to generate a further filial generation. This low mating success is consistent with the known eurygamy of this species [38,39]. Other aspects of *An. funestus* development and fitness in the laboratory were also impaired. Notably, the very long larval development period (2-3 weeks) observed for *An. funestus* in the laboratory led to a low probability of survival to adulthood. The larval development period for *An. funestus* in the lab was considerably longer than reported for other African vector species (e.g. *An. gambiae* sl. 9-11days). Although my attempts to colonize a Tanzanian *An. funestus* population were not successful, they provided general knowledge on the major barriers to colonization (e.g. mating) and help focus investigation on how to overcome them.

6.1.2 Surveillance methods of outdoor vectors of malaria transmission

Surveillance of vector populations is of great importance for assessing the effectiveness of interventions and monitoring the behavior of mosquito species in a given setting. The most important entomological indicator of malaria transmission is the entomological inoculation rate (EIR) [361]; calculated as the product of mosquito biting and infection rates. Human landing catches (HLCs) are the gold standard for estimating biting rates and the EIR in both indoor and outdoor environments. However, this method is increasingly restricted due to ethical concerns arising from the exposure of collectors to potentially infected mosquito bites. Whereas several exposure-free alternatives to the HLC have been evaluated for *An. gambiae* s.l. (HLC); there is less understanding of which methods are best

for *An. funestus*; particularly in outdoor settings. A trap evaluation study comparing the performance of HLC and six exposure-free alternative traps for outdoor settings was conducted in places where both *An. gambiae s.l.* and *An. funestus* are found. Here, I analyse the data and generate associations between the alternative traps and HLC. These associations were later used to develop a calibration tool for predicting the number of mosquitoes collected by HLC if someone were to use one of the alternative trap evaluated.

Relationships between collections made by the HLC and alternative methods may not accurately reflect their association across more diverse ecological contexts. If we wish to not only evaluate how an exposure-free trap performs relative to the HLC, but allow the 'HLC'-equivalent exposure rate to be predicted from it, it is necessary to identify which type of mathematical relationship (linear or non-linear) best describes their association. Here, a statistical calibration tool was developed under a Bayesian framework in the form of a *Shiny App* interface for its interrogation. The tool developed here provides predicted mean values for each alternative traps and their credible intervals which can be used to obtain estimates relative to HLC. Results suggest that trap performance is less affected by population baseline density (intra-specific) for all the species, though these findings tend to differ when the same alternative traps are evaluated in a different environment [124,288].

This analysis provided little evidence that the relative performance of mosquito traps is modified by the density of the target vector of other mosquito species in the environment. Trap catches varied between species and between model configurations, so no specific model or trap is 'best' for all vector species. The tool developed here warrants further evaluation of different alternative traps to consider how representative and consistent they are compared to the HLC or other collection methods.

6.1.3 Drivers of population dynamics of wild *Anopheles funestus*

Chapter 4 describes an innovative state-space population dynamics model of wild *An. funestus* in Tanzania. Population dynamics model have been frequently used to

understand the dynamics of the African malaria vectors in the *An. gambiae* complex [150,158,362]. Similar models have been difficult to develop and parameterize for *An. funestus* due to the limited knowledge of their fitness and demography. Consequently, the response of *An. funestus* to environmental factors and interventions is often inferred from population dynamics models derived for *An. gambiae* s.l.

Here, I used data collected during attempts to colonize *An. funestus* (Chapter 2) and additional data from the literature as the prior information to develop a biological process model of *An. funestus* population dynamics. The specification of this model allowed *An. funestus* dynamics to be influenced by both intrinsic (density dependence) and extrinsic drivers (environmental covariates). Population abundance time-series data were used to inform the observation process model (collections from CDC light trap). CDC light traps are designed to capture mosquitoes that are looking for a blood meal (host seeking), but also occasionally catch gravid and bloodfed mosquitoes. Consequently the observation model attempted to account for trap bias in collections of bloodfed and gravid mosquitoes. Further work is needed to assess the proportion of the population; whether unfed, gravid and bloodfed, that is sampled in CDC and other trapping methods. Such information will refine the observation model and accuracy of resultant demographic predictions.

In examining environmental covariates of *An. funestus* dynamics, I focused on temperature and rainfalls given these are known to be key drivers of mosquito fitness and demography. Additionally, the impact of intrinsic drivers was addressed through consideration of density dependence arising at the larval stage. The potential contribution of environmental covariates to vector dynamics was assessed through a removal strategy and see what the model would predict. The resultant model was able to reconstruct the abundance trajectories for all *An. funestus* life stages and gonotrophic stages. Notably, the relatively low uncertainties around these trajectories indicate the model was a reasonable fit to the data. These trajectories show that *An. funestus* densities are generally higher during the rainy period; with numbers peaking towards the end of the wet season. Notably, although *An. funestus* densities were low during the dry season, they

persisted throughout the full duration of this period. In contrast, *An. gambiae* s.s. and *An. arabiensis* had almost no captures during the driest period. During the dry season, the estimated mean values for *An. funestus* were quite low but sufficient to keep the population going until the first rain. The survival trajectories of larvae and adult stages were similar across seasons; indicating this species is relatively resilient to temperature and rainfall extremes. The ability of this vector to survive the driest part of the year indicates it plays a crucial role in sustaining malaria transmission throughout the rest of the year. Targeting *An. funestus* during the dry season could thus be a key to disrupting transmission during its most vulnerable point.

This modelling framework suggests that density dependence may be present in *An. funestus* populations, but makes a relatively minimal contribution to their dynamics. I hypothesised that there is an additional feature in the dynamics that regulates the population by lowering density dependence parameters. This was further evaluated in Chapter 5. Whether this is due to the nature of the breeding habitat or not, it warrants further investigation in the laboratory or semi-field systems. This species depends much on the presence of large and semi/permanent larval habitats such as rivers and large water reservoirs [54,341]. Rainfall (one week lagged cumulative) plays a crucial role in creating these habitats, and was positively associated with larval survival when the density dependence was taken into consideration.

6.1.4 Generalizability of population dynamics models

Chapter 5 describes a series of simulation exercises performed with the novel SSM model developed in Chapter 4; to explore its generalizability and ability to predict missing data. A key focus was on examining the generalizability of inferences drawn about the drivers of *An. funestus* dynamics from the original hierarchical model, by re-fitting it to a single population. Environmental covariates are key drivers of many mosquito populations, but if these are not well captured during the model development it might result in model misspecifications. The density dependence parameters were estimated to be small in both types of models fitted. Most of the demographic parameters were the same for both the hierarchical and

single population model except for the adult survival. Specifically, the survival of all the gonotrophic stages was very sensitive to changes in environmental covariates (i.e. temperature) in the single population model but not the hierarchical one.

In a further step, I evaluated the model's ability to reconstruct annual *An. funestus* population dynamics when different proportions of data from the annual time series were missing (25% and 50%). When model predictions were tested under a data removal strategy, the model was able to reconstruct up to 25% of the missing data with sensible credible intervals. However when half of the data (50%) was removed, the model's ability to reconstruct *An. funestus* dynamics was highly reduced by increasing the level of uncertainty around the predicted mean abundance. The model was able to predict the missing segment in the trajectories if it learns from both wet and dry seasons. Finally, I was able to test various features other than density dependence parameters that could be contributing to population regulation by increasing or decreasing the flexibility of the density dependence priors in the single population model. Even with high level of flexibility in density dependence priors, the model was suggesting small contribution of this parameter in regulating the dynamics. Given that the density dependence parameter was minimized regardless of the flexibility in the prior values, this implies that additional features might be driving the dynamic by reducing the density dependence's contribution to the overall dynamics. The difficultly of capturing sustained troughs and peaks purely via flexible density dependence indicates the need for additional currently missing biological processes (i.e. aestivation, diapause or migration) in the life-history of the mosquitoes.

6.2 Questions arising

This work generated several questions which warrant further investigation. First, why is the mating success of *An. funestus* so poor in the laboratory? This biological process is poorly understood in eurygamic species. Stable colonies will only be achieved through a significant improvement in the mating process under laboratory conditions. How could this be achieved? One option would be to facilitate

swarming behavior in more natural semi-field setups where wild adult mosquitos could be released and thoroughly examined. Establishment of colonies could also be assisted by modelling to predict the initial size of wild populations to achieve successive filial generations, and set targets for the required mating percentage to reach the first filial and the subsequent generation. Future research on mating can look at these issues in order to have a better understanding of the colonization process. While I was unable to establish *An. funestus* colony in this study, colleagues at IHI have subsequently had success in rearing a Tanzanian *An. funestus* strain up to 26 (F_{26})generations in the laboratory (Hape *et al*, in prep).

This work has evaluated the performance of a number of exposure-free traps for measuring host seeking *An. funestus* in outdoor settings. Notably, all of these gave an underestimation of biting density relative to the HLC gold standard. Unfortunately, *An. funestus* abundance is quite low in the current study site; hence, calibrating any additional trap against *An. funestus* will need more extensive sampling to reach over longer periods of time to generate sufficient sample sizes and statistical power to robustly characterize their relationships with the HLC. Regarding the statistical technique developed in this study, considerable effort should be invested into designing sensitive traps which can work in areas of low density vector populations. More investigation into how to improve the luring mechanism is recommended.

Modelling malaria vectors like *An. funestus* requires detailed understanding of how environmental covariates drive their dynamics. Several environmental variables expected to be of importance (temperature, rainfall) were explored during the model development process to assess their association with *An. funestus* dynamics. This produced some counterintuitive results. For example, demographic parameters (adult and larval survival) did not appear to be very sensitive to varying temperatures and rainfall in the hierarchical model; despite the well-known impact of these covariates on vector population dynamics and fitness [40,363]. The apparent low sensitivity to these environmental variables may be a genuine ecological property of these populations within this particular study site, or the result of high stochasticity in the mosquito data used to inform the model; generating too much noise to detect clear signals of these parameters. This raises

further questions about the sources of stochasticity in mosquito catch data. For example, how much impact does the presence or movement of human in the sample houses have on *An. funestus* catch rates? It is known that this species is highly endophagic and anthropophilic in nature. Thus the presence of humans will ultimately attract mosquitoes into houses for feeding. Previous work on the same study area shows that the number of people in the sampled houses was positively correlated with the number of mosquitoes sampled [295]. In this work, I did not consider the variations in the number of occupancy in each sampled houses.

Another unexplained feature of the mosquito surveillance data used in modelling was its apparent periodicity. The model developed here made use of daily time series data from mosquito surveillance that was made from Monday to Friday over one year period skipping weekends. The unexpected feature of this data was an apparent weekly periodicity; with catches appearing to decrease throughout the sampling week. The possible biological or environmental reason for this is unknown. One possibility is that *An. funestus* population within a household is limited, and gets progressively depleted by trapping with daily catches replenishing over weekend break. There is evidence which suggests "mass trapping" in houses can reduce the size of mosquitoes population and maybe malaria transmission [364]. In this case, a potential concern that would arise is whether mosquito populations might keep decreasing even if there was no weekend break. Would this be a potential mechanism to estimate the population size via removal sampling as previously documented by Service [365]?

6.3 Implications of the findings

6.3.1 *Anopheles funestus* colonization bottlenecks

To address ongoing residual malaria transmission, a comprehensive ecological understanding of all vectors that play a role in transmission is required. This study provides the first documentation of attempts to colonise *An. funestus* in Tanzania. It laid a foundation for continuing efforts in Tanzania to colonise this species, which have recently been successful (Hape *et al*, in prep). By studying all the barriers to colonization outlined in the second chapter, another team of scientists

at IHI has managed to reach 26 (F_{26}) laboratory generations of *An. funestus* from a local population (Hape *et al,* in prep). Learning from my experience, this team changed strategy to not just collect maternal population (F_0) at a single point; instead they repeatedly collect wild females over several weeks and months and cumulatively add them into the same laboratory founder population cages. This meant that much larger numbers of females (>2000) were present in each of multiple laboratory cages. This generated substantially larger founder populations than available for my experiments (Chapter 2 [24]). Similar to my observation, the initial mating success in these larger laboratory population was very low over first few generations. However, the larger size of these founder populations proved sufficient to generate enough offspring even with low mating success to seed further generations. Eventually, this strain started to adapt to the laboratory conditions, leading to a rise in mating success after 4-5 generations and establishment of a stable line (Hape *et al,* in prep).

6.3.2 Reliability of the mosquito sampling tools

The trapping evaluation I conducted has several implications for improving the surveillance of *An. funestus*, particularly in outdoor settings. I found that trap catches for *An. funestus* were independent of the abundance of other mosquito species in the area. This implies that there is no saturation effect in trap performance; at least across the range of densities surveyed here. In general, the relationship between the HLC and other trapping methods could be described by a simple linear relationship. Also this work demonstrated that there can be a considerable number of *An. funestus* host seeking in outdoor environments. While these *An. funestus* populations were relatively low density (compared to other vectors), the high infection rates of this vector (e.g. ~85%, [88]) means that the number host seeking outdoors contribute to residual transmission. Outdoor exposure to *An. funestus* is usually ignored in conventional surveillance activities, with only indoor sampling conducted. This focus on indoor sampling must change if we want to better quantify and tackle residual transmission. Given that people in this study area are clearly exposed to *An. funestus,* trapping methods are required that can reflect outdoor exposure as estimated by the HLC. Here, no trap was

extremely highly correlated with the HLC for *An. funestus* outdoors; however the two best candidates were the M-Trap (MTR) and M-trap with additional CDC (MTRC) inside. Therefore, if someone were to use either of these traps to sample outside biting mosquitos, the predicted mean values from this calibration method could be used to calculate the human biting rate as estimated by the HLC. Before making a decision on which outdoor trap to use, the *Shinny App* interface may be used to explore the possible range of values and how wide the credible intervals for different methods.

6.3.3 Model parameterization and validation

When modelling mosquito population dynamics, not all data or parameter uncertainty can be accounted for in the model development process. In general, population dynamic models address only four aspects of population change (i.e., mortality, birth, immigration and emigration). In mosquitoes, all these demographic process could be influenced either by vector control intervention currently in place or changes in environmental conditions [40,363]. Malaria vector species vary in their response to environmental variability [363], thus necessitating development of species-specific models.

A wider insight from this work is into application of stochastic State-Space Models for analyzing the dynamics and demography of wild vector populations. SSMs have been used in many fields of ecology but rarely used to study the dynamics of malaria vectors [332,366]. These SSMs account for two distinct types of stochasticity: sampling imprecision and biological variation. In general, combining these two stochastic processes yields better estimates of ecological quantities of interest than modelling only one stochastic source directly. An advantage of this approach is its ability to indirectly estimate the otherwise hidden demographic rates and processes that could plausibly generate the observed dynamics. Many of these demographic rates are very difficult to directly estimate in the field (fecundity, daily survival etc.), thus this approach provides a useful alternative. Population trajectories reconstructed by these models could be used to identify seasonality in abundance and the underlying demographic rates that give rise to it (survival, fecundity, and larval development rates). However, the mosquito

collection devices used (i.e., traps) and human errors during processing (i.e. counting) can have a significant impact on the quality of observation data. Careful consideration and adjustment of trapping methodology and processing thus may improve the general applicability and reliability of these types of dynamic models.

6.3.4 Selection of optimal vector control package

The potential impact of vector control intervention can be estimated from its impacts on vector demography and fitness parameters. A realistic population dynamics model enables the impacts of these intervention effects on the size and stability of a vector species to be predicted. With further development, the modelling framework developed here can be used to evaluate the anticipated response of *An. funestus* to various vector control interventions, either individually or in combination. Uniquely, this framework could enable identification of optimal environmental conditions or times of the year to deploy interventions for maximum impact. Rainfall patterns play a big role in intervention deployment strategies like IRS and LSM. For example LSM is applied during the driest period when the habitats are fixed and few [95,137,163] unlike IRS which is applied during the transmission seasons [367,368]. This framework can be used to simulate standard and variable seasonal implementation of these and other interventions, to identify which optimal package can generates the maximum negative impact on *An. funestus* survival. Despite its complexity, this framework may be expanded in the future to accommodate additional ecological and behavioral processes that may impact *An. funestus* dynamics such as host choice, oviposition behavior, and processes such as migration, diapause or aestivation.

6.4 Limitations of the study

This study provides a detailed understanding of the ecology and the drivers of the dynamics of *An. funestus* in the wild population. As described in the previous chapters, there are several limitations associated with different parts of this work; of which a few general ones will be highlighted here. Firstly, several of the direct estimates of *An. funestus* fitness and demography used in my modeling work were

derived from an unstable population. Specifically, many of the fitness parameters that served as prior values for my population dynamics model were derived from unsuccessful attempts to colonize *An. funestus* in the lab (Chapter 2). Here, detailed individual measurements of larval development time, larval and adult survival, and fecundity were measured from cohorts of F_0 females brought into the lab and their F_1 offspring. Given these individuals were not maintained past the second filial generations in the lab, their fitness values may have been impaired and thus unrepresentative of likely values in the wild. Additionally, F_0 were fed on the blood of an unnatural host in the insectary (chickens) to produce eggs for the next generation. The subsequent fecundity and survival of *An. funestus* after feeding on this host may not be reflective of their fitness after human feeding. Lyimo et al have shown that, fecundity and survival are not impaired when *An. arabiensis* is not fed on their natural host [78,81]. Also the age and mating status of the F_0 *An. funestus* females could not be estimated at capture, and may have introduced another source of variability. It is known that mosquitoes need several generations to adapt to insectary conditions [58,170], whereas I only had data for the first two generations due to poor mating success. This does not necessarily mean that all the fitness estimates derived from these adapting mosquitoes are unrealistic. For example their survival rates were relatively high and consistent with values estimated by Okoye et al [222,330]. The mean values generated here, were later used in the Bayesian framework with a wider prior range to make sure the model priors were not unnecessary precise and influential. Certainly, further model development using either fitness parameters from a stable *An. funestus* colony, or as directly estimated in the field, would be useful.

Secondly, the calibration tool developed here to predict human landing catch (HLC) - equivalent values from alternative exposure-free traps was only done for outdoor collections. Thus, we do not yet have a similar tool for calibration of indoor traps. Given that the majority of *An. funestus* bites still occur indoors, expanding this framework to indoor settings is a priority. Human landing catches are the gold standard for sampling host seeking mosquito both indoors and outdoors. In my analysis of outdoor sampling, some traps provided estimates with very wide credible intervals; with this not being due to poor fitting but rather very low numbers of *An. funestus* at the time of sampling. *Anopheles funestus* are

found at relatively low abundance in the study area. Further evaluation of this type of calibration approach in areas with higher *An. funestus* densities is needed to refine the calibration tool and make it more generalizable to data from different settings.

In addition to the previous issues mentioned, another important limitation of the population dynamics modelling work was its reliance on data from one sampling method, the CDC light trap, which is specifically targeted host seeking mosquitoes (unfed). This method is not effective for sampling other adult female life stages (i.e. blood-fed and gravid), but since sampling effort needs to be standardized for this type of modelling, I had to introduce a parameter in the observation model to account for trap bias. This meant that estimates of the abundance of blood fed and gravid females were derived from an inefficient method, and are likely to be less robust than those for unfed. Additionally, there are limitations with using CDC light traps to infer population abundance trends, even of unfed females. It is uncertain what proportion of the mosquito population, whether unfed, blood fed or gravid, and are sampled in a single CDC trap. This could potentially be explored in semi-field experiments where the number of individuals caught in a CDC trap from a population of known size could be estimated.

6.5 Further work

Ecological studies of *An. funestus* must continue if we are to control residual malaria transmission in African countries where the disease stills a public health problem. The barriers to colonization and possible solutions are described in Chapter 2, which also offers a full overview of the mating process in captivity. Currently there is an ongoing colonization process which started after my work was completed. Several generations have been maintained in Tanzania's ongoing effort, in addition to the one filial generation created through this work. When the colony is stable, this will aid in the estimation of demographic parameters and used in testing new traps, different insecticides formulation, spatial repellents to genetically modified mosquitoes.

The use of modelling to understand the dynamics of the local population of *An. funestus* species in Tanzania was demonstrated in Chapters 4 and 5. The role of modelling can change to address different questions at each step of its development process. The population model established here, for example, was first used to illustrate how environmental variables and intrinsic factors (density dependence) influence *An. funestus* dynamics and to estimate their demographic and fitness parameters in the wild. The next step will be to utilize the same model to look into the effects of different vector control interventions on adult survival and reproduction, and the resultant impact on population size and stability. As I have access to the whole range of posterior means generated by this model (i.e., 100,000 samples), I will be able to minimize the degree of uncertainty while simulating multiple intervention options. With additional data, this population dynamic model will be expanded to include other potential regulatory processes that I hypothesized could be present in Chapter 5 (i.e. mosquito aestivation, diapause, and migration). I will also explore developing this SSM into a meta-populations model which could be used to describe different, connected subpopulations of *An. funestus* across a landscape. Technically, the modelling framework's computational efficiency will have to be optimized to allow for low uncertainty in observational data collection, which will eventually lead to more robust prediction. At the same time, regular high quality data collection is critical for both decision-making and the validation of the population model used to select optimal intervention packages.

6.6 Conclusions

The work presented here covers several aspects of *An. funestus* ecology that are critical to understanding the species' stability and response to interventions. This work covers the colonisation process, vector surveillance, and modelling of population dynamics of *An. funestus* in Tanzania. The findings here will greatly contribute to a general understanding of *An. funestus* ecology, how this vector responds to environmental covariates, and colonisation barriers. Knowledge on *An. funestus* biology will continue to be impeded until the barriers with getting them to mate in the lab are overcome. The role of outdoor biting is often neglected in

An. funestus, but could be a key component of residual transmission. Until recently, we did not have a reliable alternative to HLC that is effective for sampling *An. funestus* outdoors. Population dynamics model confirms relative stability of demographic rates throughout the seasonal cycle of extreme temperature and rainfalls. The findings will also help future modelling efforts which aim at selecting the optimal vector control intervention package which can disrupt the survival of *An. funestus*. As a result, any additional efforts in Tanzania aimed at *An. funestus* could result in the eradication of malaria.

7.0 Chapter 7: Addendum of Chapter 3

7.1 Rationale

The main aim of this addendum is to correct for the error found in chapter 3. In the original chapter3, I modelled the mosquito catches by alternative traps using a mixture of gamma-Poison distribution with a log-link function. The choice of "log" violates the linearity assumption as "log" tends to plateau at high numbers. This means the simple linear model assumption is violated because the "log" function will not hold a linear relationship when there is a high number of mosquito catches.

In order to address this issue, I have restructured the negative binomial model by using an "identity-link" function instead of the "log-link" function. This will allow me to accurately assess the relationship between HLC and alternative traps catches, as the "identity-link" function does not violate the linearity assumption. In addition to this, I have also changed the prior specification to match the current model structure and assumptions.

This modification to the original model will help to provide more accurate and reliable results, and will allow for a better understanding of the relationship between HLC and different alternative traps catches. The use of the identity-link function in place of the log-link function will ensure that the model is able to capture full range of possible outcomes as hypothesized in chapter 3 (i.e. intra-specific or inter-specific density dependencies). By carefully considering the choice of link functions and adjusting the model structure, updating all tables and figures as needed, I am confident that the revised model will be able to provide more accurate and reliable results.

7.2 Model fitting

The main goal of the analyses was to create a calibration tool to evaluate outdoor mosquito traps and to validate the tool by comparing the performance of candidate trapping methods relative to HLC, the "gold standard". In particular, I

wanted to test the shape of the association curve linking the numbers of mosquitoes collected by each trap type with those collected by the HLC. First, I pooled all the hourly collections into a single collection cup per trap per night. Then, for each of the focus mosquito groups (*Culex* genera, *Anopheles funestus* s.l and *Anopheles arabiensis*), I modelled nightly HLC catches as a function of the catching rate of each alternative trap.

Four linear models were developed within a Bayesian model fitting framework to allow me to test for linear and non-linear associations through increasing levels of complexity. The Bayesian approach allowed specific constraints on parameters based on biological plausibility, in the form of priors and uncertainty when converting the counts from alternative traps into HLC equivalent values in the form of full posteriors.

For any given trap and mosquito group, I defined the response variable (N_i) as the number of female mosquitoes on every i^{th} sampling night. The final model did not account for other environmental covariates at specific trap location (e.g. temperature, humidity). Initial analyses suggest that, environmental covariates did not improve model fitting. I fitted the model following a negative binomial distribution using "identity-link" function with parameter p_i and r such that;

$$N_i \sim NegBin\,(p_i, r)$$

$$p_i = \frac{r}{r + \lambda_i}$$

Where λ_i is defined by the shape of the relationship between N_i and the number of mosquitoes collected with the alternative trap n_i (Table 7.1). Since the algebraic form of this relationship is not known, I made three assumptions with specific mathematical definitions, as follows; 1) that the relationship must start at the origin (i.e. when HLC catches zero mosquitoes, the alternative traps will, on average also collect zero mosquitoes), 2) that the relationship is positive (i.e., no negative relationships between trap catches), and 3) that any given trap could potentially suffer from a density effect (i.e., the slope of the relationship Is not constant and it can change according to the abundance of mosquitoes, either just of the same mosquito group or of all mosquitoes).

To define λ_i, I therefore formulated four possible scenarios to describe the relationship between HLCs and other trapping methods as summarized in Table 6.1. In model 1, I considered a simple linear relationship between N_i and n_i (Table 6.1). In model 2, I tested if the efficiency of the alternative trap was dependent on the density of the focal mosquito (e.g. intra-specific density dependence) by adding a quadratic term n_i^2 (Table 6.1). In model 3, I tested if the captures of a given group by a given trap were dependent on the abundance of the other taxonomic group (e.g. "inter-specific" density dependence) by adding, an interaction term between n_i and the number of all the females from other mosquito groups collected with the same trap (m_i) (Table 6.1). Model 4 was similar to Model 3, but I considered all the other K_i taxonomic groups separately. Therefore it included all the pair wise interaction terms between n_i and the number of females of each k^{th} mosquito group (s_{k_i}) (Table 7.1). My analysis mainly focused on three mosquito groups, but I collected a higher number of species hence $K > 3$).

Table 7.1: Description of models used to investigate the relationships between female mosquito catches by human landing catch and alternative traps.

Model	Structure
Model 1	$\lambda_i = \beta_1 n_i$
Model 2	$\lambda_i = \beta_1 n_i + \beta_2 {n_i}^2$
Model 3	$\lambda_i = \beta_1 n_i + \beta_2 n_i m_i$
Model 4	$\lambda_i = \beta_1 n_i + \sum_{k=1}^{K} \beta_k n_i s_{k_i}$

This analysis was performed in the statistical environment R, version 4.0 [246], with Bayesian model fitting to the data done using the program JAGS [301] interfaced within R via the package *rjags* [302]. For parameters β_1, β_2 and β_k I used gamma prior (shape = 0.1, rate = 0.1). The prior for the size r was defined from uniform distribution (min = 0, max = 10^5). The prior for β_1 was chosen to ensure a positive relationship between n_i and N_i and a positive effect of the quadratic and

the interaction terms for β_2 and β_k. To achieve convergence, the models were run for up to $3\,x\,10^4$ iterations. Means of posterior distributions with corresponding credible intervals were obtained for each model coefficient β. I compared different models by their Deviance Information Criteria (DIC). To further validate the results, the data was randomly divided into two sets: training set (75%) and a test set (25%). The root-mean-square error (RMSE) was then calculated for each model as the average prediction error on the test set. This allowed us to assess the performance of each model on unseen data and determine which model was the most accurate and reliable

7.3 Interactive calibration tool

I designed a look-up table (Table 7.3) containing means of posterior predictions for different combinations of mosquito taxa, trap types and models. This allowed me to predict the expected number of a given mosquito taxa from an HLC (with credible intervals) based on the number caught in the alternative traps. I also developed an interactive online tool, in the form of an *R Shiny* App [303] to facilitate these evaluations. This tool provides users with an interactive graphical user interface (GUI) to select the number of captured mosquitoes for a group of interest by trap type, and to explore the predicted number of mosquitoes caught in an HLC by method.

7.4. Results summary

Following the reanalysis of the data using the negative binomial distribution with identity-link function rather than the Poisson with log-link as was previously done in chapter 3, we have made the following key observations:

- Out of the four models that were fitted to the six different traps used for the development of the calibration tool, the simple linear model is still the one that was the most preferred. Among all the models, as previously mentioned in Chapter 3, there were no big differences in deviance explained for *Anopheles funestus* and *Anopheles arabiensis* (Table 7.3).

- Across the range of mosquito catches, there is no saturation effect or non-linearity as it was hypothesized in model 2 (Table 7.2). This means there is no evidence of intra-specific density dependence. This is similar with model 3 which assess the effect of other females mosquitoes caught by specific trap. There was no clear evidence of inter-specific density dependence for all the six alternative traps and all the three species (Table 7.3). This was also clearly shown by the β_2 coefficient which is very small for all the models.

- *Anopheles funestus* poor calibrations were mainly affected by the very low catches for all the alternative traps. The wider credible intervals were mainly driven by very low catches of this mosquito species.

- All figures (Figure 7.1, Figure 7.2 and Figure 7.3) and the lock-up tables (Table 7.4) were produced based on the simplest linear model (Mode 1).

Table 7.2: Coefficients of each parameter (β_1, β_2) used to explain the relationship between HLC catches and alternative traps for all the four models.

Species	β	SUN	BGS	ITT-C	MMX	MTRC	MTR
***a) Culex* spp.**							
Model 1	β_1	4.34 [3.54, 5.37]	4.78 [3.85, 5.94]	2.97 [2.33, 3.75]	10.95 [8.93, 13.47]	2.26 [1.92, 2.67]	2.00 [1.68, 2.37]
Model 2	β_1	4.35 [3.54, 5.34]	4.79 [3.83, 5.91]	2.98 [2.32, 3.76]	10.97 [8.91, 13.47]	2.25 [1.90, 2.67]	1.99 [1.68, 2.38]
	β_2	2.3e-4 [0.0, 0.002]	1.8e-4 [0.0, 0.002]	1.3e04 [0.0, 0.001]	1.3e-3 [0.0, 0.01]	4.8e-5 [0.0, 5.1e-5]	2.8e-5 [0.0, 2.6e-4]
Model 3	β_1	4.32 [3.54, 5.33]	4.78 [3.86, 5.92]	2.98 [2.32, 3.81]	10.96 [8.80, 13.61]	2.26 [1.90, 2.68]	1.99 [1.67, 2.34]
	β_2	2.1e-4 [0.0, 0.002]	8.4e-4 [0.0, 0.01]	5.5e-4 [0.0, 0.05]	3.5e-3 [0.0, 0.023]	8.7e-4 [0.0, 8.8e-4]	6.0e-4 [0.0, 0.01]
Model 4	β_1	4.22 [3.39, 5.19]	4.56 [3.68, 5.66]	2.95 [2.31, 3.79]	10.78 [8.81, 13.35]	2.12 [1.73, 2.57]	1.93 [1.63, 2.30]
	β_2	NA	NA	NA	NA	NA	NA
b) An. arabiensis							
Model 1	β_1	9.97 [7.92, 12.52]	12.98 [9.89, 16.77]	12.25 [9.43, 16.13]	13.83 [9.88, 19.31]	6.65 [5.39, 8.26]	7.41 [6.25, 8.88]
Model 2	β_1	10.01 [7.90, 12.59]	12.97 [9.97, 17.06]	12.27 [9.27, 16.12]	13.76 [10.05, 19.03]	6.67 [5.42, 8.19]	7.41 [6.23, 8.79]
	β_2	0.004 [0.0, 0.04]	2.5e-4 [0.0, 0.002]	6.2e-4 [0.00, 0.007]	0.004 [0.0, 0.04]	8.2e-4 [0.0, 0.006]	3.2e-4 [0.0, 0.003]
Model 3	β_1	9.98 [7.89, 12.45]	12.98 [9.93, 17.14]	11.59 [7.70, 15.85]	13.73 [9.59, 19.03]	6.66 [5.34, 8.19]	7.39 [6.02, 8.83]
	β_2	2.4e-4 [0.0, 0.002]	3.7e-4 [0.0, 0.003]	0.02 [0.00, 0.14]	4.3e-3 [0.0, 0.04]	1.41e-4 [0.0, 0.001]	2.8e-4 [0.0, 0.03]
Model 4	β_1	9.97 [7.81, 12.49]	10.96 [7.48, 15.35]	11.47 [7.65, 15.79]	11.07 [7.09, 16.58]	5.97 [3.91, 7.96]	7.30 [6.04, 8.82]
	β_2	NA	NA	NA	NA	NA	NA
c) An. funestus							
Model 1	β_1	4.83 [2.44, 9.36]	2.23 [1.56, 2.99]	1.66 [1.04, 2.59]	2.73 [1.28, 5.45]	2.20 [1.54, 3.19]	1.99 [1.21, 3.08]
Model 2	β_1	4.77 [2.38, 9.01]	2.23 [1.57, 2.99]	1.62 [0.99, 2.56]	2.72 [1.25, 5.57]	2.21 [1.52, 3.14]	1.99 [1.25, 3.13]
	β_2	0.007 [0.0, 0.08]	0.009 [0.0, 0.09]	0.03 [0.0, 0.19]	0.006 [0.0, 0.06]	0.003 [0.0, 0.03]	0.003 [0.0, 0.03]
Model 3	β_1	4.85 [2.40, 9.16]	2.23 [1.59, 3.02]	1.64 [1.03, 2.52]	2.71 [1.25, 5.46]	2.19 [1.50, 3.14]	1.97 [1.25, 3.10]
	β_2	2.1e-4 [0.0, 0.002]	1.3e-4 [0.0, 0.001]	1.6e-4 [0.0, 0.002]	6.2e-4 [0.0, 0.01]	1.3e-4 [0.0, 0.001]	6.4e-5 [0.0, 8.5e-4]
Model 4	β_1	4.27 [1.43, 9.46]	2.01 [1.17, 2.90]	0.94 [0.69, 1.22]	0.11 [0.0, 0.79]	2.15 [1.42, 3.22]	1.59 [0.001, 2.96]
	β_2	NA	NA	NA	NA	NA	NA

*NA = Individual interaction between species of interest (i.e. *An. funestus, An. arabiensis* and *Culex* spp.) and each of 8 females species captured during the study period. This generates 8 different values of β_2 for the most complex model (model 4)

Table 7.3: Summary (DIC and RMSE values) of models used to investigate the relationship between the numbers of female mosquitoes collected with human landing catch (HLC) and the six alternative outdoor traps. See Table 6.1 for description and models formulation.

Species and Model	SUN			BGS			ITT-C			MMX			MTRC			MTR		
	DIC	ΔDIC	RMSE	DIC	ΔDIC	RMSE	DIC	ΔDIC	RMSE	DIC	ΔDIC	RMSE	DIC	ΔDIC	RMSE	DIC	ΔDIC	RMSE
a) *Culex*																		
Model 1	1437.9	0.12	129.2	1490.2	1.65	150.4	1099.5	0.04	203.3	1167.9	0.00	120.6	1699.4	0.10	107.7	1612.9	2.22	81.6
Model 2	1438.2	0.44	130.3	1490.6	2.03	150.8	1099.6	0.22	207.6	1168.2	0.29	122.1	1699.3	0.03	109.6	1612.9	2.29	86.4
Model 3	1437.7	0.00	129.4	1490.2	1.61	151.4	1099.5	0.08	202.6	1168.4	0.51	122.5	1699.3	0.00	108.0	1612.7	1.99	84.7
Model 4	1437.9	0.18	138.6	1488.6	0.00	177.1	1099.4	0.00	205.9	1168.7	0.79	121.7	1699.5	0.20	117.7	1610.7	0.00	86.5
b)*An. arabiensis*																		
Model 1	1009.1	0.00	174.0	767.8	0.00	153.9	757.6	0.00	158.1	544.7	0.67	178.7	1415.3	0.00	140.1	1437.6	0.09	99.6
Model 2	1100.1	1.06	177.1	768.0	0.22	155.7	757.9	0.25	159.4	544.4	0.38	178.2	1415.7	0.37	140.2	1437.6	0.14	100.6
Model 3	1099.6	0.54	177.0	768.1	0.28	155.3	757.8	0.22	153.8	544.8	0.77	180.8	1415.6	0.31	141.3	1437.5	0.00	100.1
Model 4	1101.4	2.35	184.1	768.1	0.33	156.3	758.1	0.48	150.8	543.9	0.00	275.9	1417.4	2.11	154.4	1438.3	0.86	101.2
c) *An. funestus*																		
Model 1	90.1	0.00	9.41	61.6	0.21	3.83	118.3	11.9	2.41	44.6	0.00	4.15	155.0	0.00	7.63	132.7	0.27	4.47
Model 2	90.5	0.39	10.25	61.8	0.38	3.85	119.3	12.8	2.43	44.6	0.01	4.22	155.1	0.08	7.69	132.6	0.18	4.61
Model 3	90.4	0.24	10.67	61.4	0.00	3.83	118.4	11.9	2.41	45.9	1.29	4.14	155.3	0.33	7.65	132.5	0.00	4.58
Model 4	91.8	1.69	10.86	62.7	1.26	2.87	106.5	0.00	2.41	56.6	12.0	1.33	156.9	1.86	12.9	134.6	2.18	11.21

SUN: Suna trap, BGS: BG-Sentinel trap, ITT-C: Ifakara Tent Trap version C, MMX: Mosquito Magnet trap, MTRC: M-trap combined with CDC Light, MTR: M-trap;

Table 7.4: Predicted values for estimating the expected mosquito catches by human landing catch and alternative traps, according to the linear model (Model 1). Numbers in the first column refer to the mosquitoes collected with a given trap. To obtain the estimate of the equivalent number that one would collect with HLC, refer to the column corresponding to the trap itself. Numbers in brackets are (95% credible intervals).

		Expected HLC					
	Collected	SUN	BGS	ITT-C	MMX	MTRC	MTR
a) *Culex* spp.	10	43 (35-54)	48 (39-59)	30 (23-37)	110 (89-134)	23 (19-27)	20 (17-24)
	20	87 (71-107)	96 (77-119)	59 (47-75)	219 (179-269)	45 (38-53)	40 (34-47)
	30	130 (106-161)	143 (116-178)	89 (70-112)	329 (268-403)	68 (57-80)	60 (51-71)
	40	174 (142-215)	191 (154-238)	119 (93-150)	438 (357-538)	90 (77-107)	80 (67-95)
	50	217 (177-269)	239 (193-297)	149 (116-187)	548 (446-672)	113 (96-134)	100 (84-119)
	60	260 (213-322)	287 (231-356)	178 (140-225)	657 (536-806)	136 (115-160)	120 (101-142)
	70	304 (248-376)	335 (270-416)	208 (163-262)	767 (625-941)	158 (134-187)	140 (118-166)
	80	347 (284-430)	382 (308-475)	238 (186-300)	876 (714-1075)	181 (153-214)	160 (135-190)
	90	391 (319-484)	430 (347-535)	267 (210-337)	986 (804-1209)	204 (172-241)	180 (152-213)
	100	434 (354-537)	478 (385-594)	297 (233-375)	1095 (893-1344)	226 (192-267)	200 (168-237)
b) *An. arabiensis*	10	100 (79-125)	130 (99-168)	122 (94-161)	138 (99-193)	67 (54-83)	74 (62-89)
	20	199 (158-250)	260 (198-335)	245 (189-323)	277 (198-386)	133 (108-165)	148 (125-178)
	30	299 (237-376)	390 (297-503)	367 (283-484)	415 (296-579)	200 (162-248)	222 (187-266)
	40	399 (317-501)	519 (396-671)	490 (377-645)	553 (395-773)	266 (215-331)	297 (250-355)
	50	498 (396-626)	649 (495-839)	612 (471-807)	691 (494-966)	333 (269-413)	371 (312-444)
	60	598 (475-751)	779 (594-1006)	735 (566-968)	830 (593-1159)	399 (323-496)	445 (375-533)

	70	698 (554-877)	909 (693-1174)	857 (660-1129)	968 (692-1352)	466 (377-579)	519 (437-621)
	80	798 (633-1002)	1039 (792-1342)	980 (754-1291)	1106 (791-1545)	532 (431-661)	593 (500-710)
	90	897 (712-1127)	1169 (891-1510)	1102 (848-1452)	1244 (889-1738)	599 (485-744)	667 (562-799)
	100	997 (792-1252)	1298 (990-1677)	1225 (943-1613)	1383 (988-1931)	665 (539-826)	741 (625-888)
c) *An. funestus*	10	48 (24-94)	22 (16-30)	17 (10-26)	27 (13-54)	22 (15-32)	20 (12-31)
	20	97 (49-187)	45 (31-60)	33 (21-52)	55 (26-109)	44 (31-64)	40 (24-62)
	30	145 (73-281)	67 (47-90)	50 (31-78)	82 (38-163)	66 (46-96)	60 (36-93)
	40	193 (98-374)	89 (62-120)	66 (41-104)	109 (51-218)	88 (62-128)	79 (49-123)
	50	241 (122-468)	112 (78-150)	83 (52-130)	136 (64-272)	110 (77-160)	99 (61-154)
	60	290 (146-561)	134 (94-180)	100 (62-156)	164 (77-327)	132 (93-191)	119 (73-185)
	70	338 (171-655)	156 (109-210)	116 (73-182)	191 (90-381)	154 (108-223)	139 (85-216)
	80	386 (195-748)	178 (125-240)	133 (83-208)	218 (103-436)	176 (123-255)	159 (97-247)
	90	434 (220-842)	201 (140-269)	149 (93-234)	246 (115-490)	198 (139-287)	179 (109-278)
	100	483 (244-936)	223 (156-299)	166 (104-260)	273 (128-545)	220 (154-319)	199 (121-308)

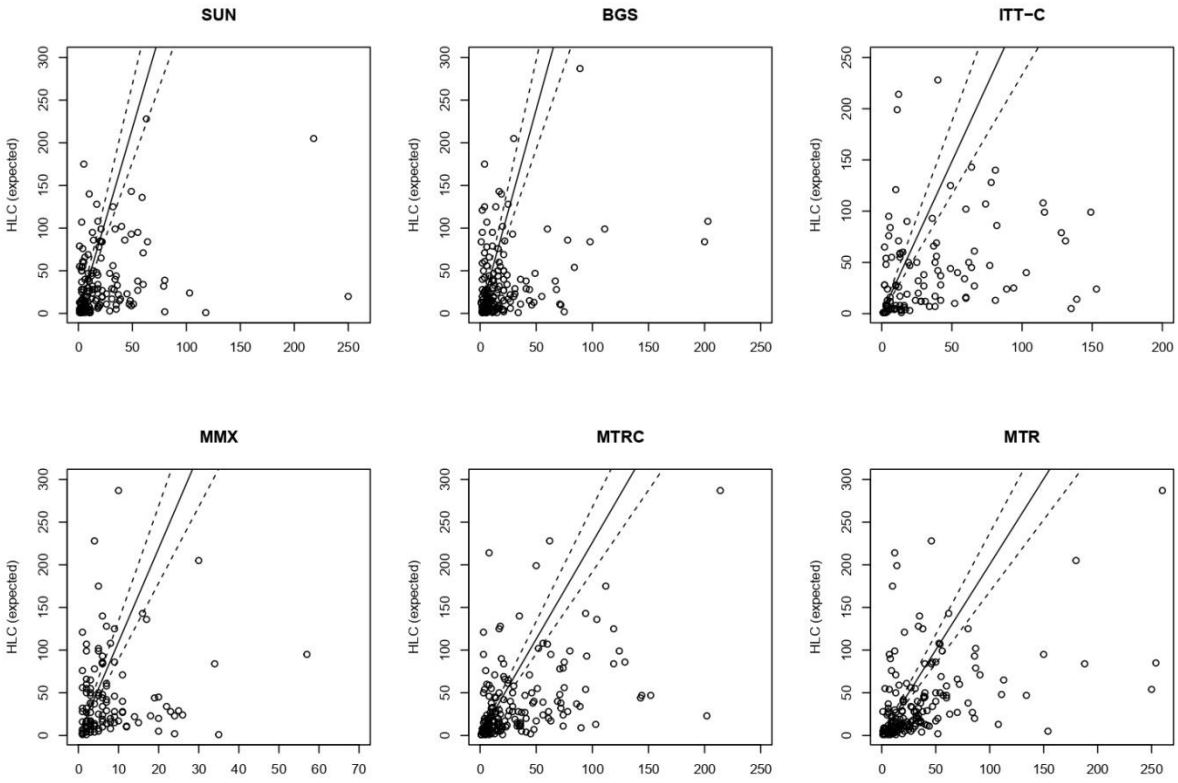


Figure 7.1: Expected number of female *Culex* spp. mosquitoes collected with HLC (y-axis), given the number of females collected with alternative traps (x-axis). Continuous line is the prediction of a normal distribution model assuming a linear relationship; dashed lines are 95% credible intervals and black circles are the actual observed values.

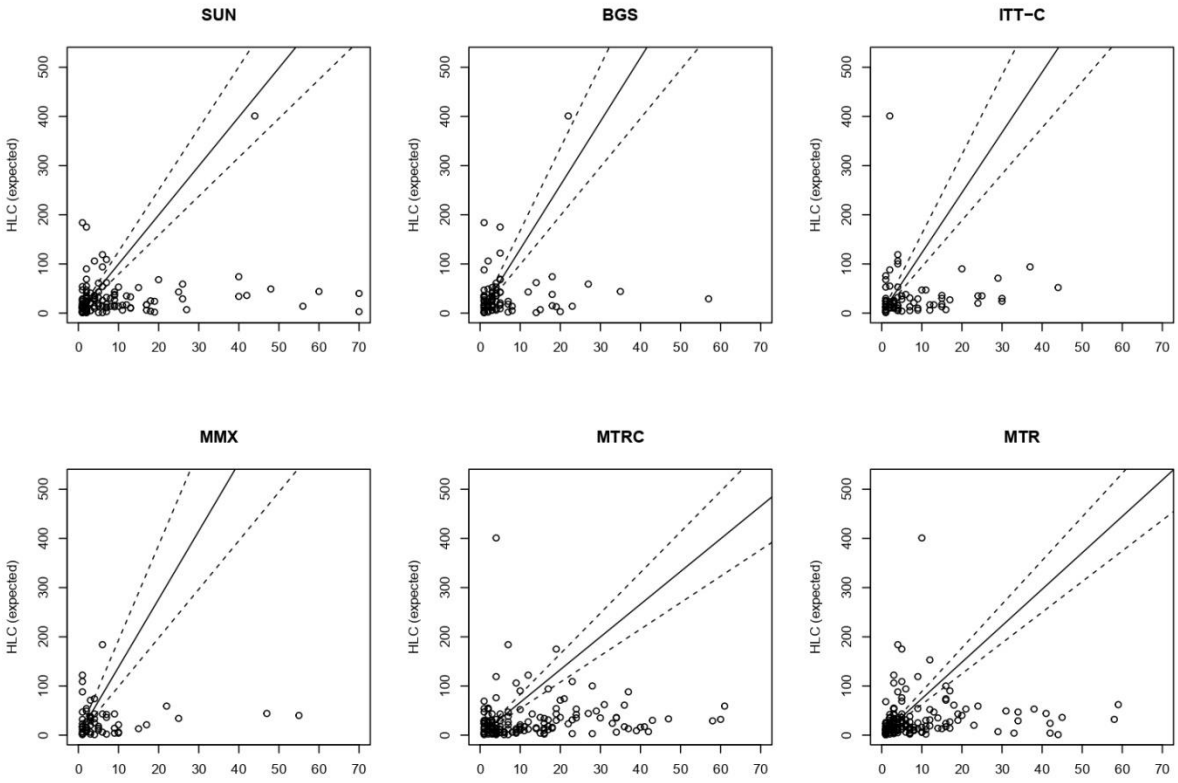


Figure 7.2: Expected number of female *Anopheles arabiensis* mosquitoes collected with HLC (y-axis), given the number of females collected with alternative traps (x-axis). Continuous line is the prediction of a normal distribution model assuming a linear relationship; dashed lines are 95% credible intervals and black circles are the actual observed values.

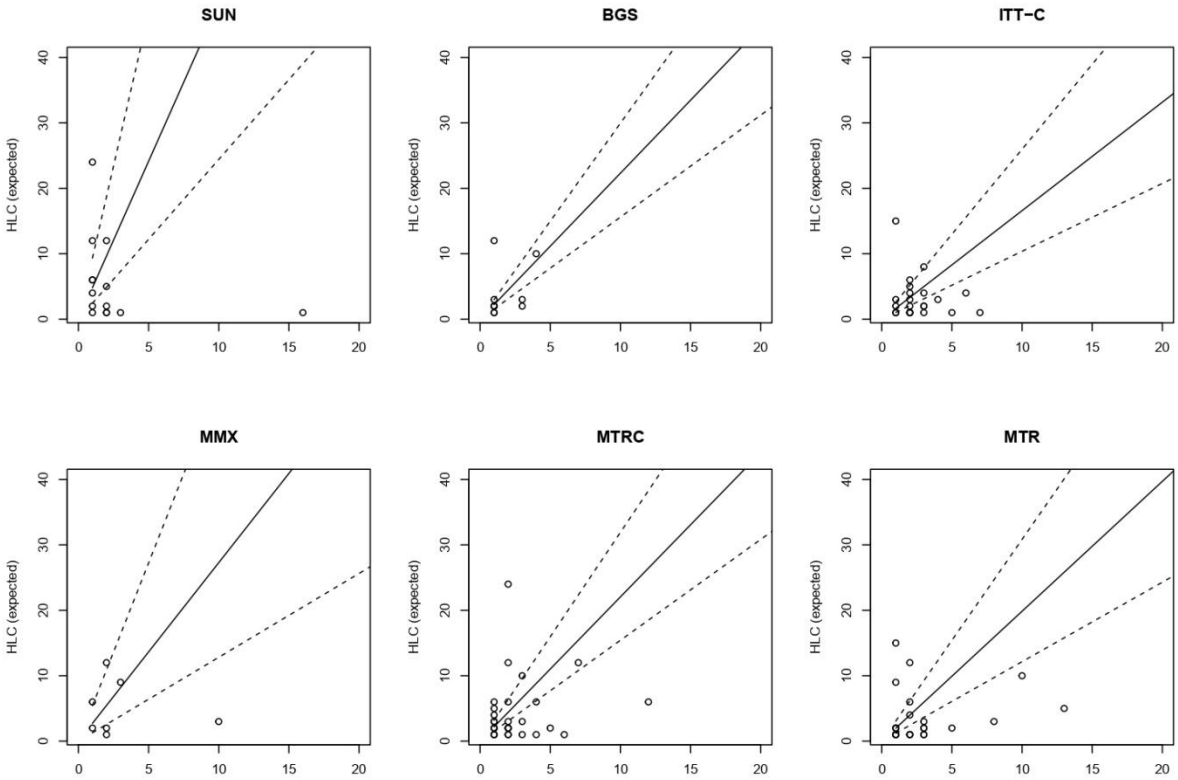


Figure 7.3: Expected number of female *Anopheles funestus* mosquitoes collected with HLC (y-axis), given the number of females collected with alternative traps (x-axis). Continuous line is the prediction of a normal distribution model assuming a linear relationship; dashed lines are 95% credible intervals and black circles are the actual observed values.

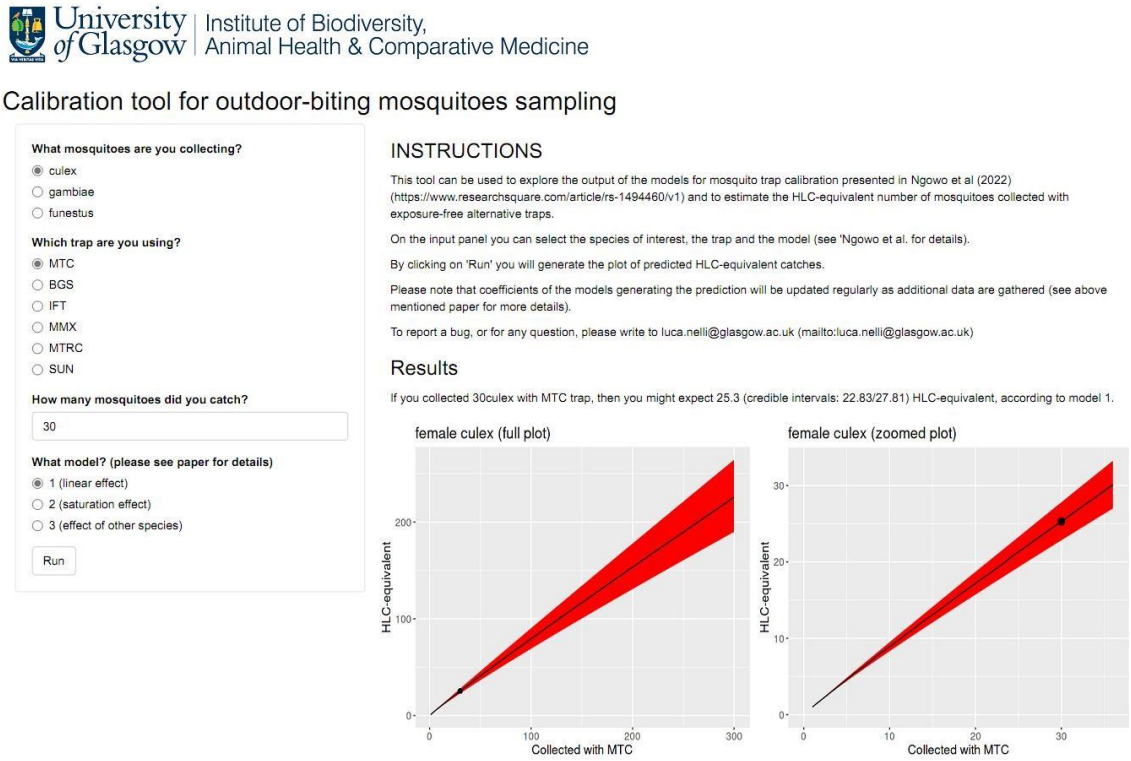
7.5 Conclusion

After reanalyzing the data, it became clear that there were no substantial differences between the two model fitting techniques within the catch range values. The current approach remains valid, as it was primary implemented to address errors in the previous one. It was not possible to identify a single model which performs better for all the species and all the alternative traps. As a result, I selected the most parsimonious model (i.e. model 1) which is the simple linear model. All figures and tables were created based on the simple linear model. Overall, I believe that the revised version of the work represent a significant improvement over the original chapter 3.

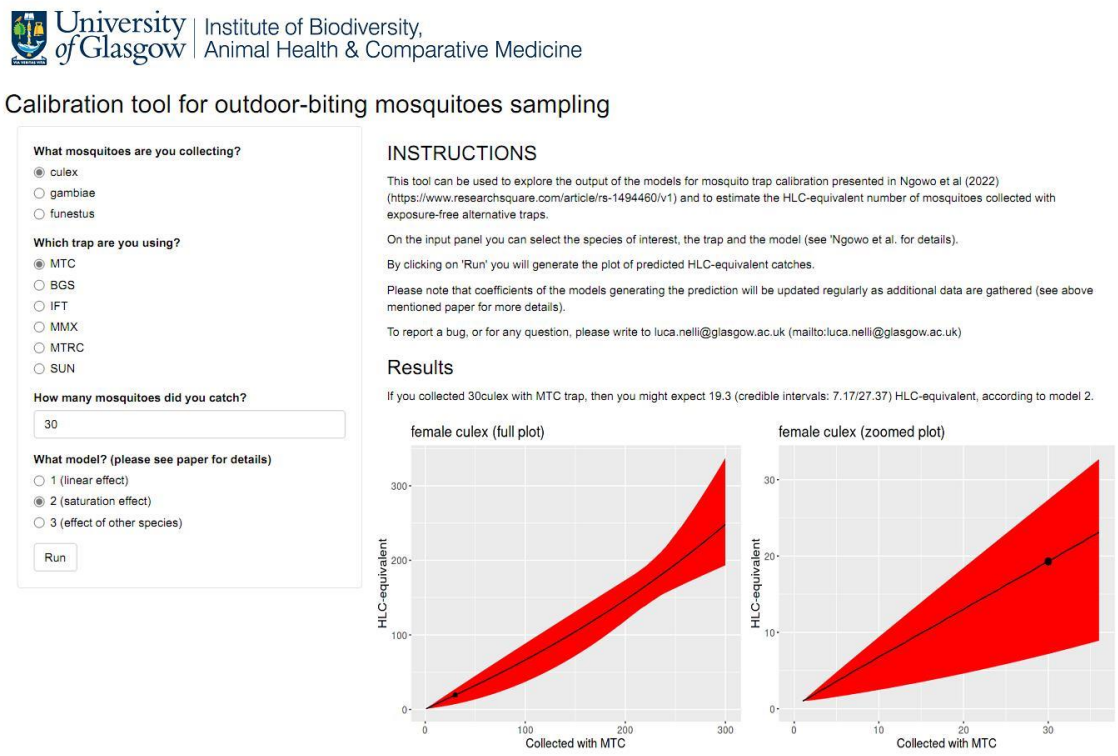
8.0 Appendices

8.1 Appendix 1: Shiny App Interfaces

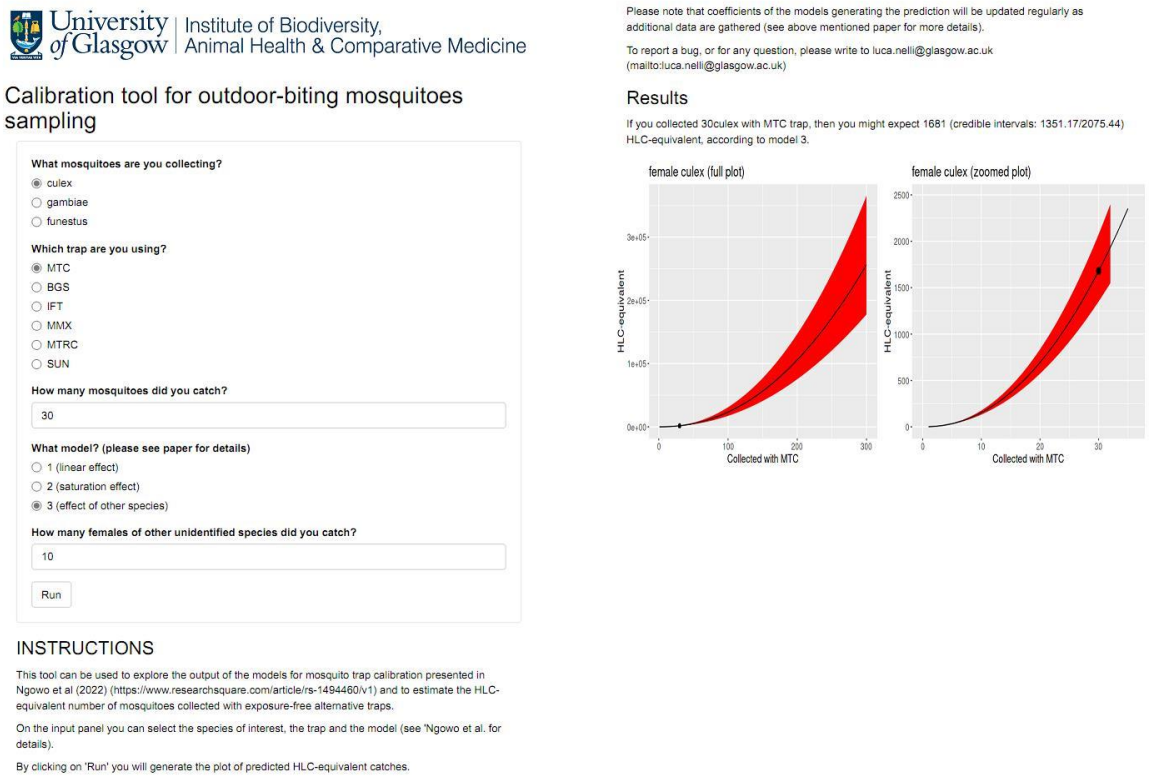
8.1.1 Shiny App dashboard showing one example of a linear relationship (model 1) between HLC and an alternative trap (i.e., MTC) for *Culex* spp



8.1.2 Shiny App dashboard showing one example of a saturation effect (model 2) between HLC and an alternative trap (i.e., MTC) for *Culex* spp



8.1.3 Shiny App dashboard showing one example of a model which account for other species (model 3) between HLC and an alternative trap (i.e., MTC) for *Culex* spp



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