Bioinformatics Systems And Mathematical Models For Improved Understanding Of Malaria Transmission, Control, And Elimination

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BIOINFORMATICS SYSTEMS AND MATHEMATICAL MODELS FOR IMPROVED UNDERSTANDING OF MALARIA TRANSMISSION, CONTROL, AND ELIMINATION

by

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ABSTRACT

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The leading malaria vector control strategies (i.e., long-lasting insecticidal nets and indoor residual spraying) can reduce indoor transmission, but these tools alone are insufficient to eliminate it. Strategies that target adult mosquitoes when they feed on humans or animals outdoors or target mosquito immature stages are also needed to achieve malaria elimination. Improved data systems for integrating diverse experimental observations and research groups, as well as process-explicit mathematical models for evaluating them are both essential to achieving these goals.

We have developed a generic schema and data repositories for the studies of malaria vectors that encompass a wide variety of different experimental designs that rapidly generate large data volumes. We extended a malaria transmission model to examine the relationship between transmission, control, and the proportion of blood meals a vector population obtains from humans: Assuming the lower limit for this indicator of human feeding preference enabled derivation of simplified models for zoophagic vectors. We present differential equation models to describe the biological processes that mediate novel strategies to control malaria vectors by autodissemination of pyripoxyfen (PPF) as it is transferred from treated stations to the gravid mosquitoes and then to the aquatic habitats where it inhibits mosquito emergence.

Data from most of the mosquito studies we reviewed conformed to our generic schema with four tables recording the experimental design, sorting of collections, details of samples, and additional observations. Our corresponding online repository includes 20 experiments, 8 projects, and 15 users at two institutes, resulting in 10 peer-reviewed publications. For zoophagic vectors, the results from model can be used to forecast the likely immediate and delayed impacts of an intervention using only three field-measurable parameters. For the autodissemination of PPF, sensitivity analysis indicates success of the strategy is plausible because the $\geq 80\%$ coverage of aquatic habitats with PPF appears achievable with modest, biologically plausible values of field-measurable input parameters.

Therefore, we have applied two of the computational sciences aspects (i.e., research data preparation using computer systems and scenario analysis with mathematical models) to address obstacles to the control and elimination of malaria.

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DEDICATION

This dissertation work is dedicated to my wonderful parents (Mr. Sifael Kiware and Mrs. Eliaita Kiware) and to my own family, beautiful wife (Ms. Doreen) and daughters (Gabby and Georgia) - I'm so thankful for the support and encouragement throughout my Ph.D. program from my parents and my Dee.

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CHAPTER 1

Thesis Introduction

This chapter introduces the context of this thesis and the problem being addressed.

1.1 Statement of the Problem

Malaria is an infectious blood disease caused by a parasite that is transmitted from one human to another by a bite of an infected *Anopheles* female mosquito. A study published by the medical journal *Lancet* suggests that 1.24 million people died from malaria in 2010 [6]. Mosquitoes that primarily feed upon humans (anthropophagic) have been the overwhelming focus of malaria research and control to date. Field observations [7, 8, 9, 10, 11] and models simulations [1, 2, 12, 13, 3] both show that a high coverage of personal protection measures such as indoor residual spraying (IRS) and long-lasting insecticidal nets (LLIN) dramatically reduce malaria transmission where vector populations depend upon human blood. However, most primary malaria vectors outside of sub-Saharan Africa can be classified as zoophagic, meaning they feed occasionally ($< 10\%$ of blood-meals) upon

humans [14, 15], so personal protection measures have negligible impact upon their survival [7, 11, 16]. How can we better understand malaria transmission and control specifically for zoophagic vectors and outdoor biting mosquitoes? We propose three malaria-based research aims;

1. Bioinformatic systems for improved understanding of malaria transmission and control;
2. Mathematical models for vector control impact upon malaria transmission by zoophagic mosquitoes; and
3. Mathematical models for the autodissemination of insecticides by mosquitoes.

These aims all support the efforts to eliminate malaria transmission with a focus on zoophagic vectors. The bioinformatics systems not only can be used to provide data to parameterize both sets of our models, but it also is an important contribution to the wider malaria research community. Both sets of models proposed for this work are based on the mosquito life cycle but with different applications. This chapter summarizes problems, current status, methods of each, which be presented in much more detail in self-contained chapters. We consider each of the aims in turn.

1.1.1 The Bioinformatics Systems for Improved Understanding of Malaria Transmission and Control

Innovative control strategies that target the entire mosquito life cycle, rather than only when mosquitoes are host-seeking inside of houses, may be required to achieve malaria elimination. Researchers need to analyze huge quantities of ecological data collected from multiple experiments to understand malaria transmission for the development of control strategies. However, while standardised schemas, databases, and even public data repositories exist for epidemiological and genetic data for malaria parasites, humans, and mosquito hosts [17, 18], systems for ecological studies of the mosquitoes which mediate transmission are only now starting to emerge.

1.1.2 Mathematical Models of Vector Control Impact upon Malaria Transmission by Zoophagic Mosquitoes

A malaria transmission model to examine the relationship between transmission, control, and the baseline proportion of blood-meals obtained from humans (human blood index) specifically for zoophagic vectors does not exist. Can we develop simple models that control practitioners may use to predict the immediate and delayed impacts of an intervention upon transmission using field-measurable parameters? Can we use the models to illustrate whether personal protection measures confer community-level protection against zoophagic vectors as

they do against anthropophagic vectors? Can the model suggest coverage and efficacy thresholds required by a personal protection measure to attain epidemiological impact? In addition, can we present biologically meaningful indicators for eliminating malaria transmission?

1.1.3 Mathematical Models for the Autodissemination of Insecticides by Mosquitoes

As an effort to develop innovative control strategies that complement long-lasting insecticidal nets (LLINs) and indoor residual spraying, semi-field system (SFS) experiments were conducted to evaluate the potential for the autodissemination of Pyriproxyfen (PPF) (i.e., a juvenile hormone analogue (JHA) that interrupts normal development and metamorphosis of targeted mosquitoes) from resting sites to the aquatic habitat by *Anopheles* adult mosquitoes into their breeding sites and its impact on adult mosquito emergence. These SFS experiments indicate a potential for this novel strategy; however, no mathematical models are yet developed to predict the scenarios in which the autodissemination of insecticide strategy may be a success in the actual field conditions.

This work is aimed at contributing to ongoing research efforts for malaria elimination. Indirectly, this work may help save millions of lives from malaria.

1.2 Status of the Problem

We present the current status of each of the research aims presented in section 1.1.

1.2.1 Bioinformatics Systems for Improved Understanding of Malaria Transmission and Control

White et al. [19] describe that data scarcity related to transmission is among the key issues to consider for malaria elimination strategies. Also, the improvements in the prediction accuracy of models of malaria transmission and control depend on reliable data on transmission [20]. A brief literature review shows that various bioinformatics and ecoinformatics systems exist [21, 22, 23, 24]. Although they are not those of mosquito biology, some of the ideas presented might be important to the work proposed here. Moreover, data repositories stored in standardized schema which are publicly accessible via the web exist for genetic data for malaria parasites as well as their human and mosquito hosts, e.g., GenBank [17] and VectorBase [18], but none exist for mosquitoes which mediate transmission.

1.2.2 Mathematical Models of Vector Control Impact upon Malaria Transmission by Zoophagic Mosquitoes

Indoor residual spraying (IRS) and long-lasting insecticidal nets (LLIN) dramatically reduce malaria transmission [25]. Both approaches extend the benefits of personal protection by also providing levels of community-wide protection for users and non-users alike once reasonably high coverage is achieved (30%-60%) [26, 27]. High demographic coverage of humans can dramatically reduce the density, longevity, and infection prevalence of mosquito species that primarily feed indoors (endophagic) upon humans (anthropophagic) such as *Anopheles gambiae* and *An. funestus* from sub-Saharan Africa [7, 11] or *An. punctulatus* and *An. koliensis* from the Pacific [28]. The importance of community-level transmission suppression for realizing the full potential of both IRS [29] and LLINs [27] using contact insecticides is well established and reflected in global universal coverage targets for these interventions [30]. Consistent with field observations and previous model simulations [7, 8, 1, 9, 2, 12, 10, 11, 13, 3], high coverage with an insecticidal personal protection intervention is predicted to have a huge immediate impact on malaria transmission where mosquitoes primarily feed indoors upon humans. However, mosquitoes which feed upon animals (zoophagic) are the primary malaria vectors in many tropical countries [14, 15], increasingly will dominate transmission in the future [10, 31], and can dominate residual

transmission in settings where high demographic coverage of LLIN or IRS has successfully suppressed previously predominant anthropophagic species [7, 10, 11].

1.2.3 Mathematical Models for the Autodissemination of Insecticides by Mosquitoes

The idea of using adult mosquitoes to transfer insecticides to their breeding site has been studied before. Devine et al. [32] present studies on the dengue vector which show that the natural behaviours of *A. aegypti* can be exploited to transfer larvicides between their resting sites and aquatic habitats. Several mathematical models have previously described the mosquito feeding cycle [1, 2, 12, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42], or the full mosquito cycle including eggs, larvae, and adults [43, 44, 45, 4] to present the impact of various interventions on malaria transmission, but none of them has incorporated the autodissemination of insecticide technique for malaria vectors. A previous model formulation [46] crudely described the relationship between the effective coverage of adult resting sites (C_r) and larval habitats (C_h) with PPF using a simple exponential function of the time over which contaminated habitats persist but remain unproductive (U), the number of ovipositions (O) by the adult population, the number of larval habitats (H), and the number of contaminating events needed to make a single habitat unproductive (Ω). However, in the actual sense it is

impossible to estimate the reasonable values for each of these parameters, C_r, H, O, and Ω are not measurable in the field, and because of nature, U remains to be an unknown parameter in the model.

Fortunately, our semi-field (SFS) experiments performed using female *Anopheles arabiensis* demonstrated a potential for this strategy using malaria vectors. We however recommended that further studies are required to demonstrate its effectiveness in the actual field as well before its full potential can be accessed. In addition, the mean time that artificial habitats remain unproductive following manual contamination with PPF has been evaluated at several doses in the SFS. Moreover, an innovation of [47] is a new scalable method for surveying oviposition contacts of mosquitoes with aquatic larval habitats by trapping them on glue-covered plastic sheets. This method will help estimate the number of contaminated ovipositing mosquitoes. These experiments may be used to estimate some of the input parameters for the autodisemination of insecticide model proposed in this work. They also provide improved understanding of the autodissemination of insecticide processes.

1.3 Statement of Materials

The research environment includes, but is not limited to, a networked Dell Windows 7 personal laptop computer with Office 2010, WinEdit 6, LaTeX, Berkeley

Madonna, MySQL Server 5.1, SQLyog Ultimate, Pendragon 5.0, MATLAB R2012a, and previously published mosquito data for model parameterizations. Other data will be obtained from Ifakara Health Institute (IHI) in Tanzania. IHI is one of Africa's most eminent health research organizations, an independent, non-profit organisation, registered in Tanzania and led by Tanzanians [48]. IHI conducts a wide range of health-related research, including biomedical and environmental studies, trials of drugs, vaccines and diagnostics, health-systems research, and monitoring and evaluation [48]. The Marquette library, GasDay lab space and computers, IHI facilities and space are used to conduct the research. The web space provided to graduate students by the MSCS is used to host our bioinformatic systems during the development and testing phases; eventually the system will be hosted by IHI.

This research involves only secondary data (no human subject is involved) collected via two IHI-based projects; Autodissemination of Insecticides by Adult Mosquitoes (ATD) and Malaria Transmission Consortium (MTC), both funded by the Bill & Melinda Gates Foundation. The IHI's Institutional Review Board performs critical oversight functions for research conducted on human subjects that are scientific, ethical, and regulatory. The study approval for ATD was granted by the Ifakara Health Institute Institutional Review Board (IHRDC/IRB/No.A-32) and the National Institute of Medical Research (NIMR/HQ/R.8a/Vol. IX/764).

IHI has several facilities including but not limited to office space, several field

sites, and semi-field systems which have attracted several local and international collaborations allowing research scientists from around the world to bring their grants to IHI and use it as their research platform. The information regarding ownership, availability, and sharing of the data at IHI can be found via

`http://ihidata.org/`

1.4 Statement of Methods

This work will involve three research aims: the bioinformatics systems for improved understanding of malaria transmission and control, mathematical models for vector control impact upon malaria transmission by zoophagic mosquitoes, and mathematical models for the autodissemination of insecticides by mosquitoes. More details on methods are provided in each of the following self-contained chapters. We will summarize the methods of each in turn.

1.4.1 Bioinformatics System for Improved Understanding of Malaria Transmission and Control

We have presented a bioinformatics system for improved understanding of malaria transmission and control. This research topic is in two parts 1) a presentation of generic schema and data collection forms that are applicable to diverse entomological experiment and surveys and 2) a database web-based application that

can be used by entomologists to link, store, share, and generate reports for malaria transmitting mosquitoes independent of a given option for data collection forms. We present this research topic in self-contained Chapters 2 and 3, and we summarize their methods here.

Generic schema and standardized data collection forms that are applicable to diverse entomological experiments and surveys

Although research in mosquito biology involves an unlimited number of possible experimental procedures, we have presented the same fundamental structure used by most mosquito entomology-based experiments [49] to develop a generic schema. All mosquito survey and experimental data could be described by a generic schema with only four tables that record: 1) Experimental design (where, when and how each group of mosquitoes was collected with a single capture), 2) Sorted content of collections (the morphological, physiological, behavioral or survival attributes used to sort them into subgroups), 3) Details of how samples are pooled or subdivided, and, 4) Observations of samples, such as taxonomy, infection, age, size, or genotype. Paper-based data collection forms were designed according to this generic schema and were applied to diversity field and laboratory investigations without any need to redesign forms or databases for each new experiment or survey. Standardized terminologies for various collection variables were set to make sure data collected

across multiple experiments are consistent and comparable [49]. So far, these forms have been implemented in one local and three national-level malaria control programmes in Africa, all of which now have consistent, comparable data sets.

Data recorded on these forms can be entered and linked with any relational database software. Proposed data collection forms and associated documents are freely available via `http://www.mscs.mu.edu/~skiware/SOM/som.html` or `iebs.ihi.or.tz/som.html`.

IEBS: A generic approach to link, store, share, and generate reports based on entomological field and lab data retrieved from data collection devices and forms

An application-specific schema was developed based on experimental procedures commonly used by mosquito entomologists; the design of the experiment, followed by sample sorting, observation, constitution, and archiving. Using that application-specific schema, we have designed customizable .csv data entry templates that allow quality-controlled upload into a MySQL relational database with a web-based application, a secured application developed using PHP known as Ifakara Entomology Bioinformatics System (IEBS) [50]. Currently, mosquito samples are traced from the point of collection through laboratory and storage facilities using corresponding unique identifiers as recorded in the data collection

forms. An improvement is made so that a short unique code attached to and read from the sample container on pre-printed bar-coded sticker generated using a system that also monitors the code generated is used instead of a sample label. Linked data requested for various analyses from the system can be provided as a .csv file that can be imported to any database or analytical software for analysis. Local and global data sharing is made possible once a user is granted access by a project investigator. The IEBS currently hosts data from multiple projects and experiments, including several mosquito information (e.g., where, when, and how they were collected and their sorted information) recorded on user-customized paper forms and sent from mobile phones or PDA designed to adhere to the generic schema. The system can upload data collected using any natural language but adhering to standardized terminologies. The proposed data upload templates can be accessed via: `http://www.mscs.mu.edu/~skiware/SOM/som.html` or `iebs.ihi.or.tz/som.html`. IEBS can be accessed via `http://www.mscs.mu.edu/~skiware/IEBS` or `iebs.ihi.or.tz/` - access credentials are available from the author upon request.

1.4.2 Mathematical Models of Vector Control Impact Upon Malaria Transmission by Zoophagic Mosquitoes

This research aim is presented in self-standing chapters 4 and 5. The methods presented in detail there are summarized here.

Simplified Models of Vector Control Impact Upon Malaria Transmission by Zoophagic Mosquitoes

We have extended a published malaria transmission model [2] to explore the dependence of malaria transmission and control upon the proportion of all blood meals obtained from humans before any intervention is introduced (baseline human blood index). Specifically, the impact of personal protection measures upon the baseline malaria transmission intensity was compared in a range of vector behavior scenarios. The full possible range of host preference for mosquitoes was simulated by modifying field estimates for cattle and human encounter rates. The human blood index was expressed in terms of a human encounter rate assumed to approach zero, which biologically means a situation where mosquitoes are not attracted to human blood so the attractiveness or availability of human blood is close to zero. Then, we expressed malaria transmission and control as a simplified function of baseline human blood index by taking the limit as human blood index approaches zero (a case for zoophagic vectors). At the end, the model results were used to explain how immediate and delayed impacts of personal protection measures can be predicted using potentially field measurable parameters. In addition, the model may be used to assess the likely extent and mechanism of the community-level impact of such personal protection measures upon human malaria exposure for the zoophagic vectors and to suggest coverage and efficacy thresholds required for a protection

measure to attain epidemiological impact. In summary, we have presented simplified models for vector control impact upon malaria transmission by zoophagic mosquitoes [4] by producing a model that examine the relationship between transmission, control, and the baseline proportion of blood-meals obtained from humans by zoophagic vectors.

Biologically meaningful coverage indicators for eliminating malaria transmission

Mosquitoes which evade contact with long-lasting insecticidal nets and indoor residual sprays by feeding outdoors or upon animals are primary malaria vectors in many tropical countries. They also can dominate residual transmission where high coverage of these front-line vector control measures is achieved. Complementary strategies, which extend insecticide coverage beyond houses and humans, are required to eliminate malaria transmission in most settings. The overwhelming diversity of the world's malaria transmission systems, and optimal strategies for controlling them, can be conceptualized and mapped across two-dimensional scenario space defined by the proportion of blood meals that vectors obtain from humans and the proportion of human exposure to them which occurs indoors.

1.4.3 Mathematical Models for the Autodissemination of Insecticides by Mosquitoes

A previous model formulation [46] crudely described the relationship between

the effective coverage of adult resting sites (C_r) and larval habitats (C_h) with PPF using a simple exponential function of the time over which contaminated habitats persist but remain unproductive (U), the number of ovipositions (O) by the adult population, the number of larval habitats (H), and the number of contaminating events needed to make a single habitat unproductive (Ω),

$$C_h = 1 - e^{-\frac{C_r U O}{\Omega H}}. \tag{1.1}$$

Here, we revise and reformulate the previously published model [46] and adapt some formulations from a recently submitted model [51] to enable the modelling of a range of alternative approaches to the autodissemination strategy; first from one of several possible resting sites (clay pots, inner walls of houses, cattle shelters) that could act as targets for initial delivery to the adult mosquito population, and then from these gravid mosquitoes to the ultimate target of the aquatic habitat. We make sure that the model parameters are field-measurable. We also perform sensitivity analysis on the model to explore the conditions at which autodissemination of insecticide strategy may be a success in the field. We briefly describe field experiments that will be required to parameterize our models. We discuss this aim in detail in Chapter 6 using a more specific title 'Predicting scenarios of success for the autodissemination of pyriproxyfen by malaria vectors

from their resting sites to aquatic habitats; Description and sensitivity analysis of a field-parameterizable model'.

1.5 Organization of this Thesis

This work is organized in such a way that each of the consecutive chapter is a complete one (i.e., has its own introduction (including background information), methods, results, and conclusions) as published or submitted (or in preparation) for a publication consideration in a peer-reviewed journal. The last chapter presents general conclusions and suggestions for future work.

CHAPTER 2

A Generic Schema and Standardized Data Collection Forms That Are Applicable to Diverse Entomological Studies of Mosquitoes

This chapter [52] will be submitted to *Bioinformatics: Oxford Journals* for publication consideration as an 'original paper'.

Abstract

Motivation: Standardized schemas, databases, and public data repositories are needed for the studies of live malaria vectors that encompass a remarkably diverse array of different designs and rapidly generate large data volumes, often in resource-limited tropical settings lacking specialized software or informatics support.

Results: Data from the majority of mosquito studies conformed to a generic schema with only four tables recording the experimental design, sorting of collections, details of sample pooling or subdivision, and additional observations. Generically applicable forms with standardized attribute definitions enabled rigorous, consistent data and sample management with generic software and minimal expertise. System use now includes 20 experiments, 8 projects, and 15 users at 3 research and control institutes in 3 African countries, resulting in 10 peer-reviewed publications.

Availability and Implementation: Standard operating procedures (Supplementary Online Materials File 2) and generically applicable data collection forms (Supplementary Online Materials File 1) are available at `mscs.mu.edu/~skiware/IEBS/SOM` or `iebs.ihi.or.tz/som/` . Data recorded on these forms can be entered and linked with any relational database software.

2.1 Introduction

To understand the dynamics of vector-borne diseases such as malaria, empirical data is required to develop an in-depth knowledge of relevant ecology, genetics, risk factors, infection rates, and clinical outcomes [53, 54]. The leading vector control strategies (i.e. long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS)) can reduce indoor malaria transmission, but these tools alone are insufficient to eliminate malaria, especially from intensely endemic regions [31]. To achieve malaria elimination, control of indoor transmission with LLINs and IRS must be improved [55, 56, 57, 58] and supplemented with vector control strategies that target adult mosquitoes outdoors or at source in their aquatic habitats [31, 59]. To effectively develop and evaluate interventions for malaria, especially new ones designed to exploit the ecology of target species, a holistic and multidisciplinary approach is necessary, with multiple researchers collaborating, sharing, and synthesising data across multiple studies and laboratories.

Standardised schemas, ontologies and databases have been used across many scientific fields from genetics to epidemiology and, more recently, ecology to improve scientific output. If well-structured, user-friendly, consistently applicable informatics tools are adopted early in the research process, individual researchers and the broader research community accumulate increased benefits over the long term, including reduced time from data collection to dissemination, facilitation of data sharing, streamlining of multisite collaborations, and enhanced retrospective analysis [21, 23, 24, 60, 61, 62]. Standardised schemas, databases, and public data repositories exist for genetic data for malaria parasites and for their human and mosquito hosts [17, 18], and similar controlled and standardised systems are available for epidemiological studies of malaria-infected human beings. However, equivalent systems for studies of the mosquitoes which mediate transmission are only now emerging [63, 64, 65].

Significant challenges are presented by the variety of data formats, ecological structures, experimental designs and sampling methods used in studies of mosquitoes, which often collect very large volumes of data and adaptively change experimental design over periods as brief as months, weeks or even days. Despite this level of data complexity and variability, experimental and survey data describing mosquitoes often are collected using experiment-specific forms that require frequent, error-prone redesign. Such cursory data management leads to

badly or inconsistently structured data, frequent transcription errors, difficulty in sharing or linking data, and information loss [66]. Improved informatics tools for malaria vector (also generalizable for other vector-borne diseases) studies are required to provide structure to data at the point of data collection and to streamline the use of databases that consistently link field and laboratory data. Therefore, we have developed a generic schema for recording taxonomic, abundance, and phenotypic data, as well as processing associated samples derived from surveys of malaria vectors caught in the field or manipulated in enclosed experimental systems. These tools were developed specifically for application in lower-income tropical countries with limited access to specialized software and expert informatics support.

2.2 System and Methods

In keeping with the goal of making this system widely available and practicable in resource-limited developing countries, all forms are available as Microsoft Excel templates (Supplementary Online Material File 1) which were used in accordance with the standard operating procedures document (Supplementary Online Material File 2). Some users subsequently entered the recorded data using specially tailored

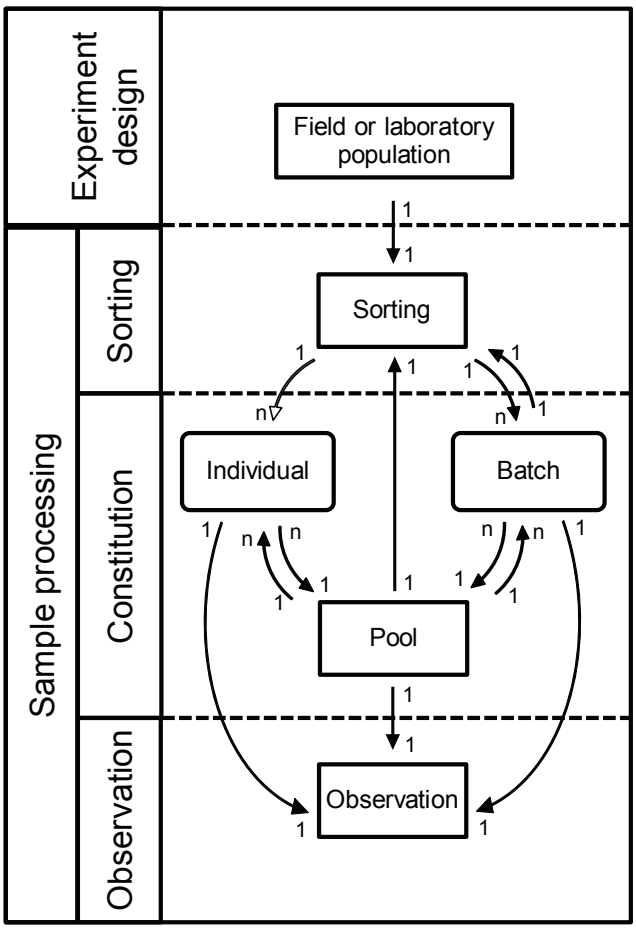

Figure 2.1: Data collection is based on a simple foundation of recording the experimental design followed by sample processing.
Sample processing (dashed boxes) involves the sorting and observation of mosquito samples. As mosquito biology experiments are highly variable in structure, there are many possible ways in which to move between the generic schemas. The arrows indicate the direction and function (e.g., one-to-many: $1 \ldots n$) of the relationships between the entities.

applications on laptops or mobile devices chosen and implemented at their own discretion. However, the data is entered directly into Excel structured consistently with the generic schema described in Figure 2.1. By using the attribute names from the forms as headers the data entered could be imported into readily available relational database software, for example Microsoft Access, and then linked and cleaned using the primary and alternative keys described below.

Algorithm

2.2.1 A Generic Schema for Recording Data from Mosquito Surveys and Experiments

Although research in mosquito biology involves an infinite number of possible experimental and survey procedures, the vast majority can be described within a single fundamental structure (2.1). Essentially, each experiment commences with a defined *experimental design*, followed by *sample collection, sorting, constitution,* and *observation.*

Experimental design and Sample collection: Before starting data collection for a given experiment, a researcher has to make sure that an experiment is well designed. A *collection* is defined as a group of mosquitoes from one sampling or trapping effort. The mosquitoes could be at any stage in the life cycle (i.e., egg, larvae, pupae, or adult) and are collected from a natural population in the field or

from a captive insectary/semi-field colony. It is critically important to know where, when, and how each collection was executed [67, 68], so these experimental or survey design attributes must be recorded before or immediately after each is completed. In some cases, mosquitoes are collected in the context of an *experiment* in the true sense, meaning that the field or laboratory environment of the mosquito population is deliberately manipulated. However, in other cases, collections within the context of a *survey* merely obtain samples of the mosquito population without any deliberate manipulation of that population by the researcher.

Sample sorting: After the collection of mosquitoes is made, the sample is sorted on the basis of specific, directly observed attributes. A sample *sort* is defined as the process by which a collection or sample is broken into subgroups on the basis of specific categorical attributes defined by direct observation at the point of collection, with or without specific experimental manipulations to reveal specific phenotypes. For example, a collection of mosquitoes from one trapping effort can be broken into subgroups of pre-defined taxon, sex, abdominal status, and the number in each subgroup is observed by counting. In fact, most experiments that are conducted by entomologists generally, and mosquito biologists in particular, rely on sorting samples into pre-defined categories based on the observed attributes of individual insects. While this sorting process is almost always followed by counting of mosquitoes in each category, this enumeration is a subsequent observation of the

sample that is distinct from those observations used to define and prepare it by sorting. The observed attributes used to sort collections of wild-caught insects always include some level of taxonomic classification. For mosquitoes, it also is typical to include their sex and abdominal status. Experimental manipulation of captive or wild mosquitoes also may be used to enable sorting based on classification of specific response phenotypes. A common example is a 24-hour survival analysis of mosquitoes after they have been exposed to an insecticide [69]: the researcher sorts the mosquitoes by 'dead' or 'alive' after completion of the 24-hour holding period, and the number of mosquitoes in each subgroup is then observed by counting.

The observations used to designate the sorting of mosquitoes into sub-groups must be recorded as attributes with continuous measurements classified into categorical strata defined before the experiment was conducted. Categorical sorting observations, such as alive versus dead within a sequence of pre-defined holding periods, are identified so their range of possible attributes values can be pre-filled into the sort form. Values for a continuous variable that is recorded based on scalar observations or measurements, such as time of copulation, may be directly observed and recorded as a continuous attribute during an experiment or analytical assay. However, such a continuous attribute cannot be used only to sort mosquitoes into samples containing single individuals unless pre-defined ranges of these measures are assigned as nominal or ordinal categories into which several insects can be classified.

Alternatively, such continuous attributes may be recorded in ordinal, discontinuous format by either observing intermittently or measuring by assignment to specific strata with defined boundaries. For example, time of death is clearly a continuous quantity but may be recorded by removing dead insects over a sequence of exposure durations that need to be designated by the researcher before commencing the experiment.

Sample constitution: After the collection is sorted and the number of mosquitoes in each subgroup has been observed, the mosquitoes can be used to constitute samples as individuals or batches. An *individual* is defined as one mosquito, and a *batch* is a group of two or more mosquitoes created from one source collection. Individuals or batches may be merged together to form *pools* of mosquitoes that are defined as a group of mosquitoes assembled from more than one source individual, batch, and/or collection.

Sample observation: An *observation* is a direct scientific observation of a defined attribute for a single whole sample, for example, the counted number of individuals in it. For individual mosquitoes, observation may include sibling species identification [70], blood-meal identification [71], sporozoite stage [72], ovarian dissection to determine gonotrophic age class [73], or visual measurement of wing length [74]. Additionally, researchers may make observations of mosquito genotype

[75] and then could link to the semantics of gene ontology [17] using complementary databases such as VectorBase [18].

A common mistake is to confuse the observed attributes used to define and prepare a sample by sorting with those assigned to that sample based on subsequent observations of it. This can be a difficult concept to grasp at first, and one that we commonly confused while designing this schema. However, the foundation of a *sort* is the process by which one sample (collection, batch or pool) is broken into many based on observation of categorical or continuous *sort attributes*, whereas a *sample observation* is a direct observation or measurement of a property of a single sample. For example, a knock-down insecticide assay of a batch or pool of mosquitoes begins with a sorting process, where the original sample is sorted into subgroups and samples based on observed survival attributes following a sequence of pre-defined holding periods. Afterwards, the number of individuals in each sorted subgroup is observed by counting them. However, this quantity is an attribute of that sample that is observed after it is prepared, rather than an attribute used to prepare it by sorting.

Table 2.1: Details of the primary tables of the generic schema that are used as the foundation of the relational database and are reflected in the data collection forms

Schema category	**Table**	**Description**	**Unique ID**
Informed consent record	Informed consent record	Details of written informed consent forms	IC1
Experimental design	Field collections	Records the design of experiments collecting mosquitoes in the field	ED1
	Batch and/or pool experimental assay	Records the design of experiments using colony mosquitoes or pre-existing batches or pools	ED2
Sample sorting	Adult field collection	Records the process where a field collection of mosquitoes is sorted into pre-defined subgroups based on taxon, sex and abdominal status	SS1
	Immature field collection	Records the process where a field collection of mosquitoes is sorted into each specified combination of taxon and body-part (which incorporates developmental stage)	SS2
	Batch and/or pool experimental assay	Records the process wherein a batch and/or pool or mosquitoes is experimentally sorted into pre-defined categories	SS3
Sample observation	Laboratory analysis	Scientific observations made using laboratory analyses of mosquito samples	SO1
	Dissection and wing length	Scientific observations made to measure the parity status (females only) and wing length of dead individual mosquitoes	SO2
	Individual experimental assay	Various scientific observations of individual mosquitoes made in the field or entomology laboratory	SO3
Sample constitution	Pools	Records the process where multiple samples are pooled into a single sample	SC1
	Box record	Records the long-term storage of sample boxes in the laboratory storage facility	ST

2.2.2 Generic, standardized data collection forms

The majority of entomological studies of tropical vector-borne diseases are conducted in lower-income countries where access to specialized software and expert informatics support is often limited, so we designed a limited number of generic, standardized paper-based data collection forms. Our six categories of data collection forms (Table 2.1) are informed consent record (IC), experimental design (ED), sample sorting (SS), sample observation (SO), sample constitution (SC), and sample storage (ST) (Supplementary Online Material File 1). The IC and ST forms are not novel and can be applied generically respectively to recording details of informed consent for human participant, and for sample storage location in any type of study rather than just entomological ones. However, the ED, SS, SO, and SC forms are designed specifically for recording the relevant details of entomological sample collection, sorting, observation, and constitution, respectively (Figure 2.1). Within each category, there are up to three different form designs to accommodate a wide variety of experimental procedures, only one of which is required for a specific individual experiment. Each experiment commences with an experimental design (ED), followed by sample sorting (SS). The ED form can be just as readily applied to recording where, when, and how mosquitoes are collected [67] as part of a survey of an un-manipulated population as it can to an experiment in which a population is deliberately manipulated. If required, additional forms can record further sample

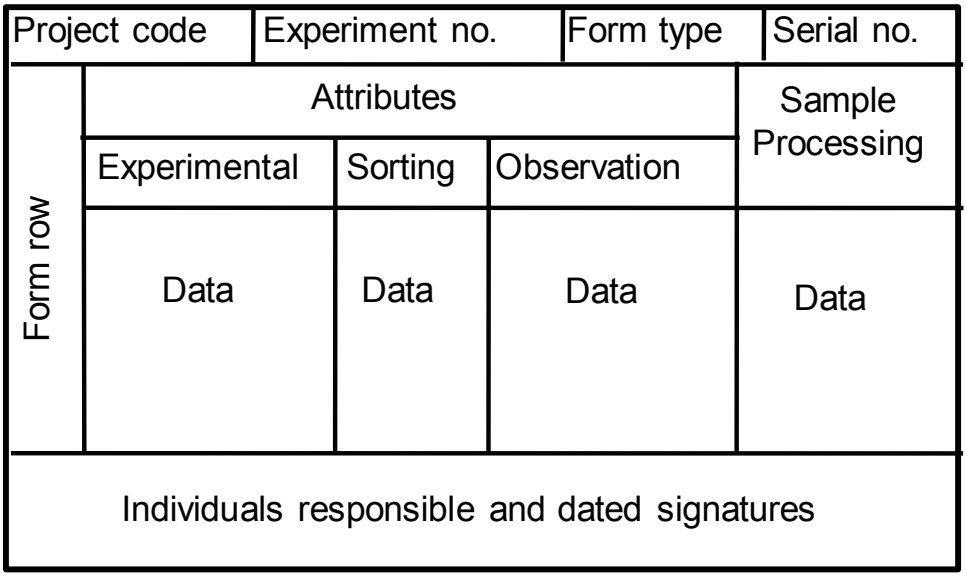

Figure 2.2: The generalised structure that was used as the foundation for designing each of the data collection form.
This figure presents a generic structure used to design each of the data collection form. The top rows record information that uniquely identifies each form, and the central grid records the actual data and observations under each attribute. To preserve the integrity of the data, managing responsible personnel, and facilitating external audits, both the supervisor and the responsible personnel can initial and sign the bottom section of the form.

observations (SO), constitution (SC), and sample storage (ST), as well as informed consent numbers for human participants (IC).

Each data collection form (Supplementary Online Material File 1) was designed using the same generic structure shown in Figure 2.2. The top rows record the *project code, experiment number, form type,* and *serial number* attributes that uniquely identify each form, as well as additional variables that are specific to each form type, such as ethical approval number (IC), study site (ED), or body part (SS). The actual data and observations are recorded in the central grid on the form.

Listed along the top of the grid are the names of the various attributes that can be used to record the experimental design, sort criteria, or direct observation. A comprehensive list of attributes has been created, a minority of which are earmarked as mandatory for rigorous data collection. However, to provide flexibility to the user, most attributes are optional. Some attributes are termed generic because they are widely understood and accepted so they can be used across all experiments in the same manner. However, experiment-specific attributes also are provided which are user-defined and only have context within the bounds of the experiment in question. The short, two or three-letter, capitalized acronym for each attribute should not be changed and is used to label each attribute in the form and each variable in the electronic data table. However, the full names of each attribute can be edited in the form template for context-specific use, including translation into the local language, so long as the meaning of the edited version is not altered.

The response category for each attribute uses numerical codes because entering data in string format is usually slower and more error-prone. The generic attributes have standardized codes that should be used by all users and are printed on the bottom or back of the forms, so it is preferable to record as many attributes as possible using these carefully standardized options to ensure comparability of data from different studies, teams, or countries. Nevertheless, columns for experiment-specific attributes, which are not captured by the generic options, also

allow the user to define codes for these additional variables. While some experiment-specific attributes, such as *experimental round, replicate* or *treatment*, are common features of diverse studies and are pre-filled as options available to the end-user, these can be over-written, and additional blank columns are also available for new user-defined attributes.

Auditable data and sample handling is very important, but often overlooked in entomological research because many studies rely on the high fidelity exchange of samples and data between distinct individuals, teams, and facilities responsible for distinct components of the process, working separately with correspondingly separate forms. Creating an auditable trail in the data record allows the user to move succinctly within the system and trace each datum and responsible individual back to the original document. Such an auditable data trail is essential for data cleaning, preserving the integrity of the data, managing responsible personnel, and facilitating external audits. The same principle is followed by financial accountants who need to be able to follow the trail from the balance sheet to individual voucher.

This is achieved by 1) the researcher clearly pre-entering the experimental design and specifying required attributes, and 2) at each stage in the experimental process, both the supervisor and the responsible personnel can sign the bottom section (Figure 2.2). The bottom section of the forms records transfer of sample handling and decision-making responsibilities between individuals at each point in

the experiment, thus creating a clear chain of communication and accountability for all responsible personnel. An auditable trail for the data and samples themselves is created with a unique identifier, termed *serial number,* at the top of each form and unique *row* numbers to identify the individual components of the data. Thus within an experiment, each row of data can be identified uniquely using the minimum amount of information, specifically the combination of the *form serial* and *row* numbers. Many ED forms are completed in each experiment, each line of which results in completing an associated SS form, and optionally, additional SO, SC, and ST forms also may be associated with the SS form. For any pair of associated forms, the *source form* is defined as the form which defines the composition of a collection or sample, while the *destination form* is defined as a subsequent form describing the next sort, observation, or re-constitution step. As an example, for any associated pair of ED and SS forms, the ED is the source form for the SS form data, while SS is the destination form for the ED form data but represents the source form for any SO, SC, or ST destination forms recording subsequent sample observation, constitution or storage data. To provide an identifier that uniquely identifies each linkage between associated rows of data in separate forms consistently with Figure 2.1, the serial number of the destination form is recorded on the source form. To enable cleaning of data for this unique identifier, the *serial* and *row* numbers of the relevant data row from the source form also are recorded on the destination form to provide an alternative identifier. Appropriate sample storage involves not only clear

labeling of each sample, but also a record of where, when, how, and by whom the samples were stored. Therefore, the long-term archiving of samples is recorded using the ST sample storage forms, allowing samples to be located easily at a later date, based on the system of sample labeling described below.

2.3 Sample Labelling and Storage

Before each experiment is commenced, the collection cups used to contain each mosquito collection are labelled clearly and meaningfully. The label should include all-important information that uniquely identify each cup at each experimental time point (e.g., *household number, time,* and *trap type*) for use by the researchers when conducting the experiment. For some complex experiments, large numbers of collections will need to be handled during each experimental unit at a given time (e.g., replicate night). To maintain order during such large experiments, we recommend grouping the collection cups by information-rich data in separate holding boxes, ideally with each box corresponding to one experimental design form. In addition to the information-rich label, the cup should also be labelled with the corresponding *serial number* and *form row* identifiers from the form. The researcher should use the form *serial* and *row* numbers to sort the collections cups sequentially, thus enforcing a structured order to the data record. Although the form *serial* and *row* numbers are sufficient in themselves to uniquely identify collection and derived samples, information-rich details that have intuitive meaning

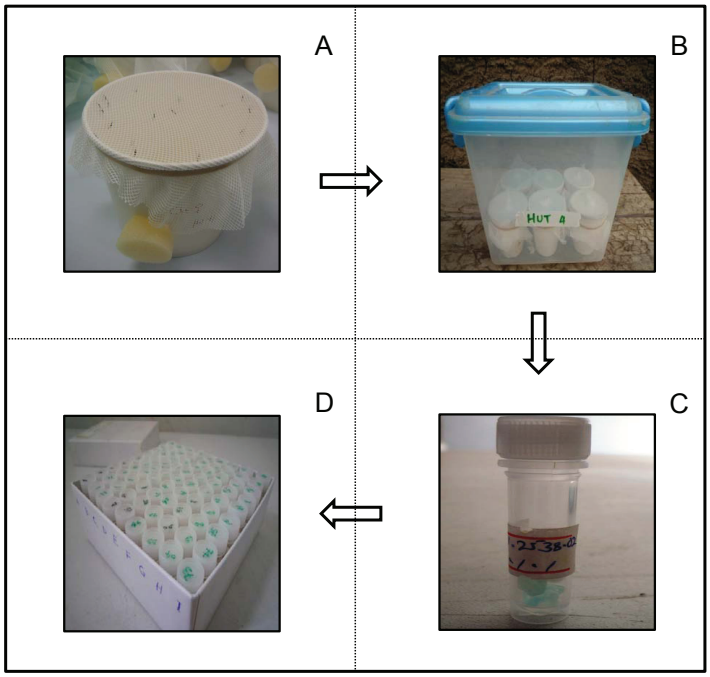

Figure 2.3: Systematic labeling of collection cups and mosquito samples. The cups containing each mosquito collection are labelled clearly and meaningfully to uniquely identify each cup at each experimental time point (e.g., *household number, time,* and *trap type*) (**A**) and then placed in a container (**B**). Mosquitoes are sorted and placed in a tube, which is identified uniquely by combining sorting *form type, form serial number, form row, body form* (to distinguish intact from carcass samples) *sample type* (to distinguish individuals, batches, and pools), and *sample identifier* (to distinguish distinct samples of a single type) (**C**). Labelled tubes with samples are then placed inside a storage box along with the SO form (**D**).

to field personnel also should be included so they can readily cross-check and correct errors in the sample labels or corresponding data on ED and SS forms (Figure 2.3).

Collections are usually sorted into several derived samples, some of which may be split into sub-samples from a single sort category for further processing and storage. Furthermore, these samples and subsamples may be processed for further observations (SO form) or re-organized into new samples with re-defined

constitution (SC form), so it is essential to trace the exact identity and origins of each individual sample. Therefore, each sample of intact mosquitoes is identified uniquely by combining *form type, form serial number, form row, sample type* (to distinguish individuals, batches, and pools) and *sample identifier* (to distinguish distinct samples of a single type) (Figure 2.3, C) to generate a primary key which takes the user to the exact place on the form where the sample was created. However, one sample of intact mosquitoes may be split into multiple body components during the observation processes, such as dissection or preparation for molecular analysis, e.g., the head and legs may be stored and processed separately, so the *body form* attribute also is recorded on both the SO form and the sample label to distinguish these sub-samples of the insect carcass. From here, the user can link to all recorded experimental design, sorting, or observation attributes for that sample. An alternative key for uniquely identifying samples may be recorded at the user's discretion as a single *sample label code* attribute on both the paper-based form and the sample label. The sample label code (SLC) may take the form of any unique code the user chooses, generated by whichever automated or manual system is available. However, we suggest using the 'current date' (in the format yyyymmdd), to distinguish one sample from another, three digits can be added in front of the current date starting from 001 onwards, depending on the number of samples needed to be labelled for that particular day. For example, if one SS1 form has three rows with data, where the sorting was done in February 20, 2013, the

SLCs are 20130220001, 20130220002, and 20130220003, respectively. This approach is preferred because it is an easy one to implement and it does not require prior knowledge of the label code used. The international standard for dates ('yyyymmdd') is suggested, that way, lexical order and time order match.

The sample storage box record form (ST) is uniquely identified by a *serial number,* which records sample storage information for each storage box containing labelled samples and filled-in SO form such as *Box & form serial number* (to distinguish distinct storage boxes from the same or different experiments and/or projects), *number of samples, storage temperature, crate/freezer/fridge number,* and *rack or carton number*.

Data collected using the forms described above, once linked and stored in a given relational database, may be linked easily with environmental or any other demographic data for a given geographic area. This is possible because the ED form captures the unique house number, where available. For example, using a unique house number recorded using ED1, data from the demographic surveillance system (DSS), which also contain a unique house number for the same location, can be linked with entomology data.

2.4 Implementation of the Schema and Data Collection Forms

2.4.1 Illustrative Examples

Figure 2.1 defines the direction and function of the relationships between each experimental stage. Clearly, there are an infinite number of possible experimental designs that could be followed, so selected examples are provided to illustrate how the generic forms and underlying schema were applied to achieve specific experimental objectives. The step-by-step procedures involved in the four experiments described below, with the three additional experiments given in the appendix, show how data collection forms were filled with data for specific attributes (Supplementary Online Material File 2, last section).

Example 1: A Demographically Representative Survey of Indoor Human Exposure to Malaria Transmission

This longitudinal survey of a mosquito population was designed and implemented to evaluate the quantitative relationships between mosquito ecology, coverage of long-lasting insecticidal nets (LLINs) as a vector control measure, and entomological indicators of malaria transmission intensity [11, 76]. The intensity of human exposure to malaria transmission was estimated as the entomological inoculation

rate (number of infectious bites by sporozoite-infected mosquitoes per person per year) [77, 78].

In Africa generally [79], and in this rural Tanzanian study site specifically [10], the main malaria vectors primarily feed upon humans while they are asleep indoors, so CDC light traps placed beside bed nets occupied by people are a reliable, widely-practiced means to collect them. After each night of collection in houses selected at random from a demographic sampling frame consisting of a village household list (recorded using ED1), the mosquitoes caught in each trap were placed in labelled cups, killed, sorted, and counted to enumerate each mosquito category and yield defined samples (SS1). Samples of individual mosquitoes were then observed visually in the field with a microscope to measure wing length and to determine gonotrophic age following ovarian dissection [73](SO2). Then the samples were transferred to a separate laboratory team, who determined sporozoite infection status for each specimen using enzyme-linked immune-absorbent assay (ELISA) [71], and sibling species identity of the *An. gambiae* complex specimens were determined using Polymerase Chain Reaction (PCR) [70] (SO1). The DNA and carcasses of the mosquito samples were archived for long-term sample storage, with their placement in 81-cell storage boxes and location of boxes in the laboratory recorded using the *box record* form (ST).

2.4.2 Example 2: Survey of Immature Mosquitoes from Natural Field Habitats

It is also common to collect immature mosquitoes in their natural aquatic habitats as part of field surveys or experiments, similarly to the way adults were surveyed in example 1. In this example, routine surveillance of larval habitats in urban Dar es Salaam in Tanzania was conducted to monitor effectiveness of a city-level larval source management program and to identify strengths, weaknesses, and opportunities for improvement in the routine internal monitoring systems of that programme [80, 81, 82]. The details of where, when, and how each collection of aquatic stage mosquitoes was obtained by dipping in carefully catalogued habitats in well-mapped enumeration areas [83, 84, 85] were recorded as date, enumeration area, compound/plot, habitat number, habitat type, collection method, and number of dips attributes in a single row of an experimental design form (ED2), based on prototypes [46] that have been refined through practical use over several years. After collection, the larvae were sorted into predefined categories based on taxon (Anopheles spp., Culex spp. Aedes spp,) and body form (egg, early stage larva (instars 1 & 2), late stage larva (instars 3 & 4)), attributes that are pre-filled into the sort form for field collections of immature stages (SS2). In this case, all collected immature mosquitoes were discarded, but if they were not discarded, then the sort category, constituent number of specimens, and identity attributes of samples

retained for experiments, observations, and storage may be readily recorded in SS2, which links to optional additional sample observation (SO1, SO3), constitution (SC1), and storage form (ST) just as described for adult mosquitoes and associated sort forms (SS1/SS3).

2.4.3 Example 3: Experimental Hut Assays of Adult Mosquito Susceptibility to Insecticides

This example illustrates the design of a small-scale field evaluation of the efficacy of several combinations of alternative LLIN and indoor residual spray (IRS) products against natural populations of mosquitoes in Zambia, under realistic but well-controlled field conditions, using experimental huts [69, 86, 87]. The procedures applied to this experiment are essentially identical to published studies from Tanzania, in which several alternative vector control product combinations were assessed, comparing their deterrency, mortality, blood-feeding inhibition, and induced exophily (house exit) in mosquitoes [58], all of which were used as input parameters for simulations of expected community-level impact [58]. *Date, enumeration area (village), method, indoor/outdoor, start time, finish time, round, house/hut, volunteer initials, treatment,* (LLIN or untreated net), and *experimental day* attributes for each of several separate collections from within each hut was recorded on a separate line of an experimental design form (ED1). To assess delayed mortality amongst the captured mosquitoes, all live mosquitoes from each collection

were then held for 24 hours in a separate holding container with a supply of glucose solution in a field insectary. After the holding period, each collection of mosquitoes was sorted into subgroups using the categorical attributes *dead, taxon, sex,* and *abdominal status.* The number in each subgroup was counted, and the derived samples of mosquitoes were placed in labelled storage tubes, all details of which were recorded in a single sort form (SS3) for each collection. These samples then were passed to a separate laboratory team, who determined sporozoite infection status by ELISA [72] and sibling species identity by PCR [70] and recorded these attributes on a sample observation form (SO1). The remaining carcasses of the mosquito samples then were archived for long-term sample storage (ST).

2.4.4 Example 4: Insecticide Susceptibility Bioassay under Laboratory Conditions

Before insecticides can be used for controlling wild vector populations in the field, it is essential to determine the optimal formulation and dosage to maximize efficacy and residual activity through laboratory experiments [69]. In this example, the mortality response of adult mosquitoes when exposed to entomopathogenic fungi was tested under insectary conditions [88]. The experiment was conducted by creating multiple collections from an insectary colony of *An. gambiae*, each of which is a single batch (usually >20) of live mosquitoes, each of which is assigned to one experimental replicate for which the source of mosquitoes (colony code), sex and

abdominal status, age, number of mosquitoes, start date, treatment, and replicate attributes were recorded in one row of an experimental design form (ED2). Each batch was treated in the same manner, except that mosquitoes in different batches were exposed to different experimental treatments, specifically a range of concentrations of fungal conidia. After pre-defined holding periods at intervals of 24 hours, the mosquitoes were sorted on the basis of being dead on that experimental day or still alive at the end of the experiment. In this example, the duration of each sequential holding period defined by the experimental design was recorded in a sort form for batches or pools (SS3) as the finish date for each holding period, but this also can be more directly recorded as the holding period attribute. The number of mosquitoes in each category of holding period and survival status was observed by counting from each collection/experimental unit, and the number in each category was recorded on one SS3 form. In this example using insectary mosquitoes from a known, presumably homogenous genetic and environmental background, no samples were retained for storage or further observation.

2.5 End-User Uptake

The generic schema and forms initially were developed and piloted at the Ifakara Health Institute (IHI) in Tanzania in 2008 and subsequently evolved through interaction with end-users adopting it for specific projects. The subsequent demand for these generic, broadly applicable schema and data collection tools are

demonstrated by growth of the user base over the following 5 years to encompass 20 experiments, 8 projects, and 8 project investigators working on a wide range of vector ecology and control issues at IHI and the National Institute for Medical Research (NIMR) as well as collaborating national malaria control programmes in mainland Tanzania and Zambia, resulting in 10 peer-reviewed publications [11, 4, 80, 82, 88, 89, 90, 91, 92, 93, 94].

2.6 Discussion

The data collection forms described here provide a framework for the processing and handling of both samples and data. It is essential to record not only the processing of the samples after collection, but also the specific experimental design and methods implemented because results only have context with regard to the way the samples were collected and observations are made. This generic schema breaks down the complexities of diverse experiments into a common, consistent, simplified structure that can be conceptualized by any researcher. The broad applicability of the data collection forms enables consistent application of this schema, as well as robust standardization of attribute definitions, both within and between experiments. Furthermore, these forms eliminate the need to redesign forms and databases for each experiment – a laborious and often error-prone task which can be prohibitively resource-intensive, especially when multiple diverse, sometimes

iteratively-designed, experiments are conducted over short periods by large research groups, consortia, or communities.

Electronic data collection devices, such as PDAs or mobile phones, provide many advantages over paper forms to the user. However, these also often require a highly specialised and customised user interface that usually is tailored to the specific collection methods and/or experimental tasks [95]. Designing and supporting electronic user interfaces is a non-trivial task, and so this flexible paper-based system may be most useful to under-resourced medical entomology groups in developing countries lacking sufficient access to specialist software or expert support to develop tailor applications to each individual studies. Therefore, we recommend entomology groups in developing countries to take advantage of our proposed generic schema which can be implemented either in paper or electronic based data collection forms depending on availability of resources.

2.7 Transition to Chapter 3

In Chapter 2, we have presented a generic schema that can be used to design paper or electronic based data collections forms for entomology studies. We also presented data collections forms designed following the fundamentals outlined in this generic schema. In Chapter 3, we use the principles outlined in this schema to design an expandable database web-based application that can be used to link, store, share,

and generate reports based on entomological field and lab data. The application that works for data retrieved from any data collection devices or forms as long as they were designed following the principles outlined in our generic schema presented in this Chapter.

CHAPTER 3

IEBS: A Generic Approach to Link, Store, Share, and Generate Reports Based on Entomological Field and Lab Data Retrieved from Data Collection Devices and Forms

This chapter [50] will be submitted to *Bioinformatics: Oxford Journals* for publication consideration as an 'application note'.

Abstract

Summary

A secured, web-based application is developed to store, link, clean, share field, and lab- based data for malaria vectors. The goal is to increase research output by handling data preparation challenges facing mosquito entomologists prior to performing data analysis addressing various scientific questions in advancing malaria control and elimination.

Availability and Implementation

The Ifakara Entomology Bioinformatics System (*IEBS)*,

`mscs.mu.edu/~skiware/IEBS` or `iebs.ihi.or.tz/`, (username and password are

available from the author upon request) is implemented in PHP and MySQL on a Linux server supporting all major browsers.

Supplementary information: Examples of customized templates for data upload and the user manual are available from `mscs.mu.edu/~skiware/IEBS/SOM` or `iebs.ihi.or.tz/som/`.

3.1 Main Tex: Description of the Proposed System

The data preparation process for analysis is often time consuming and may be a very challenging task in many mosquito entomology experiments. Challenges include designing a database for data storing, linking, cleaning, and sharing for each new experiment. Fortunately, a generic schema and set of standardized data collection forms have been developed and applied to diverse studies of malaria vectors by several projects and programmes in Africa [52]. Here we describe generic, but customizable, .csv data entry templates that allow quality-controlled upload into a relational database with a correspondingly designed application-specific schema that stores, links, and shares entomological field and lab related data aimed at increasing research output. An application-specific schema (Figure 3.1) was developed based on experimental procedures commonly used by mosquito-based entomologists: the design of the experiment, followed by sample sorting, observation, constitution, and archiving [52]. Based on the schema, a relational

database was developed, and a secure web-based application (IEBS) was designed. Our IEBS is not bound or limited to any specific data collection options, but adhering to customizable data upload templates is necessary to take full advantage of our proposed application. Also, IEBS is designed to be compatible with other systems such as Demographic Surveillance System (DSS) and also to complement other third party repositories (e.g., VectorBase and ATLAS Project).

3.2 How does IEBS work?

Users are provided with an identification number by the system administrator they can use to gain access to IEBS. A project investigator (PI) can then register his/her project by specifying the project name/code, experiment number, start and end date of the experiment, etc. The PI is also required to upload a project protocol to ensure that clear instructions for conducting experiment were produced. The PI can grant access (an option to share data locally and internationally) to some of the registered users to his/her project. Only users with access to a certain project are able to upload/download data for that specific project. Finally, the PI is required to customize some of the data upload templates such as the one for experiment design or for laboratory analysis so that users uploading the data only need to deal with a .csv template with only the required attributes (abbreviated column headings). The templates contain mandatory attributes in which some of them (e.g., *project name,*

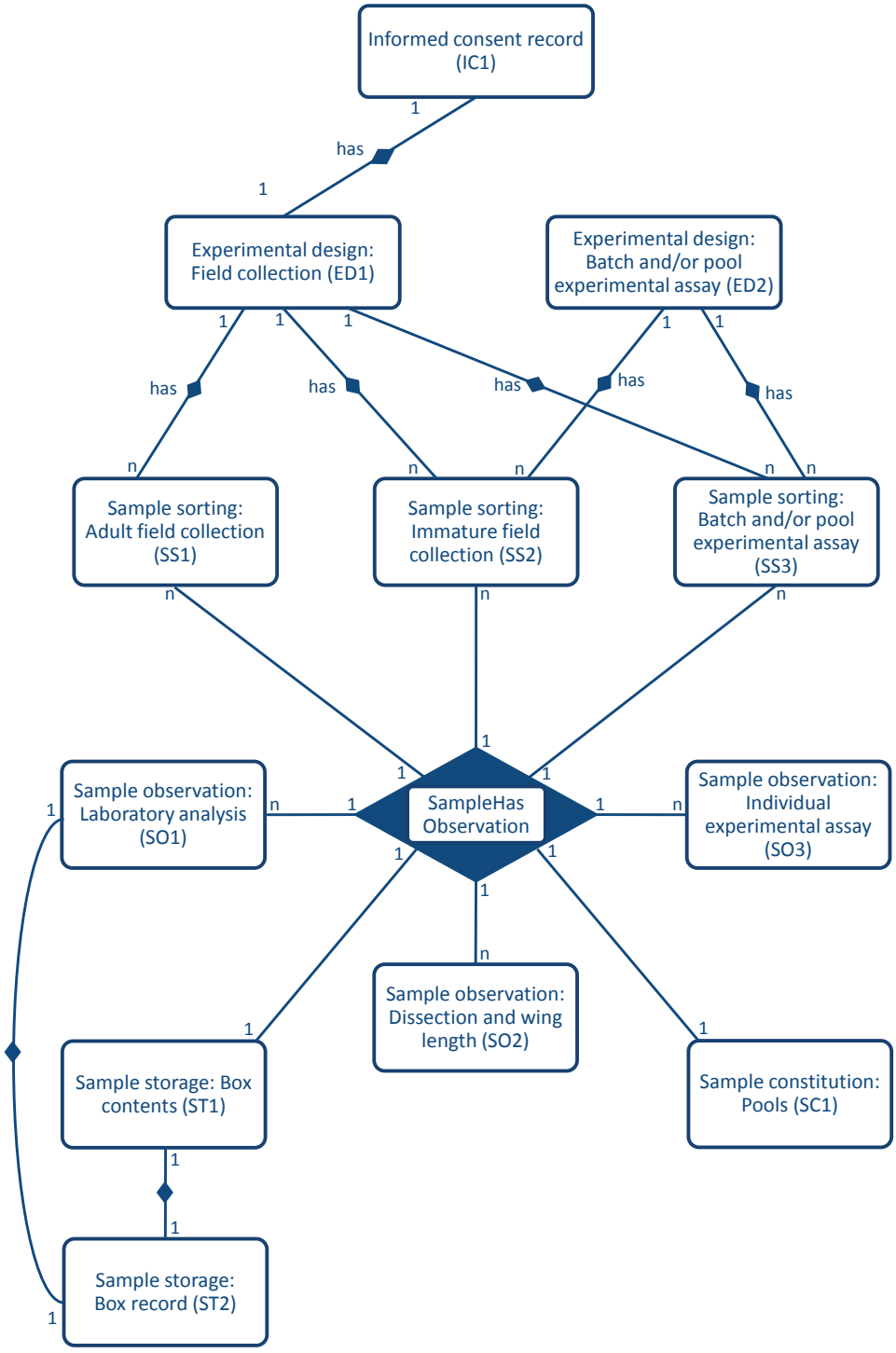

Figure 3.1: Database application specific schema.
The schema designed using the entomology commonly used procedures; experiment design (ED), sample sorting (SS), lab observation (SO), and sample storage (ST), these are the main database tables.

experiment number, form serial, and *row numbers*) are used as primary keys to link data, for example, from the experimental design to sample sorting to laboratory sample results to sample storage information. Users in the field then can enter data into customized templates from various data collections, and lab technicians can upload lab results using the required customized lab templates (customized depending on the analysis (e.g., bloodmeals or species identification) required by the PI), and the sample storage information if available can also be uploaded (see examples of customizable templates at `mscs.mu.edu/~skiware/IEBS/SOM` or `iebs.ihi.or.tz/som/`). Scientists with access to the project may choose to download a linked .csv flat file containing all or some of the data (such as mosquito densities, species type, feeding, and infectious status) to perform further analysis using any statistical software package of their choice.

Although the database for this application was designed from the generic schema previously described [52], the application still may be applicable to other data collection forms adhering to the standardized terminologies. The column names for the upload templates are abbreviated (e.g., 'me' stands for methods) to allow data collections forms to be in any natural local language (e.g., column name in a data collection form can be 'method' (me) or 'mtego' (me), a Swahili word with the same meaning as method, but both are abbreviated 'me', accompanied by corresponding abbreviations. By using the IEBS system, researchers no longer need

to re-design a database for each new experiment or to worry about how to link, store, and share data.

Data integrity is an issue among researchers, but we recognize the need for data sharing where appropriate for research improvements. Hence, the IEBS system is designed in such a way that only a responsible scientist can grant access to a collaborator for specific data depending on their agreements on data sharing. We also understand legal implications related to research data. Our recommendation for data sharing is to use a responsible scientist's institution guidelines that govern data sharing among researchers. Our IEBS makes databasing technology accessible to individual researchers with minimal resources and no specialist software so that rigorous data and sample management strategies can be incorporated into the design and execution of empirical research surveys and experiments from the outset. In contrast, many other bioinformatics tools developed merely facilitate data browsing and integration after the data has been collected [23]. IEBS will facilitate not only efficient, meaningful data sharing between multi-site collaborations, but also integration of datasets with other ecological and genetic third-party public data repositories, such as VectorBase [18], GenBank [96] and the Global Biodiversity Information Facility [97]. The publication of data through open-access public repositories has been increasing in popularity and is even considered a pre-requisite for publication, especially for genetic sequencing, in most peer-review journals.

While such systems have been established in the field of genetics and biodiversity, medical entomologists are now only beginning to understand the power of retrospective analysis, and funders are increasingly insisting that data are published using open-access repositories. Perhaps most importantly, these tools can dramatically reduce the time from data collection by diverse research and surveillance partners to dissemination of data, and even automated analytical summaries thereof, to end-users control programmes for real-time decision making. So far, the *IEBS* currently hosts data from two research and control institutes, 8 projects, and 20 experiments, including several recorded on user-customized paper forms and sent from mobile phones that nevertheless adhere to the generic schema resulting in 10 peer-reviewed publications. We highly recommend researchers in developing countries to take advantage of our free proposed secure system which requires very minimal training for users to improve research output.

3.3 Transition to Chapter 4

In this chapter, we have presented improved data systems for integrating diverse experimental observations and research groups. In the next chapters, we present process-explicit mathematical models that may be used evaluate the data sets presented in these systems to address challenges facing malaria control and elimination strategies.

CHAPTER 4

Simplified Models of Vector Control Impact upon Malaria Transmission by Zoophagic Mosquitoes

This chapter is adapted from [4], as published in *PLoS One Journal*.

Abstract

Background: High coverage of personal protection measures that kill mosquitoes dramatically reduce malaria transmission where vector populations depend upon human blood. However, most primary malaria vectors outside of sub-Saharan Africa can be classified as "very zoophagic," meaning they feed occasionally ($< 10\%$ of blood meals) upon humans, so personal protection interventions have negligible impact upon their survival.

Methods and Findings: We extended a published malaria transmission model to examine the relationship between transmission, control, and the baseline proportion of bloodmeals obtained from humans (human blood index). The lower limit of the human blood index enables derivation of simplified models for zoophagic vectors that (1) Rely on only three field-measurable parameters; (2) Predict immediate and delayed (with and without assuming reduced human infectivity, respectively)

impacts of personal protection measures upon transmission; (3) Illustrate how appreciable indirect communal level protection for non-users can be accrued through direct personal protection of users; and (4) Suggest the coverage and efficacy thresholds required to attain epidemiological impact. The findings suggest that immediate, indirect, community-wide protection of users and non-users alike may relate linearly to the efficacy of a user's direct personal protection, regardless of whether that is achieved by killing or repelling mosquitoes. High protective coverage and efficacy (80%) are important to achieve epidemiologically meaningful impact. Non-users are indirectly protected because the two most common species of human malaria are strict anthroponoses. Therefore, the small proportion of mosquitoes that are killed or diverted while attacking humans can represent a large proportion of those actually transmitting malaria.

Conclusions: Simplified models of malaria transmission by very zoophagic vectors may be used by control practitioners to predict intervention impact interventions using three field-measurable parameters; the proportion of human exposure to mosquitoes occurring when an intervention can be used practically, its protective efficacy when used, and the proportion of people using it.

4.1 Introduction to Zoophagic Vector-Based Models

Indoor residual spraying (IRS) and long-lasting insecticidal nets (LLIN) dramatically reduce malaria transmission [25]. Both approaches exceed the benefits of personal protection and provide even greater levels of community-wide protection for users and non-users alike once reasonably high coverage is achieved (30%-60%) [27, 98]. High demographic coverage of humans (C_h) can reduce dramatically the density, longevity, and infection prevalence of mosquito species that primarily feed indoors (endophagic) upon humans (anthropophagic) such as *Anopheles gambiae* and *An. funestus* from sub-Saharan Africa [7, 11, 2] or *An. punctulatus* and *An. koliensis* from the Pacific [28]. The massive importance of community-level transmission suppression for realizing the full potential of both IRS [29] and LLINs [98] using contact insecticides is well established and reflected in global universal coverage targets for these interventions [30]. Also, vector population modification by LLINs and/or indoor residual spraying (IRS) [7, 8, 10, 11, 99], has been observed since the Global Malaria Eradication Programme (GMEP) was initiated in the 1950s. For example, *An. funestus* was replaced by *An. rivulorum* and/or *An. parensis* following the introduction of IRS on at least three distinct occasions in South Africa, Kenya, and Tanzania [16, 100, 101, 102]. However, mosquitoes which feed upon animals (zoophagic) are primary malaria vectors in many tropical countries [14, 15] and can dominate residual transmission in settings where high

demographic coverage of LLIN or IRS has successfully suppressed previously predominant, anthropophagic species [7, 8, 10, 11, 16].

While LLINs confer personal protection against any mosquitoes attempting to bite while they are in use, it remains unclear whether they confer community-level protection against zoophagic vectors that feed only occasionally upon humans. Therefore, we extended a previously published static malaria transmission model [2] and applied it to explain how immediate and delayed impacts of personal protection measures can be predicted using three potentially field-measurable parameters. In addition, we simplified this model formulation by expressing malaria transmission and control in terms of a baseline human blood index [103]. Also, the model was used to assess the likely extent and mechanism of the community-level impact of such personal protection measures upon human malaria exposure for the zoophagic vectors that are primary vectors in many parts of the world [7, 8, 14] and will increasingly dominate transmission in the future [10, 31]. We also contrast these impacts and underlying mode of action with those of the anthropophagic species that have been the overwhelming focus of malaria research and control to date.

4.2 Model Description

We extended a static malaria transmission model [2] to explore the dependence of malaria transmission and control upon baseline human blood index before any

intervention is introduced. Specifically, the impact of personal protection measures such as LLINs, IRS, insecticide-treated clothing, or repellents upon the baseline malaria transmission intensity was compared in a range of vector behaviour scenarios.

4.2.1 Simulating Malaria Transmission and Control as a Function of Mosquito Host Preference

Before describing how the model simulations were performed, we present the basic input parameters and their definitions, equations and derived parameters, output from the model, description of simplified models for very zoophagic vectors, and the expression of malaria transmission and control as a function of baseline human blood index.

4.2.2 Model Basic Input Parameters and Definitions

Several subscripts are used in this model: Ω denotes an intervention package scenario consisting of a specific coverage, 0 for a baseline condition with no intervention, p for protected or u for unprotected humans (h_0), and c for cattle or other animals. Demographic or crude coverage is defined as a proportion of people using a personal protection measure as estimated in a standardized malaria

indicator surveys (C_h) [2]. Another important input is the proportion of daily exposure that a non-user typically would experience at times when a user would normally use such a personal protection measure (π). In other words, this is the maximum proportion of human exposure to mosquitoes that can be directly prevented through a personal protection by using a given measure. This is a broader definition than used previously, when the term was described as the proportion of human exposure that occurs indoors while asleep at times when LLINs can be used (π_i) [9]. This more generalized definition allows the incorporation of other personal protection interventions such as insecticide-treated clothing and repellents which can also be used outdoors. Recently, several authors [9, 104, 105] have described and discussed the importance and measurement of π_i, but the concept was also discussed during the GMEP era [106, 107], when the difficulty of controlling exophagic or exophilic vectors was described in Africa [9, 108], Asia [109], and the Americas [107]. We also introduce host-encounter rate (ε), which is the rate at which a single host-seeking mosquito encounters a given single host. The notations, $\gamma_{h,p}$, $\gamma_{h,u}$, and γ_c represent probabilities of attacking encountered protected humans, unprotected humans, and cattle, respectively, whereas $\phi_{h,p}$, $\phi_{h,u}$, and ϕ_c represent mosquito feeding probabilities upon protected humans, unprotected and cattle, respectively. The mean attack availability of individual cattle (a_c) is the rate at which a single mosquito encounters and then attacks a single cow, whereas the mean attack availability of an individual unprotected ($a_{h,u}$) human is the rate at which a

single mosquito encounters and then attacks a such single person of either protection status [2]. Mortality probability upon attacking a protected or an unprotected human or cow are denoted by $\mu_{h,p}$, $\mu_{h,u}$, and μ_c, respectively. P_{ov} denotes the survival probabilities during host-seeking and ovipisition site-seeking, which are assumed to be equal. $N_{h,u}$ and N_c are the population sizes of unprotected humans and cattle, respectively. The subscripts and the basic parameters presented here are also defined in Table 4.1, with their dimensions listed for a quick reference.

4.2.3 Model Equations for Derived Parameters

We present equations from a previous model [2] that are important to this paper relating all derived parameters in terms of the basic parameters or other already derived parameters. Although these derived parameters are defined here, their definitions and dimensions are also presented in Tables 4.2 and 4.3.

4.2.4 Protective Coverage and Baseline Human Blood Index

As previously [2], we define *de facto* protective coverage of humans ($C_{h,p}$) as the product of crude coverage (C_h) and the maximum proportion of human exposure to mosquitoes that can be directly prevented through personal protection by using a given intervention (π),

$$C_{h,p} = \pi C_h \ . \tag{4.1}$$

Table 4.1: Definition of basic parameters.

Symbol	**Definition and explanation**	**Dimension**
ε	Host-encounter rate: rate at which a single host-seeking mosquito encounters a given single hosts.	One
ε_h, ε_c	Human and cattle encounter rate respectively.	Per Time
$P_{h,u}$	Probability that a mosquito which attacks an unprotected human will successfully feed upon that host.	One
$P_{h,p}$	Probability that a mosquito which attacks protected human will successfully feed upon that host.	One
$\gamma_{h,p}$, $\gamma_{h,u}$, γ_c	represent probability of encountering protected, unprotected human and cattle respectively.	
N_h, $N_{h,p}$, $N_{h,u}$	Number of people, protected and unprotected	Human
N_c	Number of cattle	Animal
C_h	Demographic or crude coverage: Proportion of people using a personal protection measure as estimated in a standardized malaria indicator surveys.	One
$\mu_{h,u}$	Mortality probability upon attacking an unprotected human.	One
$\mu_{h,p}$	Mortality probability upon attacking an protected human	One
μ_c	Mortality probability upon attacking a cattle	One
π_i	The proportion of normal exposure to mosquito bites upon humans lacking LLINs, which occurs indoors at times when nets would normally be in use.	One
π	The maximum proportion of human exposure to mosquitoes that can be directly prevented through personal protection by using a given intervention	One
P_{ov}	The survival probabilities during host seeking and ovipisition site-seeking assumed to be equal	1/exp(Time)

The mean availability (a) of any host of any species (s) for mosquitoes to attack is the product of the rate at which individual vectors encounter that host (ε_s) and the probability that, after this encounter, they will attack the host (γ_s); $a_s = \varepsilon_s \gamma_s$ [110]. Thus, $a_{h,p} = \varepsilon_h \gamma_{h,p}$, $a_{h,u} = \varepsilon_h \gamma_{h,u}$, and $a_c = \varepsilon_c \gamma_c$ are mean attack availability of protected and unprotected humans and cattle, respectively. The mean availability of

Table 4.2: Definitions of the derived parameters.

Symbol	**Definition and explanation**	**Units**
$C_{h,p}$	Protective coverage	One
a_c	Mean availability of individual cow for attack: rate at which a single mosquito encounters and then attacks a cow or pseudo-host.	Per time per animal
a_h	Mean availability of individual human for attack: rate at which a single mosquito encounters and then attacks a human or pseudo-host.	Per time per human
$a_{h,p}$	Availability of individual protected human	Per time per protected human
$a_{h,u}$	Availability of individual unprotected human	Per time per unprotected human
A, A_h, A_c	Total availability of all hosts, all humans and all cattle, respectively: rate at which a single mosquito encounters, attacks upon these host sets	Per time
z, z_h, z_c	Mean availability of blood from all hosts, all humans and all cattle, respectively: rate at which a single mosquito encounters, attacks and successfully feeds upon these host sets.	Per time
Z, Z_h, Z_c	Total availability of blood from all hosts, all humans and all cattle, respectively: rate at which a single mosquito encounters, attacks and successfully feeds upon these host sets.	Per time
Q_h	Human blood index: the proportion of all blood meals from all hosts which are obtained from humans.	One
$Q_{,0}$	The baseline human blood index in the absence of any protection measure	One
P_γ	Probability of surviving host attack per feeding cycle	One
η_0	Oviposition site-seeking interval; number of days a mosquito takes to find an oviposition site once it starts searching for it	Time
η_v	Host seeking interval: number of days a mosquito takes to find and attack a vertebrate host	Time
P_f	The survival rate per feeding cycle	Per time
f	Feeding cycle length: measured as the number of days it takes a single mosquito to get from one blood feed to the next.	Time
E	Emergence rate of mosquito vector	Per time

Table 4.3: Definitions of the derived parameters (cont.)

Symbol	**Definition and explanation**	**Units**
β_h	The total number of infectious bites on all humans	One
β	The total number of sporozoite infected bites in all hosts per mosquito lifetime	One
EIR	Entomological inoculation rate (mean number of infectious bites that an average individual human receives per year).	Per time
$EIR_{h,\Omega}$	absolute EIR for an average community member in a given intervention scenario	Per time
$EIR_{h,u}$	EIR for non-users	Per time
$\Psi_{h,u}$	The immediately relative exposure of non-users benefiting only from communal protection	One
g	Gestation interval: number of days a mosquito takes to digest a blood meal and return to searching for oviposition site.	Time
P^g	Combined probability that a vector survives gestation	One
X	Mosquito age	Time
S_x	The sporozoite infection prevalence of mosquitoes at each age	One
κ	Human infectiousness to mosquitoes: probability of a vector becoming infected per human bite.	One
ρ	Overall proportion of personal protection against mosquito bites provide by using a given protective measure.	One
$\hat{\Psi}_{h,u,\Omega}$	The immediate impact on vector population assuming a reduction of human infectivity.	One
$P_f^{x/f}$	Estimation of daily cycle and cumulative survival of mosquitoes up to each age (x).	One

host blood (z) from a host of any species (s) is the product of the rate at which individual vectors encounter this host (ε_s) and the feeding probability upon that particular host (ϕ_s); $z_s = \varepsilon_s \phi_s$ [110]. Thus, $z_{h,p} = \varepsilon_h \phi_{h,p}$, $z_{h,u} = \varepsilon_h \phi_{h,u}$, and $z_c = \varepsilon_c \phi_c$ represent mean availability of blood from individual protected and unprotected humans and cattle, respectively.

The total availability of all hosts (A), protected humans ($A_{\mathrm{h,p}} = \varepsilon_h \gamma_{h,p} \mathrm{N_h} C_{h,p}$), unprotected humans ($A_{\mathrm{h,u}} = \varepsilon_h \gamma_{h,u} \mathrm{N_h} \left(1 - C_{h,p}\right)$), and all cattle ($A_{\mathrm{c}} = \varepsilon_c \gamma_c \mathrm{N_c}$), respectively, are the rates at which a single mosquito encounters and attacks upon these host sets [2]. These total availability parameters are related to each other and are calculated in terms of basic individual availability and host population size parameters [2],

$$A = A_{\mathrm{h,p}} + A_{\mathrm{h,u}} + A_{\mathrm{c}} \ . \tag{4.2}$$

Similarly, the total availability of blood from all hosts (Z) protected ($Z_{h,p} = \varepsilon_h \phi_{h,p} \mathrm{N_h} C_{h,p}$) or unprotected ($Z_{h,u} = \varepsilon_h \phi_{h,u} \mathrm{N_h} \left(1 - C_{h,p}\right)$) humans, and all cattle ($Z_c = \varepsilon_c \phi_c \mathrm{N_c}$), respectively, is the rate at which a single mosquito encounters, attacks, and successfully feeds upon these host sets [2] given by

$$Z = Z_{\mathrm{h,p}} + Z_{\mathrm{h,u}} + Z_{\mathrm{c}} \ . \tag{4.3}$$

The human blood index is the proportion of all blood meals obtained from both protected and unprotect humans [103]. It is calculated as a function of the total availability of blood from both categories of humans and the availability of alternative blood sources such as cattle and other animals [2],

$$Q_{\mathrm{h}} = \frac{Z_{\mathrm{h,p}} + Z_{\mathrm{h,u}}}{Z_{\mathrm{h,p}} + Z_{\mathrm{h,u}} + Z_{\mathrm{c}}} \ . \tag{4.4}$$

Changing the mean availabilities of protected humans ($a_{h,p}$) or unprotected humans ($a_{h,u}$) and cattle (a_c) correspondingly change $Z_{h,u}$, $Z_{h,p}$, and Z_c, and therefore the the human blood index (Q_h), because Z_h is directly related to a_h, whereas Z_c is directly related to a_c. The baseline human blood index in the absence of any protection measure ($Q_{h,0}$) can be used to identify vector populations which are zoophagic in terms of both their innate host preferences and their ability to exploit locally common animal hosts. This is because low values represent mosquitoes that primarily feed on animals (zoophagic), while high values represent those that primarily feed on humans (anthropophagic). Hence, when $C_h = 0$, the baseline human blood index ($Q_{h,0}$) can be derived in terms of basic parameters as

$$Q_{\mathrm{h},0} = \frac{\varepsilon_h \phi_{h,u} \mathrm{N_h}}{\varepsilon_h \phi_{h,u} \mathrm{N_h} + \varepsilon_c \phi_c \mathrm{N_c}} \ . \tag{4.5}$$

For predominantly animal-feeding mosquitoes [111], we assume that the mean encounter rate for humans (ε_h) approaches zero, so that the same is correspondingly true of the mean attack availability of humans (a_h) and the mean availability of human blood *per se* (z_h). Therefore, the total attack availability of all humans (A_h) and the total availability of all human blood *per se* (Z_h) also approaches zero.

In Equation (4.5), baseline human blood index approaches zero ($Q_{\mathrm{h},0} \to 0$) when either the denominator approaches infinity or the numerator approaches zero. The numerator can approach zero in three different ways: either when $\varepsilon_h \to 0$,

$N_h \to 0$, or $\phi_{h,u} \to 0$. It is unrealistic that the denominator would approach infinity, or that $\phi_{h,u}$ would approach 0, and it is of no interest to model malaria transmission in the situation where $N_h \to 0$. Hence, in the situations that are realistic and interesting, $Q_{h,0} \to 0$ if and only if $\varepsilon_h \to 0$. Hence, when we are interested in the situation $Q_{h,0} \to 0$, we can take the limit as $\varepsilon_h \to 0$, which biologically means a situation where mosquitoes are not attracted to human blood so the attractiveness or availability of human blood is close to zero. Therefore, the mean availability of individual humans (a_h) and the mean availability of blood from individual humans (z_h), the total availability of all humans (A_h), and the total availability of all humans blood (Z_h) including both the protected and unprotected, all approach zero as well.

4.3 Model Outputs

Malaria transmission intensity is often expressed in terms of the entomologic inoculation rate (*EIR*), which is a direct, field-measurable indicator of human exposure to bites of mosquitoes infected with transmissible sporozoite stage malaria parasites [77, 112]. Thus, the primary outputs from the model were the absolute *EIR* for an average community member ($EIR_{h,\Omega}$) and the relative exposure for non-users to the baseline condition ($\psi_{h,u,\Omega}$), both in a given intervention scenario. To help understand how the impact of a personal protection measure mediated in a given scenario (Ω), the impact upon vector population parameters, the survival rate

per feeding cycle ($P_{f,\Omega}$), human blood index ($Q_{h,\Omega}$), feeding cycle length (f_Ω), and emergence rate of adult mosquitoes (E_Ω) are plotted against $Q_{h,0}$, as intermediate secondary outputs that underlie *EIR* and changes in this primary outcomes.

We present equations from Killeen *et al.* [2] necessary to define primary and secondary outputs in terms of basic or already derived parameters. The probability of surviving host attack per feeding cycle (P_γ) is a function of the probability of surviving one complete feeding cycle (P_f). The oviposition site-seeking interval (η_o) and the vertebrate host-seeking interval (η_v) are both a function of feeding cycle length (f) and P_f, where both P_f and f are functions of emergence rate of adult mosquitoes (E) [2]. Hence, we first present equations of P_γ and the combined η_o and η_v:

$$P_\gamma = 1 - \left(\frac{\mu_{h,p}\ A_{h,p}\ +\ \mu_{h,u}\ A_{h,u} + \mu_c\ A_c}{A_{h,p} +\ A_{h,u} +\ A_c} \right)\ ,\ \text{and} \tag{4.6}$$

$$\eta_o + \eta_v = \frac{1}{A} + \frac{1}{Z} = \frac{1}{A_h\ + A_c} + \frac{1}{Z_h\ + Z_c}\ . \tag{4.7}$$

Hence, P_γ, f, and E are [2]

$$P_f = \ P^g\ P_{ov}^{\eta_o+\eta_v}\ P_\gamma\ , \tag{4.8}$$

$$g + \frac{1}{A} + \frac{1}{Z} = g + \frac{1}{A_h\ + A_c} + \frac{1}{Z_h\ + Z_c}\ ,\ \text{and} \tag{4.9}$$

$$E = \sum_{x=1}^{\infty} \frac{P_f^{x/f}}{f}\ , \tag{4.10}$$

where g is gestation period, P is the mean daily survival, P^g is the probability that a vector survives a single gestation, and P_{ov} is the survival probability for the combined host seeking and ovipisition site-seeking intervals. $P_f^{x/f}$ is the cumulative survival of mosquitoes up to a given age (x), as previously described [2]. In all cases, impact is assessed in terms of changes in the parameters under a given scenario (Ω) relative to a baseline with no protection measure (0): $\frac{P_{f,\Omega}}{P_{f,0}}$, $\frac{Q_{h,\Omega}}{Q_{h,0}}$, $\frac{f_\Omega}{f_0}$, and $\frac{E_\Omega}{E_0}$, respectively.

The number of infectious bites on humans (β_h) per mosquito lifetime is given by the product of human blood index and the sum of the products of the probabilities of surving and being infectious at each age [2],

$$\beta_h = \frac{Q_h}{f} \sum_{x=1}^{\infty} S_x P_f^{x/f} \ . \tag{4.11}$$

S_x is the sporozoite infection prevalence of mosquitoes at each age x, $S_x = S_{x-1} + (\kappa Q_h(1 - S_{x-1}))\,/\mathrm{f}$, for $x > n$. Otherwise, $S_x = 0$, where n is the extrinsic incubation period, and κ is population mean human infectiousness to mosquitoes, defined as the mean probability of a vector becoming infected per human bite. Thus, the absolute *EIR* for an average community member in a given intervention scenario is [2]

$$EIR_{h,\Omega} = \frac{\beta_h E}{N_h} \ . \tag{4.12}$$

The relative exposure for non-users ($\psi_{h,u,\Omega}$), humans who are unprotected (u) by the physical and chemical barrier of personal protection measures, but may benefit from communal protection in a given intervention (Ω) scenario, is calculated as their predicted exposure ($EIR_{h,u,\Omega}$) divided by their baseline exposure with no protection measures ($EIR_{h,u,0}$) as

$$\psi_{h,u,\Omega} = \frac{EIR_{h,u,\Omega}}{EIR_{h,u,0}} = \frac{z_{h,u}\beta_\Omega E_\Omega}{Z_\Omega} \div \frac{z_{h,u}\beta_0 E_0}{Z_0} = \frac{Z_0\beta_\Omega E_\Omega}{Z_\Omega\beta_0 E_0} \ . \tag{4.13}$$

Here, β is the number of sporozoite infected bites in all hosts per mosquito lifetime $\left(\beta = \left(\sum_{x=1}^{\infty} S_x P_f^{x/f}\right)/f\right)$, calculated as Equation (4.11), but ignoring the term Q_h [2].

4.3.1 Simplified Models for Very Zoophagic Vectors

Initial simulations suggested closer examination of the underlying mechanisms through which personal protection mediates community-level protection against malaria transmission by very zoophagic mosquitoes. We specifically define very zoophagic vectors as those which are not merely zoophagic, such as *An. arabiensis*, which readily feeds on both humans and cattle [113], but rather those which have a strong preference for animals and normally obtain 90% or more of their blood meals from animals ($Q_{h,0} \leq 0.1$). A useful example of such a vector species that can be considered very zoophagic is *Anopheles epiroticus* in the Mekong delta of Vietnam.

This mosquito population has more than an 11-fold preference for cattle over humans [109], which allows us to simulate transmission by this species by adjusting the mean encounter rate for humans (ε_h) in proportion to this relative attack rate of cattle compared with humans [2, 110, 114], but which are otherwise equivalent to those described above for *An. arabiensis* [2]. It illustrates how mosquitoes exhibiting very high levels of zoophagy at the population level ($Q_{h,0} = 0.08$) can mediate transmission intensities ($EIR = 3.1$ infectious bites per person per year) that are compatible with this mosquito's status as a primary malaria vector in the region [115].

4.3.2 Expressing Malaria Transmission and Control as a Simplified Function of Baseline Human Blood Index

We express the primary and secondary outputs in terms of human blood index ($Q_{h,0}$), because it is one of the most important determinants of overall malaria transmission locally and globally [15, 38, 103, 116, 117]. For very zoophagic mosquito populations with low human blood indices ($0 < Q_h < 0.1$) that are nevertheless sufficient to stably transmit malaria ($0 < EIR < 1$ infectious bite per year per person), we are interested in a situation where $Q_{h,0} \to 0$ to illustrate the impact of a personal protection measure on $\frac{P_{f,\Omega}}{P_{f,0}}$, $\frac{f_\Omega}{f_0}$, $\frac{E_\Omega}{E_0}$, and $\frac{Q_{h,\Omega}}{Q_{h,0}}$.

Since P_{ov} is constant, using Equations (4.6) and (4.8), we can compute $\frac{P_{f,\Omega}}{P_{f,0}}$

as $Q_{\mathrm{h},0} \to 0$ by taking the limit as $\varepsilon_h \to 0$, (so $A_{h,p} \to 0$, $A_{h,u} \to 0$, $Z_{h,p} \to 0$, $Z_{h,u} \to 0$) terms only with subscript c (for cattle) remain cancelling to 1:

$$\lim_{Q_{\mathrm{h},0}\to 0} \frac{P_{f,\Omega}}{P_{f,0}} = \frac{P^g \; P_{ov}^{\left(\frac{1}{A_{h,u}+A_{h,p}+A_c}+\frac{1}{\mathrm{Z_{h,u}+Z_{h,p}+Z_c}}\right)} \left(\; 1 - \left(\frac{\mu_{h,p}\; A_{h,p}+\mu_{h,u}\; A_{h,u}+\mu_c\; A_c}{A_{h,p}+A_{h,u}+\; A_c}\right)\right)}{P^g \; P_{ov}^{\left(\frac{1}{A_{h,u}+A_{h,p}+A_c}+\frac{1}{\mathrm{Z_{h,u}+Z_{h,p}+Z_c}}\right)} \left(\; 1 - \left(\frac{\mu_{h,u}\; A_{h,u}+\mu_{h,u}\; A_{h,u}+\mu_c\; A_c}{A_{h,p}+A_{h,u}+\; A_c}\right)\right)}$$

$$= \frac{P_{ov}^{\left(\frac{1}{A_c}+\frac{1}{Z_c}\right)} \left(\; 1 - \left(\frac{\mu_c\; A_c}{A_c}\right)\right)}{P_{ov}^{\left(\frac{1}{A_c}+\frac{1}{Z_c}\right)} \left(\; 1 - \left(\frac{\mu_c\; A_c}{A_c}\right)\right)} = 1 \; . \tag{4.14}$$

Using Equation (4.9), the same approach can be applied for $\frac{f_\Omega}{f_0}$ to get

$$\lim_{Q_{\mathrm{h},0}\to 0} \frac{f_\Omega}{f_0} = \frac{g + \frac{1}{A_{h,u}+A_{h,p}+A_c} + \frac{1}{\mathrm{Z_{h,p}+Z_{h,u}+Z_c}}}{g + \frac{1}{A_{h,u}+A_{h,p}+A_c} + \frac{1}{\mathrm{Z_{h,p}+Z_{h,u}+Z_c}}} = \frac{g + \frac{1}{\mathrm{A_c}} + \frac{1}{\mathrm{Z_c}}}{g + \frac{1}{\mathrm{A_c}} + \frac{1}{\mathrm{Z_c}}} = 1. \tag{4.15}$$

We use Equation (4.10) to drive $\frac{E_\Omega}{E_0}$ in the limit $\varepsilon_h \to 0$ by rearranging Equation (4.10) and then substituting $P_{f,\Omega}$, $P_{f,0}$, f_Ω, and f_0 from Equations (4.14) and (4.15):

$$\lim_{Q_{\mathrm{h},0}\to 0} \frac{E_\Omega}{E_0} = \frac{\sum_{x=1}^{\infty} \frac{P_{f,\Omega}^{x/f_\Omega}}{f_\Omega}}{\sum_{x=1}^{\infty} \frac{P_{f,0}^{x/f_0}}{f_0}} = \frac{\sum_{x=1}^{\infty} P_{f,\Omega}^{x/f_\Omega}}{\sum_{x=1}^{\infty} P_{f,0}^{x/f_0}} \times \frac{f_0}{f_\Omega}$$

$$= \frac{\sum_{x=1}^{\infty} \left(P_{ov}(1-\mu_c)\right)^{\frac{x}{g+\frac{1}{\mathrm{Z_c}}}}}{\sum_{x=1}^{\infty} \left(P_{ov}(1-\mu_c)\right)^{\frac{x}{g+\frac{1}{\mathrm{Z_c}}}}} \times \frac{g + \frac{1}{\mathrm{Z_c}}}{g + \frac{1}{\mathrm{Z_c}}} = 1 \; . \tag{4.16}$$

The interpretation of Equations (4.14), (4.15), and (4.15) is given in the Results section. However, the limit for the other vector population parameter does

not approach 1, indicating that human blood index is affected by personal protection measures against very zoophagic vectors that are nevertheless fractionally but sufficiently anthropophagic to put many people at risk of malaria transmission. This allows much simpler models for both immediate impacts upon malaria transmission, with and without an assumed reduction of human infectivity in the longer term, to be derived that rationalize the reduced, but nevertheless useful, impacts of insecticidal personal protective measures upon zoophagic vectors. The explanation and interpretation of what happens to the overall impact on $\frac{Q_{h,\Omega}}{Q_{h,0}}$ as $Q_{\mathrm{h},0}$ approaches zero for very zoophagic ($Q_{h,0} \leq 0.1$) vectors is provided in the Results section.

4.3.3 Simulated Scenarios

The full possible range of host preference for mosquitoes was simulated by modifying field estimates for cattle and human encounter rate, (ε_c) and (ε_h), respectively, by beginning with values typical of a mosquito such as *An. Arabiensis,* which is both anthropophagic and zoophagic [114, 117, 118, 119]. The value for ε_c was tuned down to zero to mimic highly anthropophagic African vectors such as *An. gambiae* [114], while ε_h was tuned down towards zero to mimic zoophagic mosquitoes such as *An. quadriannulatus* [118, 120] and other *Anophelines* that only occasionally feed on humans [118, 121, 122]. While *An. gambiae, An. arabiensis,* and *An. quadriannulatus* come from a single African species complex (*An. gambiae sensu*

lato), they span the full range of host choice preferences exhibited by *Anophelines* world-wide. Although *An. gambiae* typically feeds almost exclusively upon humans and has historically been the most important vector of malaria in the world [123], *An. arabiensis* is as likely to attack cattle as humans and is a correspondingly less potent but nevertheless significant primary vector [123, 124, 125]. By comparison, *An. quadriannulatus* is thought to feed rarely upon humans and transmit little, if any malaria, despite being readily infected by *Plasmodium falciparum* [126]. *An. arabiensis* is a useful intermediate example because this species has been well studied, feeds readily upon both humans and animals [113, 127], and has proven relatively resilient to control with IRS and LLINs [120].

The first scenario was simulated with no intervention by setting $C_h = 0$, while the intervention scenarios (Ω) were simulated by setting C_h for an unspecified personal protection measure to the assumed high coverage levels of 0.8, equivalent to the Roll Back Malaria targets for LLIN coverage of all age groups, with a very high proportion of human exposure to mosquitoes occurring when that protection measure can practically be used ($\pi = 0.9$).

The model was implemented with a range of values of ε_h ranging from a maximum of 1.7×10^{-3} and then decreasing to 1.1×10^{-4} encounters per day per host-seeking vector per unprotected human, with ε_c increasing from 0 up to 1.7×10^{-3} encounters per day per host-seeking vector per cow. The default value of

1.7×10^{-3} encounters per day per host-seeking vector per unprotected human, at which these two ranges coincide, is used because it is an intermediate value between field measures for ε_h of 1.3×10^{-3} and for ε_c of 2.1×10^{-3} encounters per day per host-seeking for *An. arabiensis* [2]. N_h and N_c were assumed equal (1000 for each) in all simulations, leading to $Q_{\mathrm{h},0}$ values ranging from 0.03 to 1.00.

4.4 Results

For all panels in Figure 6.1, Equation (4.5) was used to plot independent x-axis values representing simulated values of the proportion of blood meals taken from humans in the absence of an intervention ($Q_{h,0}$). Low values of $Q_{h,0}$ represent mosquitoes that primarily feed on animals, while high values represent mosquitoes that prefer to feed on humans. The y-axis for panel **A** represents the absolute entomological inoculation rate (EIR) for an average community member in which the dependent values were plotted using Equation (4.12). The y-axes for all other panels were plotted using equations given in brackets representing relative values for mosquito population parameters when compared with those expected in the absence of LLINs; panel **B**: Relative exposure for non-users $\left(\psi_{\Omega,u,0} = \frac{EIR_{h,u,\Omega}}{EIR_{h,u,0}}\right)$, Equation (4.13); panel **C**: Relative probability of surviving one complete feeding cycle $\left(\frac{P_{f,\Omega}}{P_{f,0}}\right)$, Equation (4.14); panel **D**: Relative proportion of blood-meals taken from human $\left(\frac{Q_{h,\Omega}}{Q_{h,0}}\right)$, Equations (4.4) and (4.5); panel **E**: Relative feeding cycle

length $\left(\frac{f_\Omega}{f_0}\right)$, Equation (4.15); and panel **F**: Relative emergence rate of adult mosquitoes $\left(\frac{E_\Omega}{E_0}\right)$, Equation (4.16).

Consistent with field observations [7, 8, 9, 10, 11, 1, 12, 13, 3] and previous simulations, high coverage with an insecticidal personal protection interventions is predicted to have huge immediate impact on malaria transmission where mosquitoes primarily feed indoors upon humans (Figure 6.1, panels A and B). Insecticidal personal protection is most effective against human-feeding mosquitoes ($Q_{h,0} \to 1$) because the fraction of available blood resources that protected people represent is high so that survival per feeding cycle is reduced (Figure 6.1, panel C), the length of feeding cycle is extended (Figure 6.1, panel E), and the emergence rate for adult mosquitoes is reduced (Figure 6.1, panel F) [2, 1, 13, 12].

By comparison, as previously described [7, 11, 16], insecticidal personal protection measures are less efficacious against mosquitoes that only occasionally feed upon humans ($Q_{h,0} \to 0$) because animals are not protected and remain available to feed on. Therefore, negligible impact is expected upon mosquito survival Equation (4.14), Figure 6.1, panel C, or upon feeding cycle length Equation (4.15), Figure 6.1, panel E, or upon reproduction rates Equation (4.16), Figure 6.1, panel F. Human blood index is the only parameter affected for very zoophagic vectors (Figure 6.1, panel D), so it is important to explore what happens to $\frac{Q_{h,\Omega}}{Q_{h,0}}$ as $Q_{h,0}$ approaches zero.

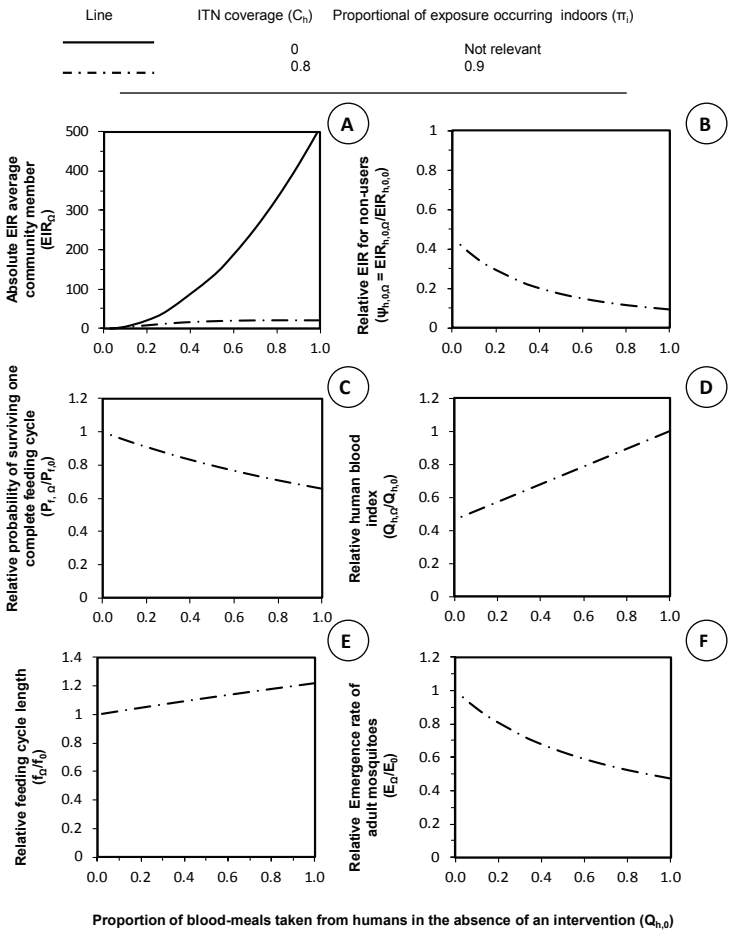

Figure 4.1: The impact of long lasting insecticide treated nets (LLINs) upon malaria vector population parameters.

Malaria vector population parameters, transmission intensity, and the impact of personal protection interventions upon them under a range of values for the proportion of blood meals obtained from humans ($Q_{h,0}$). In all panels, the x-axis is the proportion of all blood meals the vector population would obtain from humans in the absence of nets ($Q_{h,0}$). Low values of $Q_{h,0}$ represent mosquitoes that primarily feed on animals while high values represent mosquitoes that prefer to feed on humans. The y-axis for panel **A** represents the absolute entomological inoculation rate (EIR) for an average community member in a given scenario ($EIR_{h,\Omega}$). The y-axes for all other panels represents relative values for mosquito population parameters, compared with those expected in the absence of LLINs: **B**: Relative exposure for non-users, $\left(\psi_{\Omega,0} = \frac{EIR_{h,u,\Omega}}{EIR_{h,u,0}}\right)$ **C**: Relative proportion of blood-meals taken from human $\left(\frac{Q_{h,\Omega}}{Q_{h,0}}\right)$, **D**: Relative probability of surviving one complete feeding cycle $\left(\frac{P_{f,\Omega}}{P_{f,0}}\right)$, **E**: Relative feeding cycle length $\left(\frac{f_\Omega}{f_0}\right)$, and **F**: Relative emergence rate of adult mosquitoes $\left(\frac{E_\Omega}{E_0}\right)$. In all cases the intervention scenario (Ω) crude demographic coverage specified high levels of coverage ($C_h = 0.8$) and use at times when transmission would otherwise occur ($\pi_i = 0.9$).

Personal protection measures can deliver appreciable communal protection against transmission by zoophagic vectors (Figure 6.1, panel B) because they can lower the proportion of bloodmeals obtained from humans (Figure 6.1, panel D). Thus, further reducing already-low proportions of blood meals taken from humans ($Q_{h,0}$), can have a corresponding immediate impact on the exposure of non-users lacking any personal protection against malaria transmission by zoophagic mosquitoes (Figure 6.1, panel D). This is because the tiny proportion of a zoophagic mosquito population that are killed may be a large proportion of those that actually transmit human parasites such as *Plasmodium falciparum* and *P. vivax*.

4.4.1 Calculating Immediate Impact of Personal Protection upon Transmission by Very Zoophagic Vectors Using Only Three Input Parameters

Next, we illustrate how the dependence of transmission and control enables derivation of much simpler models for both immediate and delayed impacts (with and without assuming reduced human infectivity, respectively) upon malaria transmission, to be derived that rationalize the reduced, but nevertheless useful, impacts of a personal protection measure upon zoophagic vector systems that are illustrated by the intercepts on the left hand side of Figure 6.1, panels B and D.

As $Q_{h,0} \to 0$, the immediately relative exposure of non-users benefiting only

from communal protection ($\psi_{h,u,\Omega}$) (Figure 6.2, panel B), compared to their pre-intervention exposure can be computed. If we substitute equations for β and $\alpha; S_x = S_{x-1} + (\kappa Q_h(1 - S_{x-1}))/f$, into Equation (4.13), we get

$$\psi_{h,u,\Omega} = \frac{EIR_{h,u,\Omega}}{\mathrm{EIR}_{h,u,0}} = \frac{Z_0 E_\Omega \frac{1}{\mathrm{f}_\Omega} \sum_{x=1}^{\infty} \left(\frac{\kappa_\Omega Q_{h,\Omega} P_{f_\Omega}^{x/f_\Omega}}{\mathrm{f}_\Omega} \right)}{Z_\Omega E_0 \frac{1}{\mathrm{f}_0} \sum_{x=1}^{\infty} \left(\frac{\kappa_0 Q_{h,0} P_{f_0}^{x/f_0}}{\mathrm{f}_0} \right)} \ .$$

By assuming that $S_x \sim \kappa Q_h/f$ on the basis that sporozoite rates are proportional to Q_h and therefore very low for very zoophagic vectors so a mosquito only gets one chance to get infected, and if we remove all terms not affected by x from the summation and rearrange them, we get

$$\psi_{h,u,\Omega} = \frac{\mathrm{EIR}_{h,u,\Omega}}{\mathrm{EIR}_{h,u,0}} = \frac{(\mathrm{f}_0)^2 Q_{h,\Omega} \kappa_\Omega Z_0 E_\Omega}{(\mathrm{f}_\Omega)^2 Q_{h,0} \kappa_0 Z_\Omega E_0} \frac{\sum_{x=1}^{\infty} P_{f_\Omega}^{x/f_\Omega}}{\sum_{x=1}^{\infty} P_{f_0}^{x/f_0}} \ .$$

We assume that $\kappa_\Omega/\kappa_0 = 1$ in the short term because substantive changes in human infection prevalence take months or years [128, 129]. We know that by taking a limit as $\varepsilon_h \to 0$, $\frac{\mathrm{f}_0}{\mathrm{f}_\Omega} = 1$ (Equation (4.15)), $\sum_{x=1}^{\infty} P_{f,\Omega}^{x/f_\Omega} = \sum_{x=1}^{\infty} P_{f,0}^{x/f_0} = 1$ (see steps in Equation (4.16)), $E_\Omega/E_0 = 1$, and $Z_\Omega/Z_0 = 1$, since $Z_\mathrm{h} \to 0$ as $\varepsilon_h \to 0$, then $\psi_{h,u,\Omega}$ is given by

$$\psi_{h,u,\Omega} = \lim_{Q_{h,0} \to 0} \frac{\mathrm{EIR}_{h,u,\Omega}}{\mathrm{EIR}_{h,u,0}} = \frac{Q_{h,\Omega}}{Q_{h,0}} \ . \tag{4.17}$$

Now, if we substitute the definition of Q_h from Equation (4.4), rearrange, and substitute $z_{h,u} = \varepsilon_h \phi_{h,u}$ and $z_{h,p} = \varepsilon_h \phi_{h,p}$, where ε_h is human encounter rate [2],

relative exposure of non-users ($\psi_{h,u,\Omega}$) is intuitively calculated as the mean of the feeding probabilities for protected ($\phi_{h,p}$) and unprotected humans ($\phi_{h,u}$), weighted according to the protective ($C_{h,p}$) rather than simple demographic (C_h) coverage:

$$\psi_{h,u,\Omega} = \lim_{Q_{h,0}\to 0} \frac{EIR_{h,u,\Omega}}{EIR_{h,u,0}} = \frac{Q_{h,\Omega}}{Q_{h,0}} = \frac{z_{h,u}N_h\left(1-C_{h,p}\right)+z_{h,p}N_hC_{h,p}}{z_{h,u}N_h}$$

$$= \frac{\phi_{h,u}\left(1-C_{h,p}\right)+\phi_{h,p}C_{h,p}}{\phi_{h,u}} \ . \qquad (4.18)$$

In simple terms, the level of indirect communal protection afforded to all community members is equivalent to the coverage-weighted mean of feeding probabilities (Equation (4.18)). This is equivalent to the community-wide mean level of personal protection obtained as a coverage-weighted mean of personal protection. Relative exposure can also be expressed in terms of personal protection (ρ), where [2]

$$\rho = 1 - \frac{\phi_{h,p}}{\phi_{h,u}} \ . \qquad (4.19)$$

By substituting Equations (4.1) and (4.19) into a rearranged Equation (4.18), the impact upon transmission by very zoophagic vector can be expressed in terms of only three field-measurable parameters: the proportion of human exposure to mosquitoes occurring when an intervention can be practically used (π), its

protective efficacy when used (ρ), and the proportion of people using it (C_h),

$$\lim_{Q_{h,0}\to 0} \psi_{h,u,\Omega} = 1 - \rho C_{h,p} = 1 - \rho\pi C_h \ . \tag{4.20}$$

Of course, communal protection is complemented by personal protection, so the overall mean level of protection immediately obtained across all users and non-users in the community is calculated as the square of Equations (4.18) and (4.20). Consistent with previous models [2, 12, 13, 29, 38, 130, 131, 132], the immediate relative exposure of the average community member ($\psi_{h,\Omega}$) is equivalent to the ratio of the square of the pre- and post-intervention human blood index (Q_h) values,

$$\lim_{Q_{h,0}\to 0} \psi_{h,\Omega} \left(\frac{Q_{h,\Omega}}{Q_{h,0}}\right)^2 = \left(\frac{\phi_{h,u}\left(1 - C_{h,p}\right) + \phi_{h,p}\left(C_{h,p}\right)}{\phi_{h,u}}\right)^2 = \left(1 - \rho\pi C_h\right)^2 \ . \tag{4.21}$$

In direct, intuitive terms, this is because a mosquito has to bite humans twice to transmit malaria parasites.

4.4.2 Delayed Impacts Including Reduced Human Infectiousness

The relatively low transmission intensities that very zoophagic mosquitoes mediate also allow the reduction of infectiousness of the human population to mosquitoes to be approximated in a simplified manner. In addition to the direct and immediate impacts upon the vector population, reduction impacts upon infectiousness of human population to mosquitoes (κ) may also be achieved [112, 128], but only if

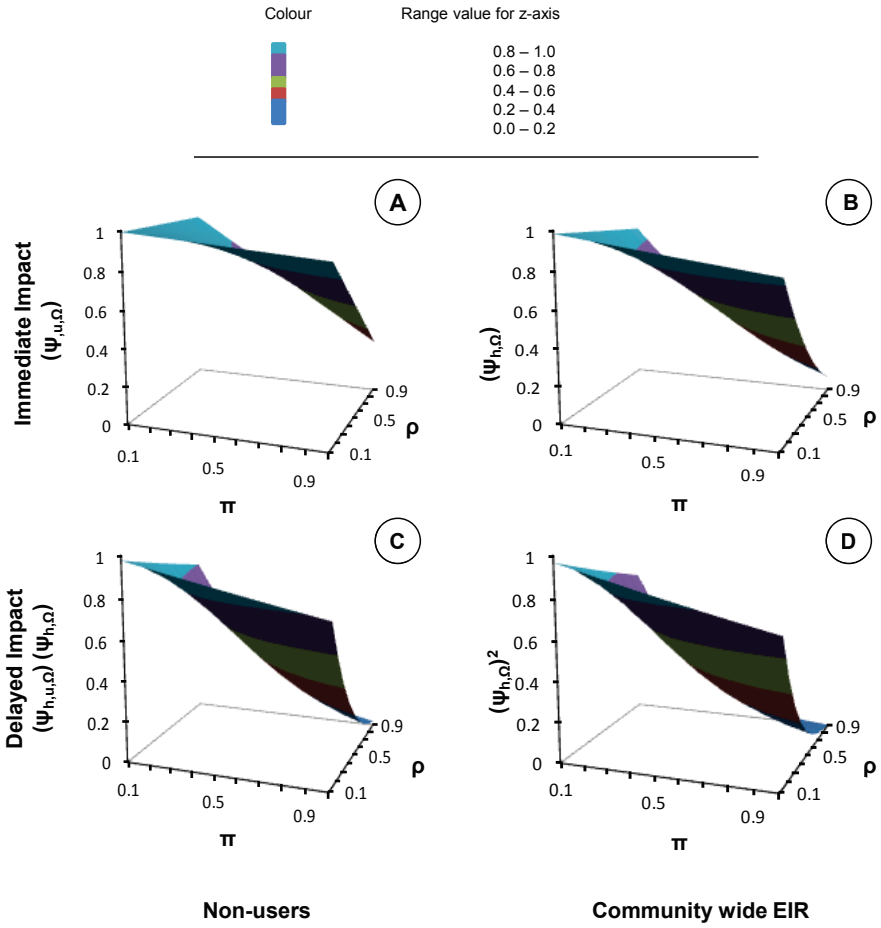

Figure 4.2: Immediate and delayed impact of personal protection upon malaria transmission intensity.

In all the four panels, the x-axis is the proportion of human exposure to mosquito bites that would otherwise occur when the protective intervention is used (π) and the y-axis represents the proportion of mosquito bites prevented by using that protective intervention (ρ). The z-axes reflects immediate (**A** and **B**) and delayed (**C** and **D**) relative exposure $\left(\lim_{Q_{h\to 0}} \psi_{h,u,\Omega}\right)$ experienced by non-users (**A and C**) and average community members (**B** and **D**).

mosquito-to-human transmission can be reduced below saturating levels ($EIR < 10$ infectious bites per person per year) [133]. In holoendemic scenarios, with highly anthropophagic vectors, getting below this threshold will require high levels of coverage $C_h \geq 0.8$ over long periods because re-equilibration of transmission and prevalence levels will take years rather than days, weeks, or months [128, 134]. At the expected intermediate levels of residual transmission ($1 < EIR < 10$ infectious bites per person per year) expected for anthropophagic vector populations exposed to high intervention coverage (Figure 6.1, panel A), the eventual impact upon EIR resulting from direct immediate impact on the vector population parameters combined with feedback upon human infectiousness is complex to predict [31, 133].

While human infectiousness is saturated at high transmission levels ($EIR \geq 10$), at the much lower levels expected for most very zoophagic vectors $EIR \leq 1$, human infectiousness to mosquitoes is thought to be directly and approximately linearly related to mosquito-to-human transmission intensity in the previous few years, $\kappa \propto EIR$. While impacts upon the vector population have an immediate effect on EIR (Figure 6.2, panel A), no immediate impact upon infectiousness is expected ($\hat{\kappa}_\Omega = \kappa_0$), and it may take a long time for a long-lived blood stage infection to be cleared from the human population and the feedback of EIR upon κ and *vice versa* to re-equilibrate [3, 13]. Assuming a linear relationship exists between these two variables at low values approaching the origin of Figure 6.1,

panel A, and that further reductions will be achieved as a result of re-equilibration between κ and *EIR*, then reduction of impact on human infectiousness to mosquitoes is expected to be greater than the immediate impact on *EIR*,

$$\frac{\hat{\kappa}_\Omega}{\kappa_0} < \psi_{h,\Omega} = \left(\frac{Q_{h,\Omega}}{Q_{h,0}}\right)^2 = \left(\frac{\phi_{h,u}\left(1 - C_{h,p}\right) + \phi_{h,p}\left(C_{h,p}\right)}{\phi_{h,u}}\right)^2 = \left(1 - \rho\pi C_h\right)^2 \quad . \quad (4.22)$$

The combination of effects mediated by the immediate impact on vector population and delayed impact on malaria parasite prevalence and mean infectiousness in the human population therefore is assumed to at least the same as the product of the two,

$$\hat{\psi}_{h,u,\Omega} < \frac{\hat{\kappa}_\Omega}{\kappa_0}\psi_{h,u,\Omega} = \left(\frac{Q_{h,\Omega}}{Q_{h,0}}\right)^4 = \left(\frac{\phi_{h,u}\left(1 - C_{h,p}\right) + \phi_{h,p}\left(C_{h,p}\right)}{\phi_{h,u}}\right)^4 = \left(1 - \rho\pi C_h\right)^4 \quad . \quad (4.23)$$

The most obvious implication of these simplified models is captured directly in Equations (4.18) and (4.20). For very zoophagic vectors, overall impact is directly related to efficacy of personal protection, regardless of whether that arises from deterrent or toxic models of action. The only other primary determinants are crude coverage (C_h) and the proportion of non-user exposure occurring when the protective measure can be used practically (π).

4.4.3 Thresholds Necessary to Attain Epidemiological Impact

In all the panels of Figure 6.2, the x-axis is the proportion of human non-user exposure to mosquito bites that occurs at times when a user would actually use the protective intervention (π), which was plotted in values decreasing from 0.9 to 0.1 in increments of 0.1. The y-axis represents the proportion of mosquito bites prevented while actually using protective intervention obtained by taking the product of $C_h = 0.8$ and the values from Equation (4.19). The z-axes reflects immediate (panels **A** and **B**) and delayed (panels **C** and **D**) impact upon relative exposure experienced by non-users. While the latter assumes that delayed effects upon human-to-mosquito transmission occur if immediate reductions in the ability of mosquitoes to mediate transmission to humans are sustained over a long time [128]. Therefore, Figure 6.2 is produced as follows: the x-axis in all panels are π values decreasing from 0.9 to 0.1, the y-axis are calculated protective ρ values from the given expression. In other hand, a different equation was used for each panel to obtain values for z-axis by using corresponding π and protective ρ values substituted into Equation (4.20) (panel **A**), Equation (4.21) (panel **B**), product of values from Equations (4.20) and (4.21) (panel **C**), and Equation (4.23) (panel **D**).

In Figure 6.2, the reader can note that the values in the z-axes only start dropping substantially at higher values of the x and y axes. Thus, Figure 6.2 illustrates how these simplified models indicate that personal protection measures

will need to be practically applicable at most times of the day when exposure can occur ($\pi \geq 0.8$), confer high levels of person protection to users ($\rho \geq 0.8$), and be used by the majority of human population ($C_h \geq 0.8$), if they are to appreciably suppress malaria transmission by zoophagic vectors.

4.5 Discussion and Conclusion

Human blood index, defined as the proportion of a mosquito population that feeds upon humans, is clearly as important a determinant of malaria transmission and control (Figure 6.1) today [111] as it was half a century ago [103]. In simple terms, the more a vector depends upon human blood, the greater will be the impact of personal protection measures upon their population density, longevity, and transmission potential, and the greater will be the advantage of pesticides which act exclusively through contact toxicity over those relying upon repellency (Figure 6.1). However, the more zoophagic a mosquito species is, the more personal protection can act simply by blocking host-vector contact (Figure 6.1) so that it becomes increasingly irrelevant whether protection is achieved through toxicity or repellency so that a wider variety of target product profiles may be considered [5].

The world's malaria vectors span the full range of baseline human blood indices considered here [15, 103], so this remains a critical parameter for national control programmes to evaluate and consider when planning vector control

campaigns. The findings from the models presented apply specifically to very zoophagic vectors, mosquitoes with a strong preference for animals which normally obtain less than 10% of their blood meals from humans, but may still mediate malaria transmission. While the simplified models developed here only apply in settings where a purely anthroponotic pathogen is transmitted by a predominantly zoophagic vector, this counterintuitive situation is remarkably widespread and important. Approximately 40% of all *Plasmodium falciparum* infections [135] and 95% of *Plasmodium vivax* infections [136] occur outside of sub-Saharan Africa, largely in parts of Asia where a wide diversity of primary vectors predominantly feed on animals rather than humans [15]. This extreme scenario contrasts starkly with the anthropophagic vectors, such as *An. gambiae, An funestus,* and *An koliensis,* that have dominated the thinking behind global malaria control policy [29, 137, 138]. However, it is important to note that many of the most important species in residual transmission systems, such as *An. arabiensis* in Africa and *An. farauti* in the Pacific, are both zoophagic and anthropophagic, so that they sit between these two extremes. Surveys of human blood indices, or underlying host preference indices such as relative availability [109, 114], relative attack rates [139], or feeding indices [140, 141] therefore should be considered as important indicators in national entomological monitoring systems.

Where such surveys confirm very low human blood indices, the minimum

immediate (Equation (4.21)) and delayed (Equation (4.23)) impacts of a personal protection measure upon transmission by very zoophagic mosquitoes can be calculated approximately with very simple models using only three parameters, which may potentially be measured in the field by National Malaria Control Programmes (NMCPs) and their supporting national institutional partners in developing countries: the maximum proportion of human exposure to mosquitoes that can be directly prevented through personal protection by using a given intervention, its protective efficacy when used, and the demographic coverage of human users. The relationship between entomologic inoculation rate (EIR), which is a direct, field-measurable indicator of human exposure to bites of mosquitoes infected with transmissible sporozoite stage malaria parasites [77, 112] and the efficacy of a personal protection measure was derived through a model that logically describe the process of mosquito feeding cycle and malaria transmission.

The suggestion that the impact of personal protection upon malaria transmission by very zoophagic vectors may be independent of the mode of action of the product has substantial implications for manufacturers and NMCPs alike. Unlike transmission mediated by anthropophagic vectors [2, 5], the impact upon malaria where zooophagic vectors predominate is a simple function of personal protective efficacy regardless of whether that arises from deterrent or toxic modes of action. Vapor-phase repellents [142, 143, 144, 145] do not require direct physical

contact with target insects. They can protect one or more individuals without comprehensively treating wall, roof, net, clothing, or skin surfaces, so high levels of personal protection may be easier to achieve in practice [5] than with the contact toxins that are clearly superior for vectors that feed indoors upon humans [2]. Such spatial repellents therefore may be particularly applicable, and even preferable to contact toxins, where malaria transmission is predominantly mediated by very zoophagic vectors, especially where transmission primarily occurs outdoors. While we present initial modeling results here, further empirical field testing of this model is essential to build solid evidence to guide malaria control programs.

In conclusion, we have extended a published malaria transmission model to examine the relationship between transmission, control, and the baseline human blood index for very zoophagic vectors. The results from model are very simple and can be used by vector control practitioners to forecast the likely immediate and delayed impacts of personal protection measures using three parameters that may potentially be measured in the field: the proportion of human exposure to mosquitoes occurring when a intervention can be practically used, its protective efficacy when used, and demographic coverage of human users. High levels ($= 80\%$) of protective coverage and efficacy are important to achieve an epidemiologically meaningful impact.

4.6 Transition to Chapter 5

The models presented in this chapter also are used to discuss biologically meaningful coverage indicators necessary for eliminating malaria transmission as discussed in chapter 5. The strategies that can be used to control zoophagic vectors discussed in this Chapter are presented in Chapter 5.

CHAPTER 5

Biologically Meaningful Coverage Indicators for Eliminating Malaria Transmission

This chapter is adapted from [146], as published in *Biology Letters Journal*.

Abstract

Mosquitoes which evade contact with long-lasting insecticidal nets and indoor residual sprays by feeding outdoors or upon animals are primary malaria vectors in many tropical countries. They can dominate residual transmission where high coverage of these front-line vector control measures is achieved. Complementary strategies, which extend insecticide coverage beyond houses and humans, are required to eliminate malaria transmission in most settings. The overwhelming diversity of the world's malaria transmission systems, and optimal strategies for controlling them, can be simply conceptualized and mapped across a two-dimensional scenario space defined by the proportion of blood meals that vectors obtain from humans and the proportion of human exposure to them which occurs indoors.

5.1 Main Text: Description of Biologically Coverage Indicators for Eliminating Malaria

Indoor residual spraying (IRS) and long-lasting insecticidal nets (LLIN) can reduce malaria transmission dramatically but will not be sufficient to eliminate it completely from most endemic tropical settings, even if effective drugs and vaccines are available, primarily because of vectors which evade contact with domestic applications of insecticides [31]. At high coverage, most of the protection conferred by these intra-domiciliary measures against malaria transmission by mosquitoes that primarily feed indoors (endophagic) or rest (endophilic) indoors, and primarily feed upon human blood (anthropophagic), occurs at the community level and arises from reduced rates of vector population survival, human blood feeding, and reproduction [27]. However, mosquitoes which can rest outdoors (exophilic) or feed outdoors (exophagic), as well as those which feed on animals (zoophagic), are primary malaria vectors in many tropical countries and are obviously less vulnerable to control with insecticides deployed to houses in the form of LLINs and IRS [31, 5, 147].

Exophagic and zoophagic vectors can therefore comprise an increasingly important fraction of residual transmission in settings where high demographic coverage of LLIN or IRS has successfully suppressed predominant species that primarily feed indoors upon humans [7, 8, 10, 11, 2, 100]. For any product conferring personal protection against mosquito bites, it is therefore critical to

measure the proportion of human exposure to mosquito bites that otherwise occurs at times when it is practical to use it (π) [4]. In the case of LLINs, this definition can be specified approximately as the proportion of normal exposure to mosquito bites upon humans lacking LLINs which occurs indoors when it would be practical to use one (π_i) and measured in the field by weighting the observed indoor (i) and outdoor (o) biting rates at each period of the night by the surveyed mean proportion of humans that are in these two compartments at that time [9, 103, 107, 148]. Where this parameter changes in response to intervention pressure, such changes typically reflect successful control and altered vector population composition so the most immediately relevant estimate of this parameter is the baseline value ($\pi_{i,0}$) in the pre-intervention scenario (Ω) before the effective scale-up of those interventions ($\Omega = 0$). *De facto* protective coverage of humans ($C_{h,p}$) with LLINs, or any other form of personal protection against indoor exposure, is therefore defined slightly more specifically than before [2, 4, 5], as the product of crude coverage (C_h; estimated as the reported nightly usage rate) and this proportion of personal human exposure which is practically and directly preventable with an LLIN [2], as shown in Table 5.1;

$$C_{h,p} = \pi_{i,0} C_h \ . \tag{5.1}$$

Obviously, the lower the proportion of exposure to a given mosquito population that

Table 5.1: Definitions and explanations for symbols & abbreviations [1, 2, 3, 4, 5]

Symbol	Definition and explanation
A	Total availability of all hosts: rate at which a single mosquito encounters and attacks all hosts.
A_h	Total availability of all hosts: rate at which a single mosquito encounters and attacks all human hosts.
$A_{h,p}$	Total availability of all protected hosts: rate at which a single mosquito encounters and attacks all human hosts while protected.
C_h	Crude coverage of humans: Proportion of people using an LLIN, or similar measure for protection against mosquitoes, each night.
$C_{h,p}$	Protective coverage of humans: The proportion of all exposure of the human population which is effectively covered by use of protective measures.
$C_{A,p}$	Protective coverage of all available blood sources: The proportion of all exposure of all available hosts which is effectively covered by use of protective measures.
μ_p or μ_u	Probability that a mosquito which attacks a host will die during the attack upon a protected or unprotected host, respectively.
N_h	Number of human hosts.
P_γ	Probability that a mosquito survives the host attack events in a single complete feeding cycle.
π	Proportion of normal exposure to mosquito bites upon humans lacking a given personal protection measure, that occurs at times when it would be practical to use it.
$\pi_{i,0}$	Baseline proportion of normal exposure to mosquito bites upon humans lacking LLINs, which occurs indoors when it would be practical to use one, before any interventions are introduced.
ρ	Overall proportion of personal protection against mosquito bites provide by using a given protective measure.
$Q_{h,0}$	Baseline human blood index: the proportion of all blood meals which are obtained from humans before any interventions are introduced.
$\psi_{h,\Omega}$	Relative exposure of the average human (h) to infectious mosquito bites in a given intervention scenario (Ω): calculated as a quotient of their exposure divided by that in the absence of any intervention.
Ω	Intervention scenario defined by coverage level with a specific intervention measure

occurs indoors, the lower will be the impact of LLINs or IRS upon the transmission it mediates, and the more persistent and prominent those populations will be in residual vector systems. Current demographic indicators of coverage for LLINs and IRS often grossly over-represent the degree of insecticidal hazard to which vector mosquitoes are exposed. A conventional demographic view of the current global target of 80% LLIN use among all age groups is presented in Figure 5.1A. However, as illustrated in Figure 5.1B, only 40% *de facto* protective coverage of humans is achieved in a scenario with 80% demographic coverage, when only 50% of human exposure occurs indoors.

However, *de facto* coverage is a biological parameter relating to the coverage of all blood resources that mosquitoes need to thrive, and is often even lower than apparent from Figure 5.1B. The baseline human blood index ($Q_{h,0}$) is defined as the population-wide mean proportion of blood meals that are obtained from humans (h), rather than animals, before the introduction of any intervention ($\Omega = 0$). This parameter can be readily measured in the field and has long been known as an important determinant of malaria epidemiology and intervention impact [103]. The impact of LLINs or IRS upon the population size and transmission potential of zoophagic vectors is attenuated, even if comprehensive protective coverage of humans is achieved ($C_{h,p} \to 1$), because killing them in sufficient numbers to suppress malaria transmission requires high protective coverage of all available

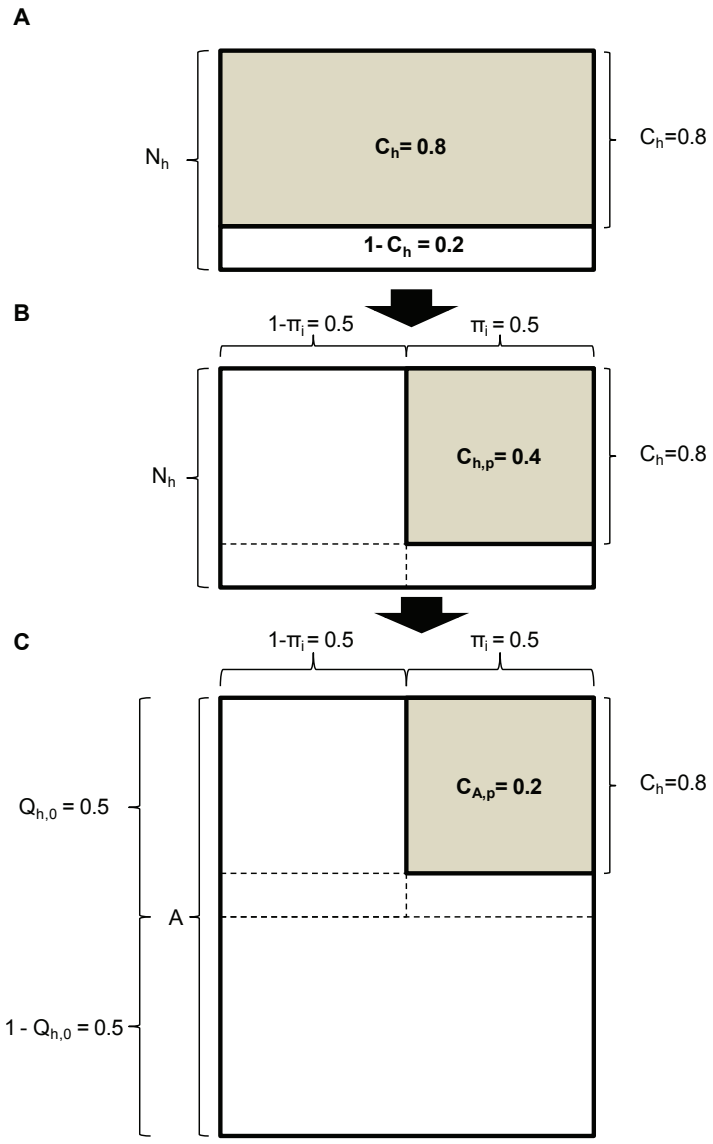

Figure 5.1: Conceptual schematic of the difference between current demographic indicators of coverage of all humans (N_h) and true biological coverage of all available mosquito blood resources.
In all panels, the proportion considered covered by the stated indicator is represented by the shaded fraction. (a) Conventional view of current LLIN/IRS target of 80% crude demographic coverage of all humans while indoor ($C_h = 0.8$). (b) Protective coverage of humans at all times when either indoors or outdoors ($C_{h,p}$; Equation 5.1) where half of human exposure to vectors occurs outdoors ($\pi_{i,0} = 0.5$). (c) Biological coverage of all blood resources ($C_{A,p}$), equivalent to the covered proportion of all available human and animal blood ($\frac{C_{h,p}A_h}{A}$; Equation 5.2) in a scenario where half of human exposure to vectors occurs outdoors ($\pi_{i,0} = 0.5$) and animals previously accounted for half of all bloodmeals ($Q_{h,0} = 0.5$).

blood sources ($C_{A,p}$), including animals. This biological indicator of resource coverage is simply the product of the pre-intervention ($\Omega = 0$) human blood index ($Q_{h,0}$) and the protective coverage of humans ($C_{h,p}$) [4],

$$C_{A,p} = \frac{A_{h,p}}{A} = \frac{C_{h,p}A_h}{A} \approx C_{h,p}Q_{h,0} = \pi_{i,0}Q_{h,0}C_h \ , \tag{5.2}$$

where A, A_h, and $A_{h,p}$ are the total availabilities or kinetic rates of encounter and feeding and attacking all hosts, all humans, and all humans while protected, respectively [2].

Figure 5.1C illustrates how 80% demographic coverage of human users could result in only 20% coverage of the total blood sources available for mosquitoes when the vector obtains half of its blood meals from animals and is equally likely to feed indoors and outdoors. The impact of LLIN or IRS interventions upon vector populations, and therefore the associated selection pressure for heritable resistance traits, are both directly related to this more biologically meaningful coverage indicator with the following simplified form of previous formulations [4]:

$$P_\gamma = 1 - (\mu_p C_{A,p} + \mu_u(1 - C_{A,p})) \ , \tag{5.3}$$

where P_γ is the probability of a mosquito surviving all host attacks in a single

feeding cycle, while μ_p and μ_u represent the mortality probabilities of mosquitoes attacking protected and unprotected hosts, respectively.

The importance of host preference behaviour is best illustrated by the numerous mosquito species that rarely feed on humans, but which do so often enough to sustain stable malaria transmission ($0 < Q_{h,0} < 0.1$) [4], and are primary malaria vectors across much of Asia and the Americas [15]. In stark contrast to settings with strongly anthropophagic vectors [2], LLINs and IRS have far less impact upon malaria transmission by highly zoophagic mosquitoes simply because human blood is of negligible importance to their survival and reproduction [4]. Nevertheless, LLINs and IRS can deliver appreciable community-level protection, for both users and non-users, against transmission by zoophagic vectors where exposure predominantly occurs indoors [4]. This is because humans are the only host for the common malaria parasites (*Plasmodium falciparum* and *P. vivax*), so the small proportion of a very zoophagic mosquito population that is killed or diverted by these insecticidal products when they encounter humans can be a large proportion of those that actually transmit malaria [4]. As malaria transmission requires at least two feeding contacts between a given mosquito and its human victims, overall minimum immediate impact upon transmission by very zoophagic vectors can be approximated as a very simple squared function of the protective coverage of humans ($C_{h,p}$; Equation 5.1) and the entomologically-measured estimate

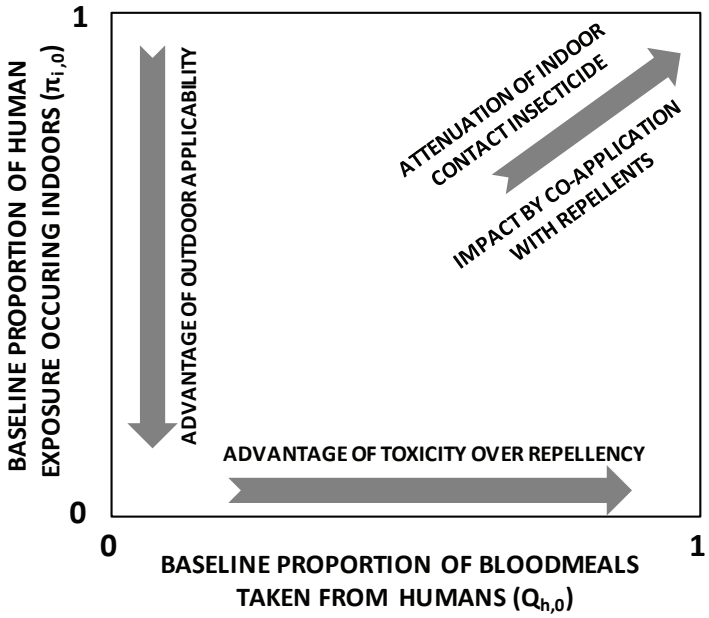

Figure 5.2: A conceptual summary on diversity of vector scenarios.
A conceptual summary of the conclusions of recent deterministic modelling analyses [1, 2, 3, 4, 5] comparing vector control product profiles with a variety of repellent and/or toxic properties in a mapped across the full range of preferences for feeding upon humans indoor versus outdoor ($\pi_{i,0}$) and upon humans versus animals ($Q_{h,0}$).

of direct personal protective efficacy against biting exposure (ρ) [4]:

$$\lim_{Q_{h,0}\to 0}(\psi_{h,\Omega}) = (1-\rho C_{h,p})^2 = (1-\rho\pi_{i,0}C_h)^2 \,, \tag{5.4}$$

where $\psi_{h,\Omega}$ is the relative rate of exposure to malaria transmission of the average human (h) community member immediately after rapidly achieving a specific vector control scenario (Ω) defined by the protective coverage and protective efficacy of LLINs or IRS, compared to the average non-user under baseline conditions before scale up [4].

LLINs or IRS are clearly insufficient in themselves to eliminate malaria transmission because *de facto* protective coverage is attenuated where mosquitoes can readily access blood resources from animals or from humans while they are outdoors (Figure 5.1C) [1, 2, 3, 4, 5]. As increasing numbers of national programmes attain and sustain high coverage of indoor spaces with IRS or ITNs, complementary strategies are increasingly needed that extend insecticide coverage beyond the house, and indeed beyond humans. Defining, measuring, and targeting blood resources other than humans inside houses, which mosquitoes depend upon for survival and which enable them to escape current front-line measures such as LLINs and IRS, are becoming increasingly important. This requires change in perspective for the responsible communities that have exclusively emphasized human and domestic targets for malaria vector control. Clear understanding of mosquito resource availability, and how to cover them with mosquitocidal measures, is required to eliminate malaria transmission by the diverse array of exophagic, exophilic and zoophagic vectors that exist worldwide. Neglected strategies, such as insecticide-treated clothes, insecticide–treated livestock, repellents, odor-baited traps, or larval source management, will be needed to complement LLINs and IRS to drive malaria parasite populations to extinction [59]. The development and implementation of these novel technologies will require a vastly improved understanding of the ecology of mosquitoes generally, rather than just the handful

of highly efficiently anthropophagic vectors that have been the overwhelming focus of research thus far [59].

Fortunately, Figure 5.1C represents a simple framework with which the overwhelming diversity of the world's malaria transmission systems, and optimal strategies for controlling them with high coverage ($C_h \rightarrow 1$) of adulticides [2-4, 12, 20, 21], can be readily conceptualized, using only two summary parameters of adult mosquito behaviour that can be readily measured in the field ($\pi_{i,0}$) [13-16] and $Q_{h,0}$ [18, 23]. For example, the conclusions of recent modelling analyses for comparing product profiles with a variety of repellent and/or toxic properties in a diversity of vector scenarios, spanning the full range of preferences for feeding upon humans indoor versus outdoor ($\pi_{i,0}$) and upon humans versus animals ($Q_{h,0}$) [2, 5, 4], can be mapped across field-measurable two-dimensional parameter space (Figure 5.2), in an intuitive format that is open to experimental evaluation by field epidemiologists, entomologists and ecologists.

5.2 Transition to Chapter 6

The concept of biological coverage presented in this chapter is extended in [51] to rationalize vector control impact based on resource (e.g., blood, resting, and oviposition sites) utilization rates. We have adapted some of the formulations in [51] to develop models that can be used to predict the scenarios of success for the

autodissemination of pyriproxyfen by malaria vectors based on their two resources (i.e., resting and oviposition sites).

CHAPTER 6

Predicting Scenarios of Success for the Autodissemination of Pyriproxyfen by Malaria Vectors from their Resting Sites to Aquatic Habitats; Description and Sensitivity Analysis of a Field-Parameterizable Model

This chapter [149] will be submitted to *PloS One* or *Transactions of the Royal Society of Tropical Medicine and Hygiene Journals* for publication consideration.

Abstract

Background: The leading malaria vector control strategies (i.e., long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS)) can reduce indoor transmission, but these tools alone are insufficient to eliminate it. Strategies that complement LLINs and IRS by targeting adult mosquitoes when they feed on animals or humans outdoors or target mosquitoes at their immature stages also are needed to achieve malaria elimination. Large-cage semi-field system (SFS) experiments indicate a potential for autodissemination of insecticide (i.e., a transfer of pyriproxyfen (PPF) (mosquito emergence inhibitor) by *Anopheles arabiensis* mosquitoes from resting sites to aquatic habitats) as one option to complement LLINs and IRS. These experiments also indicate that coverage is amplified in two steps: (1) partial coverage of resting sites with PPF contamination results in far

higher contamination coverage of adult mosquitoes because they are highly mobile and use numerous resting sites per gonotrophic cycle, and (2) even greater contamination coverage of aquatic habitats results from accumulation of PPF from multiple oviposition events.

Methods and Findings: Differential equation models are described that use only field-measurable input parameters and capture the biological processes that mediate autodissemination of PPF. Recent successes in enclosed SFS can be rationalized, and the plausibility of success in full field application can be evaluated *a priori*. The model also defines measurable properties of different prototypes that may be conveniently and rapidly optimized under controlled experimental conditions to maximize chances of successful application at ecosystem scale in full field trials. While perhaps the most obvious flaw in this model is the endogenous relationship that inevitably occurs between the output parameter and one of the input parameters if the target mosquito species is used to mediate PPF transfer, this helps illustrate the naturally self-limiting feedback loop that occurs between impact and densities of ovipositing mosquitoes mediating autodissemination, thus illustrating the potential advantages of using a different mosquito species that shares the same aquatic habitats as the primary target for contamination at selected resting sites. For autodissemination interventions to eliminate malaria transmission or vector populations during the dry season window of opportunity will require

comprehensive contamination of all aquatic habitats ($C_l \to 1$), including the most challenging subset of these that persist or retain PPF activity for as little as a week ($C_{l,x} \to 1$, where $U_x = 7$ days). The model presented here suggests that to achieve greater than 99% contamination coverage of this ephemeral aquatic habitat subset will necessitate successful contamination of most mosquitoes in the population ($C_M \to 1$), and that the quotient of the ovitrap-detectable rates of oviposition by wild mosquitoes into this subset of habitats, divided by the titre of contaminated mosquitoes required to render them unproductive, also will have to at least approach unity ($m_{l,x,z,d}/T_{l,x,z,d} \to 1$).

Conclusions: The simple multiplicative relationship between C_M and $m_{l,x,z,d}/T_{l,x,z,d}$, and the fact that their combined effect can be described as a simple exponential decay of uncontaminated aquatic habitats, allows ready application of this model by theoreticians and field biologists alike. The most important caveats and limitations to applying this model relate to uncertainties about the validity of the underlying simplifying assumptions and the natural or achievable ranges of its input parameters.

6.1 Introduction

The leading malaria vector control strategies (i.e., long-lasting insecticidal nets (LLINs) [150] and indoor residual spraying (IRS) [151]) can dramatically reduce

transmission by indoor-biting mosquitoes, but these tools alone are insufficient to eliminate it. The success of LLINs and IRS rely on their ability to control indoor-feeding mosquitoes (e.g., *An. gambiae*) that heavily rely upon human blood for survival. So, their impacts are limited by the fact that many important primary vectors across the world [152, 153, 154], and particulary *An. arabiensis* in the East African context [155, 156, 157, 158, 159, 160, 161] sometimes can evade contact with them and survive by feeding upon animals or humans outdoors. Hence, to achieve malaria elimination, strategies are needed that target mosquitoes when they feed on animals or humans outdoors or while using one of the other biological and environmental resources such, sugar, mating sites, resting sites, and oviposition sites [162]. Some of most promising strategies that might be used to complement LLINs and IRS by targeting adult mosquitoes outdoors include vapour-phase repellents [163], insecticide-treated clothing [164, 165] insecticide-treated cattle [166], and odor-baited traps [167, 168]. Another, far older strategy, that has been used to suppress vector densities in a variety of contexts, is to prevent emergence of adults at source by applying insecticides to their aquatic larval habitats [169]. While this approach has achieved some striking successes against malaria vectors and transmission, even in Africa, its applicability and effectiveness may be limited by the substantial logistical challenges and associated costs of comprehensively and continuously identifying and treating relevant breeding habitats, especially in large rural areas with sparse human population [169, 170, 171].

However, it has proven possible to deliver larvicides by contaminating adult mosquitoes when they rest inside treated containers so that when they subsequently oviposit, the insecticide is transferred to their aquatic habitats [172]. Such autodissemination of insecticide from resting sites to the aquatic habitats via adult mosquitoes requires particularly potent larvicides, such as the juvenile hormone analogue pyriproxyfen (PPF) that interrupts normal development and metamorphosis of targeted mosquitoes [173, 174]. The autodissemination strategy was first demonstrated in the ideally-suited Dengue vector, *Aedes aegypti,* which breeds in sealed containers that retain PPF and protect it against extremes of temperature and solar radiation [172]. More recently, autodissemination of PPF by the malaria vector *Anopheles arabiensis* has been demonstrated in large-cage SFS experiments, but it remains to be seen whether similar levels of success can be achieved with field populations under natural conditions [175].

It is therefore essential to understand quantitatively and verify the influence of the distinct processes that drive autodissemination phenomenon, so that this strategy can be rationally designed, optimized, and evaluated. Autodisseminition of PPF previously has been described using a mathematical model [176] that crudely describes the relationship between the effective coverage of adult resting sites (C_r) and larval habitats (C_h) with PPF contamination, using a simple exponential model of PPF accumulation and decay based on the time (in days or nights) over which

contaminated habitats persist but remain unproductive (U), the total number of ovipositions (O) by the entire adult population per night, the number of larval habitats (H), and the number of contaminating events needed to make a single habitat unproductive (Ω):

$$C_h = 1 - e^{\frac{-C_r U O}{\Omega H}}. \tag{6.1}$$

While the durations over which contaminated natural habitats persist but remain unproductive (U) in principle may be measured by direct observation of habitat persistence and sampling or pupae or emerging adults, it is difficult to define and impossible to measure absolute values for the input parameters C_r, H, O, and Ω as originally defined [176]: (1) The absolute proportion of all resting sites that have been contaminated with PPF (C_r) is inestimable because it is not feasible to define measurable units for all forms of resting sites, much less survey them [177, 178], (2) The number of hydrologically independent habitats (H) cannot be quantified simply because it is not practically possible to clearly and measurably define what constitutes genuine larval habitat, as opposed to the water bodies they are associated with [178], (3) the number of ovipositions carried out each night by the entire population across all habitats (O) or even per habitat in a sample of habitats (O/H) cannot be quantified because the only existing trap for capturing free-flying, wild *Anopheles* when they oviposit in natural aquatic habitats only samples unknown fractions of the total number of ovipositing gravid females visiting those

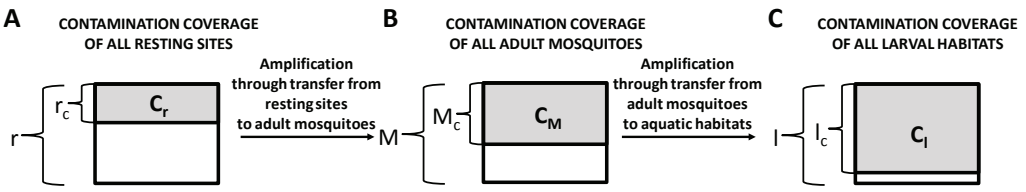

Figure 6.1: PPF double amplification.
A schematic illustration of how partial coverage all resting sites is amplified in two steps as PPF contamination is transferred to the adult mosquito population and then onwards to the larval habitats. The coverage of resting sites (C_r), adult mosquitoes (C_M) and larval habitats (C_l) is depicted as a proportion of all resting sites (r), adult mosquitoes (M) and larval habitats (l) covered with PPF contamination (c).

habitats [179], which are in themselves impossible to distinguish and quantify [178], while other prototypes may be applied only to artificial sentinel habitats [180, 181], (4) number of contaminating events needed to make even a single, selected habitat unproductive (Ω) cannot be estimated because, as described for (H), naturally occurring habitats are extremely difficult to define and distinguish [178], and because titration by introducing varying numbers of contaminated mosquitoes into such habitats within cages placed over them do not necessarily correspond to equivalents number of contamination events, which are in themselves impossible to quantify with existing oviposition traps for the same reasons as (O/H).

Furthermore, recent large-cage SFS experiments with *An. arabiensis* [175] clearly demonstrate that autodissemination via this vector species, which is behaviorally resilient to control with LLINs or IRS [155, 156, 157, 158, 159, 160, 161, 182], actually involved two coverage amplification

steps (Figure 6.1), rather than merely one as previously assumed [176]. The previous formulation [176] assumed that contamination coverage of resting sites (C_r) and coverage of the adult mosquito population (C_M) are equivalent, and that coverage amplification occurs as PPF is accumulated in larval habitats through repeatedly transfer from contaminated adults. However, these large-cage experiments [26] demonstrate how coverage amplification also occurs as PPF is transferred from the resting sites to the mosquito population (Figure 6.1): Taking the proportion of all sampled mosquitoes that were recovered from clay pots as a crude indicator of resting site coverage ($C_r = 0.17$) and contrasting this with the high proportion of mosquitoes caught outside of the pots that were contaminated ($C_M = 0.72$), illustrates how an approximately four-fold amplification ($C_M/C_r = 0.72/0.17 = 4.2$) apparently occurred. This additional amplification step presumably occurs because mosquitoes move around through several resting sites over the course of a night, as demonstrated by the direct observation of such high proportions of contaminated mosquitoes outside of the treated pots.

Here, we revise and reformulate the previously published model [176], and adapt some formulations from another more recent model that allows multiple resource utilization events per gonotrophic cycle to be measured and accounted for [177] to enable the modelling of a range of alternative approaches to implementing such a double-amplification autodissemination strategy using only input parameters

that are field-measurable. Simulation analysis explores conditions at which an autodissemination of insecticide strategy might be successful in the field.

6.2 Methods

Since the publication of the model described in Equation 6.1 [176], we have developed a broader set of generalizable models for capturing the effects of a wide variety of intervention strategies that target diverse resources and resource subsets which mosquitoes utilize [167, 177, 183, 184, 185, 186, 187]. Here we adapt the model [176] notations and definitions to harmonize them with these broadly applicable frameworks and to enable development into a far more explicit, practically applicable, and field-parameterizable form. In particular, the notations and definitions are revised to enable the modelling of a range of alternative approaches to the autodissemination strategy in a way that explicitly captures the changing levels of coverage achieved as PPF is transferred, first from one of several possible resource subsets that could act as targets for initial delivery to the adult mosquito population via contact contamination, and then from those adult mosquitoes to the ultimate aquatic habitat targets when they make contact by oviposition (Figure 6.1). First of all, the notation is adjusted (Table 6.1) by 1) substituting C_M for C_r to reflect direct dependence of larval habitat coverage (C_l) upon coverage of the mosquito population (C_M) and only indirectly upon coverage

of the resting sites (C_r), 2) substituting T_l for Ω to reflect potential for measurement by mosquito exposure titration experiments and to prevent overlap in meaning with previous uses of the symbol Ω to reflect distinct vector control scenarios [167, 177, 183, 184, 185, 186, 187], and 3) substituting l for H to enable consistent use, not only as a subscript to specify larval habitat coverage (C_l), but also as all forms of that entire specific resource ($R = l$) [177] as illustrated in Figure 6.1. Equation 6.1 is therefore reformulated as

$$C_l = 1 - e^{-\frac{C_M U O}{T l}} . \tag{6.2}$$

6.2.1 Amplification of Contamination Coverage Through Transfer of Pypriproxifen from Treated Resting Sites to Adult Mosquitoes

We have revised the definition of the coverage term on the right hand side of Equation 6.1 to represent more accurately its original conceptual basis. This coverage term was originally and mistakenly described as the coverage of all resting site resources (C_r) [176], but the conceptual basis of the equation is that it describes the coverage of the ovipositing adult mosquito population with PPF contamination (C_M). Therefore, the original formulation implicitly assumed that the proportion of all resting sites contaminated (C_r) and the proportion of the adult mosquito

Table 6.1: Symbols and their definitions.

Symbol	Definition
C_h	Demographic coverage of the human (h) subset of all available blood sources
$\pi_{h,i}$	Proportion of human exposure to mosquito bites that would occurs indoors (i) in the absence of any protective intervention
Q_h	Proportion of all available blood meals that originate from the human (h) host species subset.
C_r	The proportional coverage of *all available forms* of a given resting site (r) with PPF
α_r	Utilization rates for all available forms of a given resting site (r), defined as the rate at which individual mosquitoes attempt to utilize it per gonotrophic cycle
$\alpha_{r,x,z}$	Utilization rates for a defined subset of a given resting site that has been identified (x) and surveyed entomologically (z) in the field ($r_{x,z}$), defined as the rate at which individual mosquitoes attempt to utilize the PPF covered subset per gonotrophic cycle
ε_d	Detection efficiency of a given trapping (sticky trap) or observational method used to detect utilization of a defined (x) and entomologically surveyed (z) subset of given oviposition site ($\mathrm{l}_{x,z}$), defined as the proportion of events occurring within that subset over the survey period that are detected
$m_{r,x,z}$	Number of mosquitoes trapped or observed utilizing a surveyed sample subset (z) of any identifiable and targetable subset (x) of a given resting site ($r_{x,z}$)
$m_{v,z}$	Number of mosquitoes trapped or observed utilizing a defined, entomologically surveyed sample subset (z) of blood resources (v_z)
$M_{v,z}$	Number mosquitoes that completed a feeding cycle in an environment subset co-surveyed for both feeding and resting site resources and adjusted for protection and blood meals obtained from other sources
$m^{min}_{\mathbf{r},x,z}$	Minimum number of mosquitoes that can be detected utilizing a defined, entomologically surveyed subset of a resting site ($r_{x,z}$)

Table 6.2: Symbols and their definitions (cont.)

Symbol	Definition
$\mu_{R,c}$	Mortality probability associated with exposure to a covered form of the resource through a single utilization event
N_h	Number of humans (h) living in a defined setting
$N_{h,z}$	Number of persons sampled by an entomological survey (z) of mosquitoes attacking human (h) hosts
$N_{h,?}$	Number of persons residing in all houses sampled by an entomological survey (Ω) of mosquitoes attacking human (h) hosts
$P_{\propto_{r,c}}$	Probability of a mosquito surviving all attempts to utilize intervention-covered forms of the targeted resting site per gonotrophic cycle
ζ	Probability associated with exposure to a PPF contaminated form of the resting site through a single utilization event
R	The total availability of all forms of a given resource, defined as the rate at which individual mosquitoes encounter and attempt to utilize it per night
R_c	The total availability of all forms of a given resource that are covered with an intervention (c), defined as the per night rate at which individual mosquitoes encounter and attempt to utilize that covered resource subset
R_x	The total availability of all forms of a given resource that can be identified and targeted with an intervention (x), defined as the per night rate at which individual mosquitoes encounter and attempt to utilize that identifiable, targetable resource subset
C_l	Proportion of all larval habitats (l) which are effectively contaminated with PPF
$\alpha_{r,x}$	Utilization rate of the subset of resting sites
$c_{r,x}$	Proportion of subset of resting sites which are contaminated with PPF
U	Mean time that habitats persist but remain unproductive following contamination with PPF
$T_{l,x,z,d}$	The minimum rate at which contaminated ovipositing females are captured by sticky traps placed at a sample of aquatic habitat, that is required to render those habitats unproductive within one night
$m_{l,x,z,d}$	Rate at which oviposition events are detected by sticky traps placed at samples of natural habitats

population that were contaminated (C_M) are equivalent ($C_M \approx C_r$) because each mosquito rested in only one location per gonotrophic cycle.

Coverage of the mosquito population (C_M) may be measured directly by testing samples of individual mosquitoes for PPF contamination or its biological activity [175], or alternatively with a variety of markers [188] that can be used as more convenient, readily-detected surrogates for PPF contamination. However, as described in the following subsection, (C_M) also may be estimated from entomological surveys of the resting site utilization processes that directly mediates it.

Contamination Coverage of the Mosquito Population as a Function of Coverage and Utilization Rates of Targeted Resting Site Subsets

The assumption that a mosquito visits a resting site only once per gonotrophic cycle is questionable for many mosquito species [189]. Furthermore, recent experimental observations that a high proportion of mosquitoes caught outside the pots were treated with PPF [26] clearly demonstrate just how inaccurate this assumption is in relation to *An. arabiensis* specifically. Therefore, we introduce an additional, intermediate parameter which describes coverage of the entire adult mosquito population (M) that mediates autodissemination of PPF. In this revised formulation, coverage of the mosquito population (C_M) that mediates transfer from

the treated resource to the aquatic habitat resource (l) is assumed to be a function of the rate at which all resting sites that are covered with PPF contamination (r_c) are visited by mosquitoes ($\alpha_{r,c}$), which is in turn the product of the coverage of all available contaminated and uncontaminated resting sites [28] (C_r) and the rates at which individual mosquitoes utilize all available resting site surfaces (α_r):

$$C_M = f\left(\alpha_{r,c}\right) = f\left(\ \alpha_r, C_r\right). \tag{6.3}$$

The latter utilization rate term is defined as the mean number of times an individual mosquito makes physical contact with any contaminated or uncontaminated resting site surface during a typical gonotrophic cycle. Hence, instead of assuming the proportion of all contaminated adult mosquitoes is approximately equivalent to the proportion of all available contaminated resting sites ($C_M \approx C_r$), we present an exponential relationship relating coverage of the mosquito population (C_M) to coverage (C_r) and utilization rate (α_r) of all available resting sites (r), rather than just those that have been covered with PPF contamination (r_c). Also, the new terms for the per gonotrophic cycle utilization rate of a resource (α_R) or resource subset ($\alpha_{R,x}$) also is previously introduced [177], so that the effects of covering resources that may be utilized more than once per gonotrophic cycle can be modelled [177].

Even assuming that the proportion of the gravid mosquito population contaminated with PPF (C_M) is a function of proportional coverage of the resting sites treated with PPF (C_r) and the rates at which mosquitoes visit the resting sites (α_r), the problem remains that neither can be measured reliably, even within the confines of our SFS because it is impossible to quantify or survey all the possible surfaces mosquitoes may choose to rest upon.

Fortunately, in cases where the total amount of a given mosquito resource (R) (such as blood (v), resting sites (r), or aquatic habitat (l)) cannot be quantified, it is possible to predict the impact of conventional insecticides that directly kill adults based on the measurements of coverage ($C_{R,x}$) and utilization rates ($\alpha_{R,x}$) for any definable, targetable subset (R_x) of that overall resource [177]. The advantage of using $\alpha_{R,x}$ and $C_{R,x}$ is that both are directly measurable. It is no longer necessary to know the proportion of the total resource which the covered subset represents (C_R) or the utilization rate for all available forms of that resource (α_R). Specifically, the product of the coverage (C_r) and utilization rate (α_r) of all resting site is equivalent to the product of the corresponding terms for the insecticide-targeted subset ($C_{r,x}$ and $\alpha_{r,x}$, respectively), which are both field-measurable parameters for such quantifiable, surveyable, subsets of a resting site [177],

$$\alpha_{r,c} = C_r\alpha_r = \alpha_{r,x}C_{r,x}. \tag{6.4}$$

A previous formulation in [177] designed to predict mosquito mortality resulting from resting surfaces treated with insecticide that kill them on contact was adapted to predict mosquito population coverage with PPF (C_M) by substituting the term contamination for mortality. In this preceding formulation [177], the probability of surviving all attempts to use intervention-covered forms of the targeted resource, in this case specified as resting sites ($R = r$) per gonotrophic cycle ($P_{\propto_{r,c}}$) is calculated as a simple exponential decay function of the product of the mortality probability associated with exposure to a covered form of the resting site through a single utilization event ($\mu_{r,c}$) and the mean utilization rate for all covered forms of that resting site ($\alpha_{r,c}$) [177], which may be substituted with the product of the coverage ($C_{r,x}$) and utilization terms ($\alpha_{r,x}$) from Equation 6.4,

$$P_{\propto_{r,c}} = e^{-\mu_{r,c}\alpha_{r,c}} = e^{-\mu_{r,c}\alpha_{r,x}C_{r,x}}. \tag{6.5}$$

By definition, $P_{\propto_{r,c}}$ also may be understood as the probability per gonotrophic cycle of an individual mosquito of not being killed through contact with insecticide-covered forms of a targeted resource. This complementary definition can be adapted readily to calculate the probability of adult mosquitoes not being contaminated with PPF. Hence, replacing the mortality term with the probability of mosquito contamination resulting from a single exposure to a PPF-contaminated resting site through a single utilization event ($\zeta_{r,c}$), and then replacing the survival

probability term ($\rho_{\propto_{r,c}}$) with the probability per gonotrophic cycle of not being contaminated with PPF through contact with any of the covered resting sites, we get the equivalent formulation

$$\rho_{\propto_{r,c}} = e^{-\zeta_{r,c}\alpha_{r,x}C_{r,x}}. \tag{6.6}$$

Therefore, the proportion of contaminated adult mosquitoes is the complement of the probability of not being contaminated with PPF,

$$C_M = 1 - \rho_{\propto_{r,c}} = 1 - e^{-\zeta_{r,c}\alpha_{r,x}C_{r,x}}. \tag{6.7}$$

In addition, the contaminating probability associated with exposure to a PPF-contaminated form of the resting site through a single utilization event may be reasonably assumed to approach unity ($\zeta_{r,c} \to 1$) based on experimental data indicating that 100% of all mosquitoes caught resting within a clay pot treated with PPF are contaminated [175], so

$$C_M \approx 1 - e^{-\alpha_{r,x}C_{r,x}}. \tag{6.8}$$

Hence, the proportion of contaminated adult mosquitoes can be calculated directly using only two field-measurable parameters for the targetable, quantifiable,

surveyable subset, specifically the contamination coverage of the targeted resting site subset ($C_{r,x}$) and the population mean utilization rate for that resting site subset by individual mosquitoes ($\alpha_{r,x}$).

Calculating the Utilization Rates of Resting Sites Subset Indirectly Using Quantifiable Blood Resources

The mean utilization rate for a resting site subset ($\alpha_{r,x}$) may be estimated indirectly by comparison with the rate at which the mosquito blood feeding events occur at the population level [177]. Otherwise, it is impossible to quantify directly the rates at which mosquitoes make contact with a subset of resting sites which is definable and measurable in itself but constitutes an unknown fraction of an indefinable, un-measurable total quantity of resting sites [177]. By comparison, numbers of blood hosts of particular species can be readily quantified, as can the rates at which mosquitoes blood feed upon them and the proportion of all blood meals that each host species represents, so it is possible to estimate the rate at which blood meals or gonotrophic cycles are completed by a mosquito population (M_v) or population sample ($M_{v,z}$) [177]. Thus, if the rate at which a defined, targetable subset of resting site resources (r_x) are visited by the same mosquito population ($M_{r,x}$) or population subsample ($M_{r,x,z}$) also can be estimated, the mean rate at which individual mosquitoes visit that resting site subset per gonotrophic

cycle may be calculated as the quotient of these two quantities [177]:

$$\alpha_{r,x} = \frac{M_{r,x}}{M_v} = \frac{M_{r,x,z}}{M_{v,z}}. \tag{6.9}$$

However, sampling of resting mosquitoes ($M_{r,x,z}$) typically is conducted only once per night by aspiration in the early morning, so it systematically underestimates $M_{r,x,z}$ because many mosquitoes may rest on targetable surfaces but then leave again before they can be surveyed [177, 187]. Interestingly, this can be expressed in terms of utilization rates, so that it is apparent that the mobility which causes coverage amplification also directly causes its own underestimation through conventional snapshot surveys of resting mosquitoes. Mobile mosquitoes may visit more than one resting site per gonotrophic cycle ($\alpha_r > 1$) and therefore may have several opportunities to become contaminated (r_x), even if it is only covers a subset of all those resting sites ($\alpha_r > 1$). However, moving around between several resting sites means that the mosquitoes spend proportionally shorter periods at each resting site, and the probability that they will be detected there with a single sampling effort declines correspondingly. Therefore, it is clear that accurate estimates for $\alpha_{r,x}$ that account for the effects of such mosquito movements upon entomological survey results are needed so that the resulting level of coverage amplification from treated subsets of resting sites to the mosquito population, that such mobility between resting sites directly mediates, is captured accurately (Figure 6.1).

Here, the principles of population size estimation by repeated removal trapping [178] are adapted to estimate the full per gonotrophic cycle rates of utilization of the targeted subset of resting sites (e.g., pots) ($\alpha_{r,x}$) using an additional analytical sub-model of the rate at which the number of mosquitoes caught by each resting site sample decreases as sampling frequency increases (Figure 6.2A). By definition, the utilization rate per gonotrophic cycle for the targeted resting site subset ($\alpha_{r,x}$) is equivalent to the product of the per capita rate per night at which all mosquitoes that are present within the surveyed sample of the ecosystem (M_z) rest on the targeted resting sites resource or the nightly probability that an individual mosquito would go inside ($k_{i,x}$) the pots that were targeted in the recent experimental demonstration [175], and the duration of the gonotrophic cycle (g) in nights

$$\alpha_{r,x} = gk_{i,x}. \tag{6.10}$$

Similarly, the total number of mosquitoes present in a given sample of the ecosystem (z) can be calculated as the product of the rate at which the population completes gonotrophic cycles ($M_{v,z}$) and the mean duration of those gonotrophic cycles

$$M_z = gM_{v,z}. \tag{6.11}$$

Given estimates of the rate per night at which all mosquitoes present within

a sample of the ecosystem (z) feed and complete gonotrophic cycles ($M_{v,z}$) [177], it is possible to estimate the resting site subset utilization rate $\alpha_{r,x}$ by varying the frequency of complete and exhaustive sampling from within that targeted subset ($k_{s,x}$) and fitting the following model of its expected effect to the number of mosquitoes caught per sample ($m_{r,x,z,s}$). The nightly per capita rate at which mosquitoes alight upon the targeted resting site subset ($k_{i,x}$) and the nightly per capita rate at which mosquitoes resting on that subset of surfaces leave again ($k_{o,x}$) can be described as the rates at which individual mosquitoes go inside (i) and outside (o) of pots targeted (x) with PPF in a recent experimental demonstration. Understanding these interactions in terms of a conventional compartment model (Figure 6.2A), these processes may be described mathematically using a system of ordinary differential equations

$$\frac{dm_{r,x,z,s}}{dk_s} = k_{i,x}M_z - k_{o,x}m_{r,x,z,s} - k_{s,x}m_{r,x,z,s}. \tag{6.12}$$

Solving Equation 6.12 for rates at which mosquitoes utilize the pot ($k_{i,x}$) per capita by assuming steady state conditions ($dm_{r,x,z,s}/dk_{s,x} = 0$ at $k_{s,x} = 1$; Figure 6.2B) yields

$$k_{i,x}M_z - k_{o,x}m_{r,x,z,s} - k_{s,x}m_{r,x,z,s} = 0. \tag{6.13}$$

By substituting $gM_{v,z}$ for M_z (Equation 6.11) and performing simple algebra

Figure 2

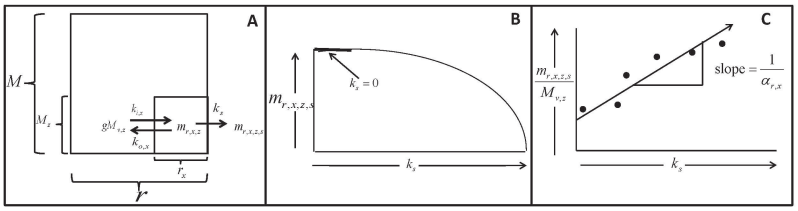

Figure 6.2: An illustration of how a true utilization rate may be estimated. **A:** Schematic illustration of how blood fed mosquitoes ($M_{v,z}$) may rest ($m_{r,x,z,s}$) and then move in ($\alpha_{r,x}$) and out by exit ($k_{o,x}$) or when removed during sampling at a rate ($k_{s,x}$) from a subset ($r_{x,z}$) of resting sites r_z in given sample per gonotrophic cycle. **B:** Simple illustration of a steady state ($dm_{r,x,z,s}/dk_s = 0$) condition at $k_{s,x} = 1$ where $\alpha_{r,x}$ and $k_{o,x}$ are constant. **C:** An illustration using a simple plot (that can eventually be plotted using experimental data and Equation 6.15) showing how utilization rate of a subset of a resting site which is given by a reciprocal of a line ($\alpha_{r,x} = 1/\text{slope}$) may be computed.

(i.e., re-arrangements of terms) in Equation 6.13, we can express $m_{r,x,z,s}/M_{v,z}$ first in terms of $k_{i,x}$, $k_{o,x}$, and $k_{s,x}$, and then of $\alpha_{r,x}$ by substituting $\alpha_{r,x}$ for $gk_{i,x}$ (Equation 6.10) as

$$\frac{m_{r,x,z,s}}{M_{v,z}} = \frac{gk_{i,x}}{k_{o,x} + k_{s,x}} = \frac{\alpha_{r,x}}{k_{o,x} + k_{s,x}}. \tag{6.14}$$

Therefore, an experiment that samples resting mosquitoes at different frequencies can be performed to alternately record the values of $m_{r,x,z,s}$ and $M_{v,z}$ using a range of different sampling frequencies ($k_{s,x}$). Host-seeking and resting samples should be alternated and separated by intervals at least as long as the gonotrophic cycle to prevent depletion of resting mosquitoes in that ecosystem

sample by host-seeking sampling and *vice versa*. Then, the non-linear model described in Equation 6.14, or the following linear form derived by rearrangement of terms, may be fitted to experimental data to estimate $\alpha_{r,x}$ and $k_{o,x}$ (Figure 6.2C)

$$\frac{m_{r,x,z,s}}{M_{v,z}} = \left(\frac{1}{\alpha_{r,x}}\right) k_{s,x} + \frac{k_{o,x}}{\alpha_{r,x}}. \tag{6.15}$$

Once full per gonotrophic cycle rates of utilization of the targeted resting site subset ($\alpha_{r,x}$) are estimated using experimental data (Equation 6.14 or 6.15), Equation 6.8 can be used to calculate the proportion of adult mosquitoes which are contaminated (C_M) by setting a high but achievable target value (e.g., 0.8) for the proportional coverage of the targeted resting site subset with PPF contamination ($C_{r,x}$).

6.2.2 Amplification of Contamination Coverage Through Transfer of PPF from Gravid Adult Mosquitoes to Aquatic Habitats

The four other parameters in Equation 6.2, namely the total nightly rate of oviposition by the entire adult population (O), the duration over which contaminated habitats persist but remain unproductive (U), the number of larval habitats (l), and the mean number of contaminating events needed to make a single habitat unproductive (T_l), all relate to transfer and accumulation of PPF in aquatic

habitats. However, as originally defined, these are not practically measurable in the field, so these components also are revised at a fundamental conceptual level.

Calculating the Minimum Number of Ovipositions by Contaminated Mosquitoes Required to Render Habitats Unproductive

The minimum rate at which contaminated ovipositing females are captured by sticky traps placed at a sample of aquatic habitat, that is required to render those habitats unproductive within one night (T_l) can be measured in a large-cage SFS with one or more artificial habitats by simple titration, accomplished by measuring the impact of PPF delivered by varying numbers of released, contaminated mosquitoes. The term Ω in the original model [176] is replaced by (T_l) to reflect that titration measurement and to avoid conflicting with previous models using the former symbol to denote vector control scenario [167, 183, 184]. The mean titre of all habitats (T_l) is defined as the minimum rate at which contaminated ovipositing females that is required to reach a targeted percentage (usually = 95% but = 99% is more appropriate for such a strategy intended to eliminate rather than merely control vector populations and the malaria transmission they mediate) of emergence inhibition of adult mosquitoes from contaminated aquatic habitats. Mathematically, T_l may be calculated as the product of the rate of utilization of habitat(s) by mosquitoes (α_l) and the minimum rate at which contaminated ovipositing females are captured by sticky traps placed

at a sample of aquatic habitat, that is required to render those habitats unproductive within one night, divided by the total quantity of habitat (l),

$$T_l = \alpha_l \frac{M_l^{min}}{l}. \tag{6.16}$$

However, the overall total oviposition event titre for all aquatic habitats present in any natural ecosystem (T_l) is impossible to measure in practice. Furthermore, titre estimates for artificially constructed habitats are of dubious relevance to natural habitats, which are far more diverse, dynamic, and variable in qualitative and quantitative terms [190, 191]. In principle, titration experiments could be conducted in natural habitats in the field by temporarily placing large cages over them and releasing varying numbers of contaminated, insectary-reared gravid females. However, the obstacle that remains to predicting impact of an autodissemination strategy is estimating the natural rates of exposure of these habitats to ovipositing females in the absence of any way to measure the total number of ovipositing mosquitoes visiting them.

Fortunately, a recently developed method [179] for surveying oviposition contacts of mosquitoes with either artificial or natural aquatic larval habitats, by trapping them on glue-covered plastic sheets, now allows an index of oviposition input to be recorded. This method probably exhibits incomplete efficiency of

oviposition contact detection through physical capture ($\varepsilon_d < 1$), but the number of oviposition events that can be observed with this sticky trap method in a given habitat sample (z) for a given titration experiment can be assumed to be proportional to total oviposition contacts if that efficiency level is consistent for each habitat type (x) category, such as puddles, river fringes, or springs:

$$T_{l,z,d} = \varepsilon_d T_{l,z}, \tag{6.17a}$$

and

$$T_{l,x,z,d} = \varepsilon_d T_{l,x,z}. \tag{6.17b}$$

Failures of the trap to capture mosquitoes that make contact with it may lead to incomplete trapping of all mosquitoes visiting a habitat. More crucially, however, each trap only surveys a sample of the perimeter of any water body where most larval habitat occurs. This is an advantage because it presents a valuable opportunity to field-parameterize these models. While it is not possible to estimate which fraction of all larval habitat (l) or subset thereof (l_x) that any given set of sticky oviposition traps ($l_{x,z}$) represent, it can be assumed to vary in proportion to the rate of oviposition input per *unmeasurable but constant* unit of quantity of habitat such traps are considered to sample ($l_{x,z}$), regardless of how much unknown, un-measurable total habitat (l) or habitat subset (l_x) is present in the ecosystem.

As described in detail below, the absolute titre estimates for samples of natural habitats can be replaced by titres of detected (d) oviposition events, measured with oviposition sticky traps [179]. This new term for the detectable oviposition contact titre ($T_{l,x,z,d}$) is expressed as the minimum rate at which contaminated ovipositing females are captured by sticky traps placed at a sample of aquatic habitat, that is required to render those habitats unproductive within one night ($T_{l,x,z,d}$).

Consider an SFS or full field experiment undertaken to measure the minimum number of ovipositing mosquitoes utilizing the habitat(s) that are required to render it unproductive (M_l^{min}) and the total quantity of habitat (l) where sticky traps [30] are used to measure the number of oviposition events by mosquitoes for a sample (z) of a categorical subset (x) of aquatic habitats ($l_{x,z}$). It is assumed that the numbers of mosquitoes caught by a single sticky trap represent a detectable fraction (ε_d) of all utilization events occurring at the unmeasurable but constant unit of habitat each one can cover ($M_{l,x,z}$), which is typically distributed along the perimeter of water bodies rather than in them, because 1) they are applied at a constant density per unit of perimeter in existing protocols, and 2) each sticky trap has a fixed area and dimensions [179]. If $M_{l,x,z,d}^{min}$ represents the mean minimum catch per night per sticky trap that results in lack of productivity following controlled exposure to contaminated mosquitoes, then the detectable oviposition titre of detected

oviposition events per night per sticky trap $T_{l,z,d}$ or $(T_{l,x,z,d})$ may be computed as

$$T_{l,z,d} = \alpha_l \frac{M^{min}_{l,z,d}}{l_z} = \alpha_l \varepsilon_d \frac{M^{min}_{l,z}}{l_z}, \tag{6.18a}$$

and

$$T_{l,x,z,d} = \alpha_l \frac{M^{min}_{l,x,z,d}}{l_{x,z}} = \alpha_l \varepsilon_d \frac{M^{min}_{l,x,z}}{l_{x,z}}, \tag{6.18b}$$

respectively, where $M_{l,x,z}$ is the number of oviposition events occuring in the aquatic habitats subsets that was surveyed with the sticky traps, and ε_d is the detection sensitivity of those events by the sticky trap.

Assuming that a sample of all habitats (l_z) or a categorical subset thereof $(l_{x,z})$ is a representative, it also may be assumed that the mean catch per night per sticky trap for a sample of that subset l_z or $l_{x,z}$ also is representative of the mean catch per night per sticky trap for the entire set (l) or subset (l_x) of habitats. Therefore, proportional to the fraction of all aquatic habitats that surveyed samples represent

$$\frac{M^{min}_{l,z}}{M^{min}_{l}} = l_z l, \tag{6.19a}$$

and

$$\frac{M^{min}_{l,x,z}}{M^{min}_{l,x}} = \frac{l_{x,z}}{l_x}. \tag{6.19b}$$

Re-arranging Equation 6.18 yields

$$\alpha_l \frac{M_{l,z}^{min}}{l_z} = \frac{T_{l,z,d}}{\varepsilon_d}, \tag{6.20a}$$

and

$$\alpha_l \frac{M_{l,x,z}^{min}}{l_{x,z}} = \frac{T_{l,x,z,d}}{\varepsilon_d}. \tag{6.20b}$$

Re-arranging Equation 6.19 yields

$$\frac{M_l^{min}}{l} = \frac{M_{l,z}^{min}}{l_z}, \tag{6.21a}$$

and

$$\frac{M_{l,x}^{min}}{l_x} = \frac{M_{l,x,z}^{min}}{l_{x,z}}. \tag{6.21b}$$

Substituting Equation 6.21 and then Equation 6.20 into Equation 6.16 yields

$$T_l = \alpha_l \frac{M_l^{min}}{l} = \alpha_l \frac{M_{lz}^{min}}{l_z} = \frac{T_{l,z,d}}{\varepsilon_d}, \tag{6.22a}$$

and

$$T_{l,x} = \alpha_l \frac{M_{l,x}^{min}}{l_x} = \alpha_l \frac{M_{l,x,z}^{min}}{l_{x,z}} = \frac{T_{l,x,z,d}}{\varepsilon_d}. \tag{6.22b}$$

Hence, even without knowing the total number of habitats (l) or the utilization rate of oviposition sites by individual mosquitoes per gonotrophic cycle (α_l), in principle, the absolute titre of all habitats may be calculated by dividing the known detectable

titre of the sampled habitats ($T_{l,x,z,d}$) by the detection efficiency of the sticky trap (ε_d). Although it is not obvious how the detection efficiency of the sticky trap could be measured, except perhaps by direct observation [192, 193], as described below. It is not essential to know the absolute oviposition input titre as long as the titration experiments use the same imperfect sampling tool as surveys of oviposition exposure of the same natural habitats to wild mosquito populations. Mathematically, this allows a fully measurable solution to Equation 6.2 because, as described in the next sub-section, the unmeasurable detection sensitivity term (ε_d) also appears in the otherwise fully measurable solution to the quotient O/l, or an equivalent term in a model for a defined subset of habitats O_x/l_x.

Calculating the Ratio between the Numbers of Ovipositions by Adult Mosquitoes and of Aquatic Habitats

The remaining terms to be addressed include only the total rate of ovipositions events per night (O) by the adult population, and the number of aquatic habitats available for them to oviposit into (l), which constitute a quotient (O/l) in Equation 6.2.

Given that the mean number of oviposition events each gravid mosquito executes per gonotrophic cycle remains unknown but clearly greater than unity for African *Anopheles* studied thus far ($\alpha_l > 1$) [194], it is not possible to measure directly or to reliably infer the population-level total rate at which these events

occurs in an entire ecosystem, even if the total rate at which mosquitoes become gravid and begin ovipositing (M_l) could be inferred from estimates of gonotrophic cycle completion based on surveys of blood utilization:

$$O = \alpha_l M_l = \alpha_l g M = \alpha_l M_v. \tag{6.23}$$

As described in the previous section, the larval habitat utilization term (α_l) is essentially unmeasurable, and it is also very difficult to define what constitutes mosquito aquatic larval habitat in a quantifiable way. Even if it is possible to quantify a sample of habitats (z), possibly within a defined subset (x) using relatively simple indicators, such as the perimeter of the water bodies with which they are associated, it is impractical to measure directly the total quantity of habitat present in an entire ecosystem (l) on village-level spatial scales that are large enough to be epidemiologically for an intervention like autodissemination that only acts at the community level [195]. However, it as discussed above, is now possible to survey oviposition events rates [179] per unit of habitat, even if that fixed unit is undefined and unmeasurable [179]. Thus, it is should be possible to relate observed oviposition rates at aquatic habitats under natural conditions to those in titration experiments in which varying numbers of contaminated mosquitoes are introduced to them, following which their productivity or lack thereof is determined, so long as the same survey method is applied in both experiments. By substituting Equation

6.23 for O, the quotient of the rate of the ecosystem-wide oviposition (O) by the adult mosquito population, divided by the number of aquatic habitats (l), is

$$\frac{O}{l} = \frac{\alpha_l M_l}{l}, \tag{6.24a}$$

and

$$\frac{O_x}{l_x} = \alpha_l \frac{M_{l,x}}{l_x}. \tag{6.24b}$$

As described in Equation 6.21, $\alpha_l M_l / l$ can be estimated by assuming a sample of aquatic habitats (l_z), or a sample of a defined subset of those habitats ($l_{x,z}$) is representative, so

$$\frac{O}{l} = \alpha_l \frac{M_l}{l} = \alpha_l \frac{M_{l,z}}{l_z}, \tag{6.25a}$$

and

$$\frac{O_x}{l_x} = \alpha_l \frac{M_{l,x}}{l_x} = \alpha_l \frac{M_{l,x,z}}{l_{x,z}}. \tag{6.25b}$$

We already know that the sticky traps under-count ovipositing mosquitoes ($\varepsilon_d < 1$), but the number of ovipositing mosquitoes observed in the trap can be assumed to be proportional to that absolute quantity, so the rate at which mosquitoes oviposit in a surveyed sample of larval habitats (l_z) or subset of habitats ($l_{x,z}$), that are detected by a sticky trap, may be described as

$$M_{l,z,d} = \varepsilon_d \alpha_l \frac{M_{l,z}}{l_z}, \tag{6.26a}$$

and

$$M_{l,x,z,d} = \varepsilon_d \alpha_l \frac{M_{l,x,z}}{l_{x,z}}. \tag{6.26b}$$

By rearranging Equation 6.26 ($M_{l,z} = l_z M_{l,z,d}/\varepsilon_d \alpha_l$ or $M_{l,x,z} = l_{x,z} M_{l,x,z,d}/\varepsilon_d \alpha_l$) and substituting into Equation 6.25, α_l and $l_{x,z}$ both cancel, leaving ε_d as the only unmeasurable term:

$$\frac{O}{l} = \alpha_l \frac{M_{l,z}}{l_z} = \frac{\alpha_l}{l_z} \frac{l_z M_{l,z,d}}{\varepsilon_d \alpha_l} = \frac{M_{l,z,d}}{\varepsilon_d}, \tag{6.27a}$$

and

$$\frac{O_x}{l_x} = \alpha_l \frac{M_{l,x,z}}{l_{x,z}} = \frac{\alpha_l}{l_{x,z}} \frac{l_{x,z} M_{l,x,z,d}}{\varepsilon_d \alpha_l} = \frac{M_{l,x,z,d}}{\varepsilon_d}. \tag{6.27b}$$

Thus, Equation 6.27 indicates that the quotient of the ecosystem-wide oviposition rate by the adult mosquito population (O), divided by the number of aquatic habitats (l), may be estimated by dividing the rate at which mosquitoes ovipositing at a surveyed sample of habitats are detected with a sticky trap ($M_{l,z,d}$) by the efficiency of that trap (ε_d), and the same applies to subsets of habitats within the ecosystem (O_x, l_x, and $M_{l,x,z,d}$), respectively.)

Fortunately, the terms O/l and T_l appear in Equation 6.2 as a quotient (O/lT_l), so the same applies to $M_{l,z,d}$ and $T_{l,z,d}$ or $M_{l,x,z,d}$ and $T_{l,x,z,d}$ and the unknown detection efficiency term (ε_d) cancel in the equivalent quotient. Substituting Equation 6.22 for T_l (and then $T_{l,x}$), Equation 6.27 for O/l (and then

O_x/l_x), we get

$$\frac{O}{l\ T_l} = \frac{\varepsilon_d M_{l,z,d}}{\varepsilon_d T_{l,z,d}} = \frac{M_{l,z,d}}{T_{l,z,d}}, \tag{6.28a}$$

and

$$\frac{O}{l\ T_l} = \frac{\varepsilon_d M_{l,x,z,d}}{\varepsilon_d T_{l,x,z,d}} = \frac{M_{l,x,z,d}}{T_{l,x,z,d}}. \tag{6.28b}$$

6.2.3 Integrating the Model Components to Obtain a Formulation Using Only Field-Measurable Parameters

Taking Equation 6.2 and substituting Equation 6.28 for O/lT_l or $O_x/l_xT_{l,x}$ and Equation 6.8 for C_M, we get

$$C_l = 1 - e^{-\frac{C_M\ U\ m_{l,z,d}}{\mathrm{T_{l,z}}}} = 1 - e^{-\frac{(1-e^{-\alpha_{r,x}C_{r,x}})\ U\ m_{l,z,d}}{\mathrm{T_{l,z,d}}}}, \tag{6.29a}$$

and

$$C_{l,x} = 1 - e^{-\frac{C_M\ U_x\ m_{l,x,z,d}}{\mathrm{T_{l,x,z}}}} = 1 - e^{-\frac{(1-e^{-\alpha_{r,x}C_{r,x}})\ U_x\ m_{l,x,z,d}}{\mathrm{T_{l,x,z,d}}}}, \tag{6.29b}$$

where C_l and $C_{l,x}$ are the respective proportions of all aquatic habitats (l) or a subset thereof ($l_{x,z}$), which are effectively contaminated with PPF. All the parameters specified in Equation 6.29 that replace equivalent terms in Equation 6.2 are field measurable. The only term that remains from Equation 6.2 (U), the mean duration over which habitats persist and remain unproductive) also may be measured directly in the field following experimental contamination of natural

habitats with at least the measured titre of live contaminated females required to render them unproductive.

6.3 Results and Discussion

Overall, Equation 6.29 enables prediction of larval habitat coverage with PPF contamination via autodissemination, using input parameters that are all field measurable and have a relatively straightforward deterministic relationship, so that recent successes in enclosed large-cage SFS [175] can be rationalized, and the potential for application in full field ecosystems can be assessed. Beyond merely assessing the prospects for any given PPF formulation and delivery method, the model also defines measurable properties of different prototypes that may be conveniently and rapidly optimized under controlled experimental conditions so that such prospects for success in full field ecosystems may be maximized. Furthermore, combining mathematical sensitivity analysis with a review of the known biological and physical constraints upon the input parameters allows assessment of the plausibility of success in full field ecosystems and threshold values or, more accurately, combinations of values for those input parameters that are required to achieve meaningful impact upon dry season malaria transmission or even the population stability of the parasite and vector populations that mediate it.

The model described by Equation 6.29 and the numerous applications

described below all depend on the implicit assumption of steady-state conditions, despite the fact that African malaria vector populations, especially those of species from the *Anopheles gambiae* complex to which *An. arabiensis* belongs, often are considered to be highly dynamic [190, 191, 196]. However, recent field observations [197] examining the hydrology of malaria in the particularly well-characterized village of Namwawalla, in rural southern Tanzania, confirms that for 2 to 3 months of the dry season, all larval habitat is continuously created and then destroyed by the receding groundwater table. The total quantity of aquatic habitat remains essentially stable but reflects a constant turnover of habitats with life-spans of days and weeks, rather than months, as the perimeter of water bodies recedes along a varying gradient [197]. The spatial distribution of optimal habitat across populations of depressions in the landscape varies from week to week as their shallowest fringes are first exposed and then drained by the dropping water table. Therefore, this can be treated as an example of a system of larval aquatic habitats and associated mosquito populations that are dynamic, but nevertheless approximate steady-state conditions, so that the parameters of Equation 6.29 all may be measured over the period and reasonably used to predict impact of autodissemination strategies during the depth of the dry season from August to October. Other studies of dry season larval habitat ecology for members of the *Anopheles gambiae* species complex describe larval habitat dynamics that are at least as stable and provide several examples of where permanent or semi-permanent

habitats are seasonally important during such annual minima of larval habitat availability and mosquito population density [190, 191, 198, 199]. Bearing in mind the limitations of any mathematical model, which is by definition a deliberately simplified representation of complex real world processes, Equation 6.29 may be reasonably applied, as described in detail below, to optimize approaches to realizing the autodissemination of PPF and to assess the plausibility of success for specific approaches, based on field measurements of its input parameters.

6.3.1 Model Parameterizability

All the parameters on the right hand side of Equation 6.29 are, as described in the preceding narrative of the methods section, measurable not only in large-cage SFS, but also in full field ecosystems: (1) The proportional coverage of the subset of resting sites which are targeted with PPF that are actually treated in practice ($C_{r,x}$) may be surveyed by direct inspection, ideally by personnel independent of the team responsible for delivery of the intervention, and reasonable operational targets for this parameter may be set based on existing precedents, such as LLINs or IRS [200]; (2) The rate at which individual mosquitoes utilize the PPF-targetted subset of resting site resources ($\alpha_{r,x}$), may be measured by comparing rates at which resting events occur with those observed for blood-feeding events, using entomological surveys with varying frequencies of removal sampling and a corresponding

differential equation model (Equation 6.16 or 6.17) to account for regular movements and associated imperfect detection of resting events by aspiration capture; (3) The minimum rate at which ovipositing females are captured by sticky traps [30] placed at a sample of aquatic habitat, that is required to render those habitats unproductive ($T_{l,z,d}$ or $T_{l,x,z,d}$) may be estimated by titration, achieved by introducing varying numbers of contaminated mosquitoes into cages placed over those habitats, within which sticky traps are placed at a standardized density; (4) The number of oviposition events detected by the same sticky traps in the same sample of habitats under natural conditions ($m_{l,z,d}$ or $m_{l,x,z,d}$) may be measured in the same way, but with the cage removed so that it is exposed to normal levels of oviposition by the wild mosquito population $m_{l,x,x,d}$; and (5) The duration over which contaminated habitats both persist and remain unproductive (U) may be measured by longitudinal observation of the habitats contaminated during the titration experiments, particularly those at the minimum effective level of mosquito exposure that defines the measured titre. The model described by Equation 6.29, not only enables field parameterization, but also directly defines the design of the experiments that need to be conducted to (1) rationalize the recent demonstrations of success PPF autodissemination in enclosed large-cage SFS [175], and (2) assess the plausibility of success in full field ecosystems, using either *An. arabiensis*, or an alternative mosquito species with which it shares aquatic habitats, to mediate PPF transfer and coverage amplification.

6.3.2 Measurable Optimization of Autodissemination Technologies and Delivery Strategies

Equation 6.29 also defines measurable properties of different prototype autodissemination strategies that may be rapidly optimized, often under conveniently controlled experimental conditions, to enhance prospects for success and maximize impact in full field ecosystems.

The most obvious of these is the detectable titre of ovipositing females required to render habitats unproductive ($T_{l,z,d}$ or $T_{l,x,z,d}$); while standardized artificial habitats created inside experimental cages may not be representative of their natural counterparts, they may nevertheless be perfectly adequate and far more convenient for comparing the level of emergence inhibition activity transferred to mosquitoes by a variety of alternative PPF formulations. While such activity measurements (by definition the inverse of titre) may not be used to predict likely impact in natural larval habitats, the formulation conferring the highest level of transferrable activity in such experimental systems is also probably the best option for full field application, unless some other considerations, such as persistence, acceptability, or cost are limiting.

The next most obvious parameter which might be maximized to enhance impact is the duration over which contaminated aquatic habitats persist and remain unproductive (U), which is in turn determined and limited by the rate at which

individual habitats are created and destroyed or by the rate at which emergence activity decays in these habitats, whichever of these two rates is fastest. To a large extent, this parameter already may have been optimized to some degree simply by choosing PPF as the larvicide, because it is a relatively persistant active ingredient. However, the disappointing brief persistence times of approximately two weeks [201] that were recently observed in artificial habitats for immature stages of mosquitoes from the *An. gambiae* complex that were exposed to natural meteorological conditions and sunlight suggest that there may yet be room to improve upon either the choice of active ingredient or its formulation, as many dry season habitats may last much longer under natural conditions [190, 191, 198, 199]. In advance of full field trials, it would be important to measure the actual frequency distributions of habitat and PPF persistence in natural target ecosystems to determine whether both are sufficiently long to enable adequate accumulation of emergence inhibition activity.

Furthermore, alternative PPF formulations and resting site subset treatment targets also may be selected and optimized to maximize coverage ($C_{r,x}$) and utilization ($\alpha_{r,x}$) parameters, based on measurements of achieved values for these two parameters in samples of the ecosystems in which they are designed to be applied. While resting site subset coverage ($C_{r,x}$) may be readily and rapidly surveyed by direct inspection, measuring the rate at which individual mosquitoes

utilize these targets for PPF treatment ($\alpha_{r,x}$), as the quotient of the rates at which resting events and blood-feeding events occur, will rely upon fitting an additional analytical model to data from quite intensive entomological experiments (Section 2.1.2). However, these target site coverage and utilization rate input parameters are used merely to predict contamination coverage of the adult mosquito populations (C_M), which is actually the direct determinant of aquatic habitat contamination levels (Equation 6.29). It would therefore be more directly predictive, and probably far simpler and more efficient, to directly measure mosquito population contamination coverage (C_M) using appropriate labels to mark insects [188] making contact with resting site surfaces that are, or would be, treated with PPF. In fact, even if it is useful to measure the target resting site subset utilization rate ($\alpha_{r,x}$) in addition to mosquito population coverage, it is probably far easier and more accurate the former as a simple function of measured values for the latter. Equation 6.8 may be rearranged so that $\alpha_{r,x}$ can be either calculated directly from single measurements of C_M, or estimated by fitting the following equation to measures of C_M at varying levels of coverage of the targeted resting site subset ($C_{r,x}$):

$$\alpha_{r,x} = -\frac{\ln(1 - C_M)}{C_{r,x}} \tag{6.30}$$

Even the number of oviposition events detected by sticky traps placed at samples of natural habitats ($m_{l,z,d}$ or $m_{l,x,z,d}$), which might initially appear to be a

fundamental property of the ecosystem in question, is also amenable to optimization by choosing the most effective approach to PPF autodissemination in the context of local community ecology of multiple mosquito species and other vector control methods that may be applied. While all successful demonstrations of successful PPF autodissemination to date [172, 175] have used the target mosquito species itself to mediate PPF transfer to its own aquatic habitat, this does not necessarily have to be the case where the target species shares its aquatic habitats with others. In fact, if we examine this choice from a mathematical perspective, Equation 6.29 is clearly endogenous if the target species is used to mediate autodissemination because contamination coverage of larval habitats is clearly dependent upon adult mosquito density, reflected in the rate at which they are caught in sticky traps ($C_l \leftrightarrow m_{l,z,d}$ and $C_{l,x} \leftrightarrow m_{l,x,z,d}$). In biological terms, the impact of the autodissemination strategy will be self-limiting ($\lim_{m_{l,z,d,0}\to\infty} C_l < 1$, where $m_{l,z,d,0}$ is the mean rate at which ovipositing mosquitoes are captured with sticky traps at the point where the autodissemination intervention is introduced) because increasing coverage of larval habitats will progressively reduce the densities of mosquitoes that enable it, unless either (1) larval populations of the target species are eliminated ($C_l \to 1$) before the adult population driving it die off and PPF contamination of those habitats persists longer than that remaining adult population so that re-infestation is prevented, or (2) A different mosquito species is used to mediate PPF transfer that co-occupies most of the target-species habitats simultaneously or before the target species, and

is ideally behaviourally and/or physiologically resilient to control with other vector control measures that may be present, such as LLINs and IRS, so that it persists and oviposits at high densities (maximum $m_{l,z,d}$) even as autodissemination progressively controls, and ideally eliminates [202], the target species.

6.3.3 Minimum Threshold Value Combinations for Measurable Input Parameters to Render Intervention Impact Plausible

If habitats are assumed to be created, destroyed, and replaced weekly, or that PPF activity lasts only a week in natural habitats [201] ($U = 7$ days), the minimum target of 90% coverage of aquatic habitats with PPF may be achieved if the contamination coverage of the mosquito population (C_M) and the quotient of the ovitrap-detectable rates of oviposition by wild mosquitoes in natural aquatic habitats contact divided by the titre of contaminated mosquitoes required to render them unproductive ($m_{l,z,d}/T_{l,z,d}$), both approach unity (Figure 6.3A). As habitats and PPF are assumed to persist for longer periods, the required thresholds of C_M and $m_{l,z,d}/T_{l,z,d}$ are less stringent, and 90% contamination coverage of larval habitats may be achieved if values for all these determinants are considerably lower (Figure 6.3 B through to D). For example, values of only 0.3 for both C_M and $m_{l,z,d}/T_{l,z,d}$ may be sufficient to effectively contaminate 90% of habitats that persist and retain PPF activity for approximately one month (Figure 6.3C).

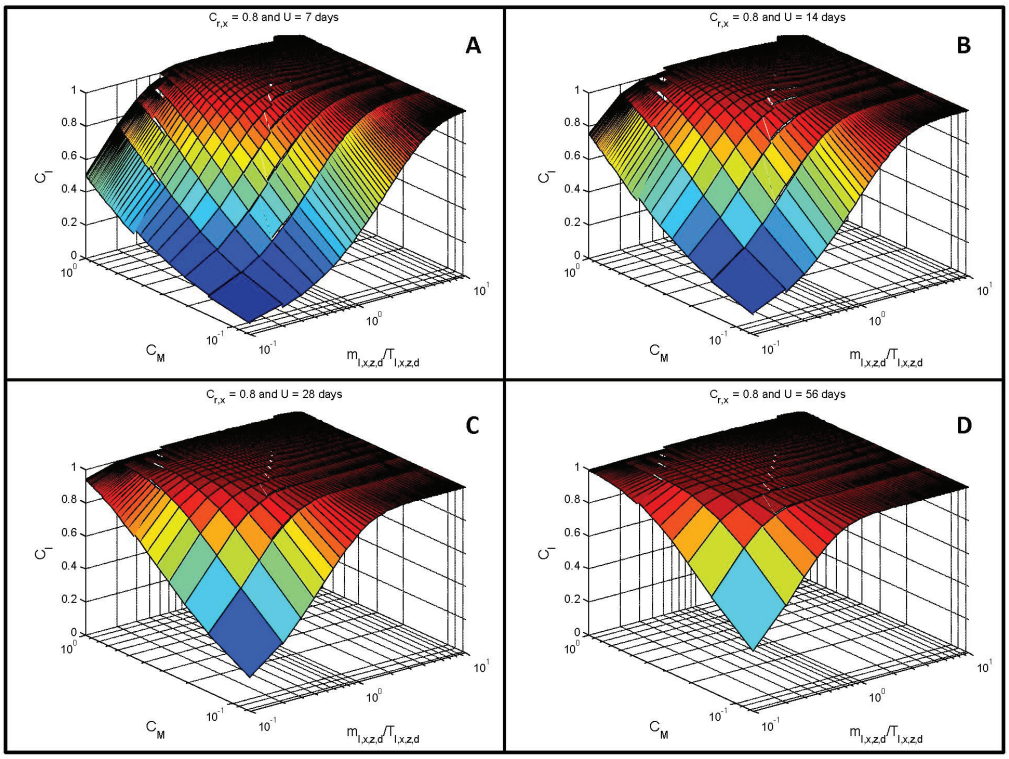

Figure 6.3: Evaluation of the model output using different values for the three main input parameters.
Evaluation of the proportion of all aquatic habitats which are effectively contaminated with PPF (C_l) at different values of the mean time that habitats persist but remain unproductive (U). Figure 6.3 presents combinations of minimum values for C_M and $m_{l,z,d}$ and $T_{l,z,d}$ that may lead to $C_l = 0.90$ or $C_l = 0.99$ at different values of U.

While infinite possible combinations of values for C_M, $m_{l,z,d}$, and $T_{l,z,d}$ exist that can result in a given level of predicted larval habitats coverage (C_l), the apparent complexity of inputs and outputs illustrated by the responses surfaces in Figure 6.3 follow remarkably simple relationships: All the panels of Figure 6.3 are symmetric about the line $C_M = m_{l,z,d}/T_{l,z,d}$ because of the simple multiplicative relationship between C_M and $m_{l,z,d}/T_{l,z,d}$ in Equation 6.29. In fact, the titre of contaminated mosquitoes required to render natural habitats unproductive appears

in Equation 6.29 as its reciprocal $1/T_{l,z,d}$, which is mathematically equivalent to the activity (A) of contaminated mosquitoes, so any increase in one of these terms can compensate exactly for a proportional decreases in the others, with the caveat that C_M is a proportion and therefore constrained to values of less than one. Their combined effect can be represented as a direct function of their product, leaving the question as to how these three parameters may be optimized to achieve predicted threshold values for their product as an open matter for debate, experimentation, and measurement. Furthermore, because the autodissemination strategy is limited in applicability to the dry season, more ambitious larval habitat coverage targets must be set ($C_l > 99\%$) that enable elimination of malaria transmission [202], or even the vector population itself [3]. Just like field measurement of progress in any elimination programme [203], visualizing simulated progress towards zero requires a corresponding change in perspective and scale. Fortunately, the combined influence of C_M, $m_{l,z,d}$, and $1/T_{l,z,d}$ upon the availability of uncontaminated aquatic habitats $(1 - C_l)$ to the vector population is described by Equation 6.29 as a simple exponential decay, so the increasing threshold values that are required to achieve these more ambitious larval habitat coverage targets can be visualized as a log-linear function of their product (Figure 6.4).

Any autodissemination intervention aiming to eliminate malaria transmission or vector populations needs to achieve comprehensive coverage of essentially *all*

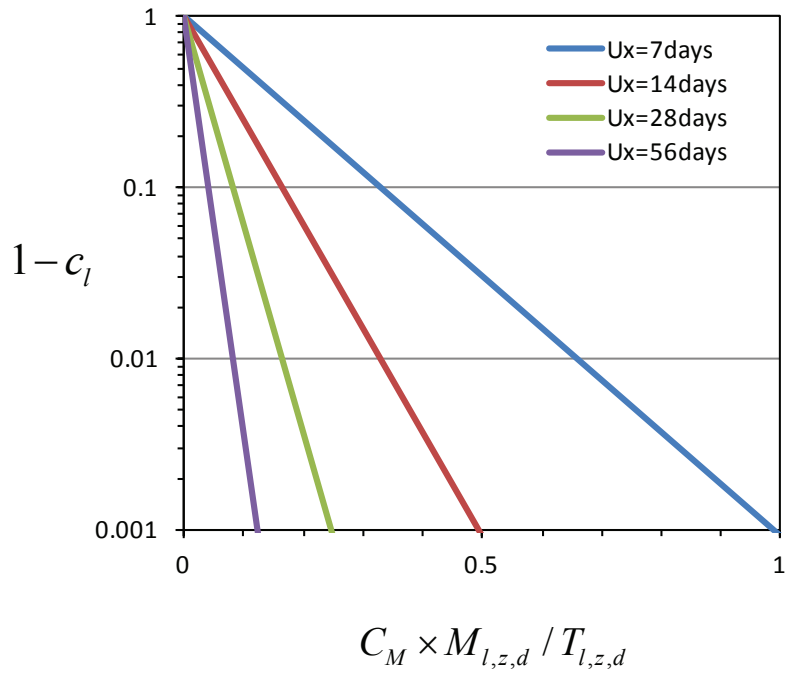

Figure 6.4: Assessing the impact of the three main model input parameters in predicting the output.
An illustration of the main three input parameters in predicting the proportion of all aquatic habitats contaminated with PPF. Figure 6.4 presents combined influence of C_M, $m_{l,z,d}$, and $1/T_{l,z,d}$ upon the availability of uncontaminated aquatic habitats $(1 - C_l)$ to the vector population as a simple exponential decay, so the increasing threshold values that are required to achieve larval habitat coverage targets can be visualized as a log-linear function of their product.

habitats $(1 - C_l < 0.01)$. Prospects for success at this high level of ambition will be limited by the most challenging, presumably ephemeral, of the subsets of targeted aquatic habitats $(C_{l,x} \to 1)$, and recent studies from Kenya suggest that PPF activity may also not last much longer than a week [201]. The predicted threshold values for the product of C_M, $m_{l,z,d}$, and $T_{l,z,d}$ for such short-lived habitats and insecticides $(U_x = 7$ days) therefore probably represent the most appropriate targets for optimizing and evaluating prototype autodissemination strategies based on field

measurements of these three input parameters. As illustrated in Figure 6.4, values for $C_M m_{l,z,d}/T_{l,z,d}$ approaching unity will be required to achieve at least 99% contamination coverage of this most challenging subset of habitats. This in turn suggests that, as achieved in the recent large-cage SFS demonstration of successful autodissemination, contamination of most mosquitoes in the population ($C_M \rightarrow 1$). Mosquito population coverage is a proportion and is therefore constrained to values of less than one, so the quotient of the ovitrap-detectable rates of oviposition by wild mosquitoes into this subset of ephemeral aquatic habitats divided by the titre of contaminated mosquitoes required to render them unproductive will also have to approach or exceed unity ($m_{l,x,z,d}/T_{l,x,z,d} \rightarrow 1$).

6.4 Conclusions

The model described in Equation 6.29 uses input parameters that are all field measurable, so recent successes in enclosed large-cage SFS can be rationalized, and the plausibility of success in full field application can be evaluated *a priori*. The model also defines measurable properties of different prototypes that may be conveniently and rapidly optimized under controlled experimental conditions to maximize chances of successful application at ecosystem scale in full field trials. While perhaps the most obvious limitation in this model is the endogenous relationship that occurs between the output parameter and one of the input parameters if the target mosquito species is used to mediate PPF transfer, this helps

illustrate the naturally self-limiting feedback loop that occurs between impact and densities of ovipositing mosquitoes mediating autodissemination, thus illustrating the potential advantages of using a different mosquito species that shares the same aquatic habitats as the primary target for contamination at selected resting sites.

For autodissemination interventions to eliminate malaria transmission or vector populations during the dry season window of opportunity will require comprehensive contamination of all aquatic habitats ($C_l \to 1$), including the most challenging subset of these that persist or retain PPF activity for as little as a week $C_{l,x} \to 1$, where $U_x = 7$ days. The model presented here suggests that to achieve at least 99% contamination coverage of this ephemeral aquatic habitats subset will necessitate successful contamination of most mosquitoes in the population ($C_M \to 1$), and that the quotient of the ovitrap-detectable rates of oviposition by wild mosquitoes into this subset of habitats, divided by the titre of contaminated mosquitoes required to render them unproductive, will also have to at least approach unity ($m_{l,x,z,d}/T_{l,x,z,d} \to 1$) .

The simple multiplicative relationship between C_M and $m_{l,z,d}/T_{l,z,d}$, and the fact that their combined effect can be described as a simple exponential decay of uncontaminated aquatic habitats, allows ready application of this model by theoreticians and field biologists alike. The most important caveats and limitations to applying this model relate to uncertainties about the validity of the underlying

simplifying assumptions and the natural or achievable ranges of its input parameters.

6.5 Transition to Chapter 7

In the next last chapter, we provide a summary for this work and discuss projects that may be considered for future work.

CHAPTER 7

General Conclusions and Future Work

Each of the stand-alone Chapters 2 - 6 includes its own conclusions. In this chapter, we summarize general conclusions together with the remaining knowledge and technology gaps which should be considered for future research.

7.1 General Conclusions

The focus on malaria research is overwhelmingly on species that primarily feed indoors (endophagic) upon humans (anthropophagic). Field observations [7, 8, 9, 10] and model simulations [1, 2, 12] indicate that high demographic coverage of humans ($\geq 80\%$) by indoor vector controls (i.e., indoor residual spraying (IRS) [29, 30] and long-lasting insecticidal nets (LLINs) [27, 30]) can reduce dramatically the density, longevity, and infection prevalence of mosquito endophagic and anthropophagic mosquitoes [7, 2, 11, 28]. However, even in areas where LLINs and IRS have been successful, malaria transmission by outdoor biting and outdoor resting vector populations remains a serious challenge for malaria elimination. To complement efforts already attained by LLINs and IRS and potentially to achieve

malaria elimination, we also need strategies that target adult mosquitoes outdoors [31, 5, 147] or that survive primarily by feeding on animals (i.e., zoophagic mosquitoes) [14, 15] or at source in their aquatic habitats [80]. As a contribution toward achieving these goals, our work addressed three projects: 1) informatics tools that can be used for data preparation of all types of mosquitoes (i.e., the ones feeding and resting indoors and/or feeding and resting outdoor and/or feeding upon humans and/or upon animals) (Chapter 2 and 3), 2) mathematical models to assess the impact of personal protection measures upon malaria transmission by zoophagic mosquitoes (Chapter 4) also resulting into a discussion on biologically meaningful coverage indicators for eliminating malaria transmission (Chapter 5), and 3) mathematical models discussing the autodissemination of insecticide aimed at targeting mosquito emergence (Chapter 6). In developing our models, we adapted some of the previous model formulations where applicable, but more importantly, we made sure that all the input parameters are field-measurable. Our models also contribute towards a broader 'in-house' set of generalizable models that may be used for capturing the effects of diverse intervention strategies.

In Chapter 2, we presented and discussed a generic schema that was used to develop standardized data collections forms implemented for the study of most entomology-based experiments. Our generic schema can be used to design paper or electronic data collection forms depending on the resources (devices, informatics

experts, etc) available. In fact, one of the projects in Dar es Salaam already is using our experimental design and sample sorting forms implemented using cell phones to collect data [204]. The informatics tools developed not only work for malaria vectors, but also should work for other vector-borne diseases such as lymphatic filariasis [205, 206] and dengue [206]. In Chapter 3, based on the fundamentals of this generic schema, we were able to develop a database web-based application that can store, link, clean, and share field, laboratory, and storage data. The application is known as Ifakara Entomology Bioinformatics System (IEBS), `mscs.mu.edu/~skiware/IEBS` or `iebs.ihi.or.tz/`, (username and password are available from the author upon request). These tools we developed with the aim to complement rather than to compete with existing global-based third party repositories such as VectorBase [18] and The Malaria Atlas Project [54]. Some of the data available in IEBS was used to parameterize the models presented in this work, and we expect that data for performing quality assurance of these models will come from or will be collected by these informatics tools. The developments of these tools ensure a collection of quality malaria vector data which necessary for the success of any malaria research. Quality data is also important in the development of mathematical models to avoid "junk in, junk out". Our generic schema and IEBS not only were important to our research work through model parameterizations, but also a contribution towards a large malaria research community ensuring collaboration among multi-site studies, hence increasing research output.

In Chapter 4, we extended a published malaria transmission model [2] to examine the relationship between transmission [207], control [208], and the baseline proportion of bloodmeals obtained from humans (human blood index) [15, 103]. The lower limit of the human blood index ($\leq 10\%$) enables derivation of simplified models for zoophagic vectors. Our models were developed in such a way that its results are very simple and can be used by vector control practitioners to forecast the likely immediate and delayed impacts of personal protection measures (e.g., indoor residual spraying (IRS) [29, 30], and long-lasting insecticidal nets (LLIN) [27, 30], insecticide-treated clothing [209] or repellents [210]) against malaria transmission by zoophagic vectors. This is achieved by using only three field-measurable parameters: the proportion of human exposure to mosquitoes occurring when a intervention can be practically used [4], its protective efficacy when used [4], and demographic coverage of human users [2]. The models indicate that high levels ($\geq 80\%$) of protective coverage and efficacy are important to achieve an epidemiologically meaningful impact. As a result of models developed in Chapter 4, we were able to discuss biologically meaningful coverage indicators for eliminating malaria transmission presented in Chapter 5.

In Chapter 5, we state that LLINs or IRS are clearly insufficient in themselves to eliminate malaria transmission because *de facto* protective coverage is attenuated where mosquitoes can readily access blood resources from animals or

from humans while they are outdoors. The *de facto* coverage is a biological parameter relating to the coverage of all blood resources (rather than just from humans) that mosquitoes need to survive [2]. We stressed that strategies to complement LLINs and IRS, which extend insecticide coverage beyond houses and humans, are required to eliminate malaria transmission in most settings. We showed and explained how the overwhelming diversity (e.g., *Anopheles gambiae* and *An. funestus* from sub-Saharan Africa [7, 11] or *An. punctulatus* and *An. koliensis* from the Pacific [28]) of the world's malaria transmission systems, and optimal strategies for controlling them, can be simply conceptualized and mapped across two-dimensional scenario space defined by human blood index [15, 103] and the proportion of human exposure to mosquitoes which occurs indoors [4]. A recently submitted model [51], extends the concept of biological coverage to rationalize vector control impact based on resource (e.g., blood, resting sites, and oviposition sites) utilization rates. Some of the formulations from this model [51] and a previous model [46] which crudely described the relationship between effective coverage of adult resting sites with PPF and larval habitats were adapted while developing the models on the autodissemination of insecticide presented in Chapter 6.

In Chapter 6, we presented mathematical models to predict the probability of success for strategies to autodisseminate pyriproxyfen (PPF) (i.e., is a juvenile hormone analogue (JHA) that interrupts normal development and metamorphosis of

targeted mosquitoes [211]) from mosquito treated resting sites to the gravid mosquitoes and then to the aquatic habitats. We made sure that the overall model is based on parameters which may be measured in the field. Our model describes a simple exponential relationship between the proportion of all gravid mosquitoes which are effectively contaminated with PPF and one minus the proportion of subset of resting sites treated with PPF with their utilization rates. Then, the model presents an exponential relationship between the proportion of all habitats which are effectively contaminated with PPF and one minus that of gravid mosquitoes, the mean time that those habitats persist but remain unproductive, the number of ovipositing females detected per sticky trap [47] placed at a sample of larval habitat when the mean minimum number required to render those habitats unproductive is titered into them with cages placed over them, and the number of oviposition events detected by the same sticky traps in the same sample of habitats under natural conditions as with the cage removed. We performed and discussed one-at-a-time sensitivity analysis [212, 213] of these field-measurable parameters using biologically plausible range of input values to show the conditions at which autodissemination strategy may be a success. The analysis indicates a success of the strategy because modest achievable input values leads to a targetable model output necessary for this strategy to be useful.

7.2 Future Work

Beyond the work presented in Chapters 2–6, we briefly present projects that should be considered for future research.

Firstly, we outline several areas in which the informatics tools presented in Chapters 2 and 3 can be extended;

1. We propose that electronic (e.g., Personal Digital Assistant (PDA) or computer tablets) based data collections forms be implemented adhering to our generic schema. This way a site can choose which data collections version to use. However, the users should be informed of the advantages and disadvantages of each version and choose which one is the most appropriate for their specific site depending on the resources and expertise available.
2. Although, the informatics tools presented are currently used by several projects and experiments, they are all from only two institutes in two countries. A part of future work, our generic schema and the Ifakara Bioinformatics Entomology System (IEBS) should be recommended for use by several other research centers in other countries. To make sure that it is acceptable and sustainable, on-site training and any support that might be needed should be provided. Our IEBS should be linked with other third party

repositories (e.g., VectorBase and Malaria Atlas Project) to complement their projects, contributing to a much larger, more effectively, research community.

3. Reports generated from IEBS should be enhanced so that a researcher can obtain more information quickly from the system. For example, the system should be able to generate reports on the number of mosquitoes collected and their infectious or resistance or feeding status for a given species from a specific project and experiment. In addition, IEBS should be able to generate graphs that can provide project investigators with summarized results from a specific experiment, for example, a graph showing a trend of different species over time.

4. Lastly, funding should be sought to help our institute in sustaining and scaling up the proposed informatics tools. This way, it is a large, on-going project, so that even if a specific site opts to run and manage its own adapted system, data from all sites further system development, and support still will be maintained.

Secondly, we outline further research projects for the zoophagic mosquitoes based mathematical models (Chapter 4):

1. We propose that empirical field testing be conducted to test these parameters against a selected personal protection measure to compare the outcome with

the one simulated from our models. This will build solid evidence for our models, which then can be used to guide malaria control programs.

2. Once the solid evidence is built, we propose building systems based on the model with user-friendly interfaces that can be used by malaria control practitioners or policy makers to take advantage of our models. This way, users can easily select different reasonable input values to predict the outcome.

Lastly, we present further research work for the autodissemination of insecticide based models (Chapter 6):

1. We propose testing and refining the model against the Semi-Field Systems experimental results, once they become available. This will be a part of quality assurance for the model.

2. We recommend using the model to produce and publish results based on field-experiment data, once they become available. Also, we propose building systems based on this model with user-friendly interfaces to allow researchers to take full advantage of the model.

7.3 Concluding Statement

In conclusion, we were able to apply two of the computational sciences aspects (i.e., research data preparation using computer systems and mathematical models) to

address malaria control and elimination challenges, which is one of the most serious world's health problems. Acquiring knowledge from other disciplines (i.e., understanding the mosquito biology) was important in developing the proposed informatics tools and mathematical models. The research was performed in such a way that its findings will directly benefit the researchers and/or malaria control practitioners by providing them with informatics tools they can use to improve research outputs and mathematical models that may actually be tested and used because the results from the models are very simple and uses input parameters that may be measured in the field. Moreover, as one would expect, our work has contributed to the research/scholar community through peer-reviewed publications and oral or poster presentations in local and international conferences.

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