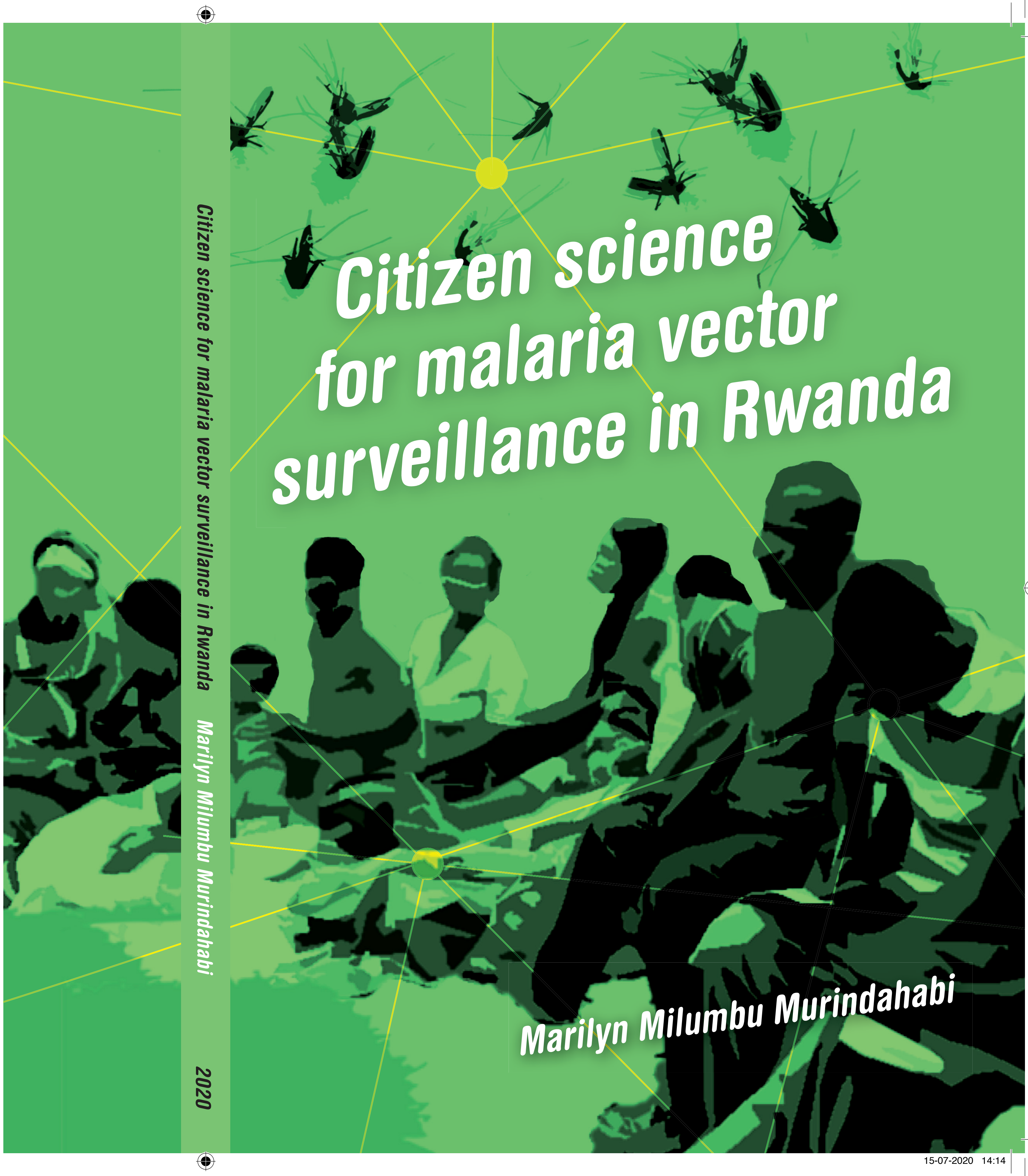


Citizen science for malaria vector surveillance in Rwanda

Marilyn Milumbu Murindahabi

Citizen science for malaria vector surveillance in Rwanda

Marilyn Milumbu Murindahabi

2020

Propositions

1. Citizen science in malaria vector control in low resource settings can only succeed when it falls within people's priorities.
   (this thesis)
2. Understanding the molecular and ecological basis of mosquito behaviour within species is key for successful vector control.
   (this thesis)
3. The role of incentives in participatory research creates undesired bias.
4. Information technology is just a tool and not a solution *per se* for education.
5. Sufficient sleep translates into sound physical and mental health.
6. PhD attainment closes career opportunities.

Propositions belonging to the thesis, entitled

Citizen science for malaria vector surveillance in Rwanda.

Marilyn Milumbu Murindahabi

Wageningen, 26 August 2020

Citizen science for malaria vector surveillance in Rwanda

Marilyn Milumbu Murindahabi

Thesis committee

Promotor

Prof. Dr W. Takken
Personal chair, Medical and Veterinary Entomology
Wageningen University & Research

Co-promotors

Dr C.J.M. Koenraadt
Associate Professor, Laboratory of Entomology
Wageningen University & Research

Dr A.J.H. van Vliet
Biologist, Environmental System Analysis Group
Wageningen University & Research

Prof. L. Mutesa,
Human geneticist, College of Medicine and Health Sciences
University of Rwanda, Kigali, Rwanda

Other members

Prof. Dr A.E.J. Wals, Wageningen University & Research
Prof. Dr M. van Vugt, University of Amsterdam
Dr G. Smant, Wageningen University & Research
Dr J. de Boer, Netherlands Institute for Ecological Research, Wageningen

This research was conducted under the auspices of the Graduate School for Production Ecology & Resource Conservation

Citizen science for malaria vector surveillance in Rwanda

Marilyn Milumbu Murindahabi

Thesis
submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus
Prof. Dr A.P.J. Mol,
In the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Wednesday 26 August 2020
at 1.30 p.m. in the Aula.

Marilyn Milumbu Murindahabi
Citizen science for malaria vector surveillance in Rwanda
186 pages.

PhD thesis, Wageningen University, Wageningen, the Netherlands (2020)
With references, with summary in English

ISBN: 978-94-6395-399-3
DOI: https://doi.org/10.18174/521433

To the people of Ruhuha

Table of contents

Chapter 1

General introduction

Malaria is one of the major public threats in the world, especially in tropical and sub-tropical countries (WHO 2019). The World Health Organization estimated 228 million new malaria cases and 405,000 deaths in 2018, with 93% of the morbidity and 94% of the mortality occurring in African regions (WHO 2019). During the last decade, huge improvements have been made in reducing the malaria burden in endemic countries around the world (Mmbando et al. 2010; O'Meara et al. 2010; WHO 2015, 2019). Between 2010 and 2018, the global incidence of malaria declined from 71 in 2010 to 57 cases in 2018 per 1,000 people at risk. Although the total number of malaria cases for 2018 decreased by 23 million compared to 2010, the data of 2016-2018 show a lack of progress as the reduction in malaria has stalled for the past few years (WHO 2018, 2019) The most vulnerable populations are children under five years of age and pregnant women. In 2018, an estimated 272,000 (67%) of all malaria deaths worldwide were children under five years of age (WHO 2019). This burden of malaria is not only felt in the health sector, but also heavily affects economic development (WHO 2019). The economic burden caused by malaria morbidity and mortality was estimated at 2.7 trillion USD in 2018 (WHO 2019). The amount invested in 2018 was less than the estimated 5.0 billion USD needed to stay on track towards the milestones of the Global Technical Strategy for malaria 2016 – 2030 (WHO 2019).

Malaria in Rwanda

In line with other African countries, Rwanda made significant progress in reducing and controlling the malaria burden from 2005 till 2011 (Karema et al. 2012; PMI 2019). This was made possible through the implementation and scaling-up of various vector control interventions (Karema et al. 2012; PMI 2013). These vector control interventions include long-lasting insecticidal treated bed nets (LLINs) and indoor residual spraying (IRS) in high endemic districts. For control of the parasite, artemisinin-based combination therapy (ACTs) constitutes the first line of defence (Karema et al. 2012). From a survey carried out in 2005, malaria was the leading cause of morbidity in children under the age of five. Malaria dropped to the third cause of morbidity in 2008, and by 2012 dropped further to the fourth cause of morbidity in children less than five years of age (PMI 2019). According to data from the Health Management Information System (HMIS) of Rwanda, between 2005 and 2011 the overall incidence of malaria decreased by 86%, and in-patient malaria deaths decreased by 74% (PMI 2019).

Despite these successes, the country experienced an eight-fold increase of reported malaria cases across the country from 2012 to 2016. Malaria incidence increased every year in the country from 48 per 1,000 population in 2012 to 403 per 1,000 in 2016 (MOH 2019). Increases of malaria cases were observed in all districts, but the eastern and southern provinces were the most affected regions with the largest increases of malaria cases (PMI 2019). A five-fold increase in malaria cases was denoted in the eastern province and a thirteen-fold increase of malaria cases was reported in the southern province (PMI 2019). An increase in malaria-related deaths was also reported during the same period from 419 deaths in 2013 to 715 deaths in 2016. Failure to effectively sustain the progress gained in malaria reduction and control achieved in the period from 2005 up to 2011 was due to multiple factors. These factors can be classified as environmental, biological, socio-economic, and institutional (see Chapter 2). Among these are an increase in rice cultivation (and hence breeding opportunities for malaria vectors), development of resistance of vectors to pyrethroid insecticides and increased rainfall, low universal coverage of Insecticide Treated Nets (ITNs) (43 percent coverage of ITNs for two people), inconsistent vector control activities, an increase in the total number of patients seeking care in health facilities, an increased number of health facilities reporting malaria cases in the health-management information system, better availability of rapid diagnostic tests (RDTs) and of ACTs (encouraging patients to seek treatment in well-stocked health facilities), (PMI 2019). In response to the dramatic increase in malaria cases over the past several years, in 2017 the Malaria and Other Parasitic Diseases Division (MOPDD) revised its National Malaria Control Strategic Plan (MSP) 2013-2020 and removed the focus on pre-elimination and reoriented the programme towards controlling malaria transmission (MOH 2017b; PMI 2019). One of the strategies of MSP aims to effectively protect 90% of the population at risk by 2024 with locally appropriate preventive and vector control interventions, based on evidence (MOH 2017b, 2019). To realize this strategy, a strong integrated vector management programme has been implemented with the aim of maintaining the effectiveness of vector control despite the threat of insecticide resistance in the country (Hakizimana 2019; Hakizimana et al. 2016; MOH 2019). In response to this plan, Rwanda has tried to reverse the general malaria situation (MOH 2019). As a result, with the end of the reporting Fiscal Year 2018-2019, malaria incidence in Rwanda reduced from 389 per 1,000 person per year in 2017-2018 to 321 per 1,000, and the number of deaths due to malaria decreased significantly as well from 392 in 2017-2018 to 264 in 2018-2019 (MOH 2019).

Malaria parasites, the vectors, and its transmission to the host

Malaria is a protozoan disease caused by *Plasmodium* parasites. The parasites are spread to a person through the bite of an infected female *Anopheles* mosquito (Maier et al. 2019; White et al. 2014). In humans, malaria is caused by one of the five species of *Plasmodium*, namely *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi* (Kantele & Jokiranta 2011). *P. falciparum* and *P. vivax* are the most prevalent malaria parasite species worldwide, with *P. falciparum* the most common across much of Sub-Saharan Africa (WHO 2019). Few infections are caused by *P. ovale* and *P. malariae* (White et al. 2014). *Plasmodium falciparum* is associated with complications such as acute kidney injury, conscious alteration, convulsions, cerebral malaria, respiratory distress, liver dysfunction and hypoglycaemia (White et al. 2014). In terms of malaria transmission, the density, longevity, biting habits and vector competence of the *Anopheles* vectors are the main determinants of the intensity of malaria transmission. There are 462 formally named *Anopheles* species, and 70 of these have been demonstrated to be competent vectors of human malaria (Hay et al. 2010; Massey et al. 2016). *Anopheles gambiae* and *An. funestus* species complexes are the primary vectors of *P. falciparum* malaria in Sub-Saharan Africa (Kelly-Hope et al. 2009). As in many Sub-Saharan African countries, the *Anopheles gambiae* complex is the primary vector species in Rwanda, consisting of the sibling species *An. gambiae* s.s. Giles and *An. arabiensis* Patton (MOH 2013; PMI 2019; Sinka et al. 2010; White 1974). As there is no non-human reservoir for human *Plasmodium*, it is considered that malaria can be eradicated with joint national and international efforts by focusing on interruption of parasite transmission. However, people coming from endemic regions can import malaria, causing recurrence of symptomatic cases (Abong'O et al. 2018; Dhiman 2019; Sturrock et al. 2015). This risk is also the primary reason why following successful elimination, ongoing rigorous surveillance is needed to identify and treat imported cases, before local vector populations can pick-up an infection and initiate the transmission.

Current strategies for malaria vector control

Vector control strategies focus on (1) adult mosquitoes when they are most active and (2) on controlling larval populations in habitats where mosquitoes are most likely to breed (Watentena & Okoye 2019). Long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS) remain the principal physical way to protect the human host from infected bites from vectors. These interventions are mostly effective in targeting malaria vectors indoors (Maharaj et al. 2019). Although these vector control interventions have contributed to approximately 70% of the total aversion of malaria cases especially in Africa including Rwanda (Bhatt et al. 2015), they are insufficient to completely eliminate or reduce the transmission of malaria. A fraction of the malaria vectors bites outdoors and therefore these interventions do not provide protection to individuals when they are outside their homes (Maharaj et al. 2019). There are currently insufficient tools to provide protection when people are outdoors (Killeen 2014). Failure to maintain the progress made in malaria control has been attributed to operational and financial challenges, growing insecticide and drug resistance, as well as to a dynamic response of malaria vectors in terms of avoiding vector interventions by an evolutionary change in their biting behaviour (Akogbéto et al. 2020; Durnez & Coosemans 2013; Ferreira et al. 2017; Hakizimana et al. 2016; Killeen 2014; Russell et al. 2011). These developments emphasize the need for continued mosquito and insecticide resistance surveillance.

Mosquito surveillance is critical for the success of any malaria control and elimination programme (Alonso et al. 2011; Dykstra, 2008; Feachem et al. 2010; Tanner & Savigny 2008; WHO 2019). Surveillance systems for the management of vector-borne diseases depend on collaboration with skilled scientists, including entomologists, epidemiologists, and statisticians (Coleman & Hemingway 2008). However, these skills are rarely represented in the average malaria control programme (Coleman & Hemingway 2008). In Rwanda, disease surveillance is done through early diagnosis combined with prompt and effective treatment both at a health facility and community level using 30,000 Community Health Workers (CHWs) (MOPDD 2013). Entomological surveillance is carried out by the National Malaria Control Programme (NMCP) and includes malaria vector collections in sentinel sites by means of active surveillance to assess vector bionomics, the resistance of the vector to insecticides and bioassays for quality control of residual efficacy of IRS campaigns and LLINs (MOH 2019). The Government of Rwanda is currently working on a revised national strategy for vision 2050 (PMI 2019). However, the Rwanda Malaria Strategic Plan targets to keep malaria trends on a downward trajectory (PMI 2019). To achieve this goal, control of transmission occurring indoor with LLINs and IRS must be improved and supplemented with vector control interventions that target adult mosquitoes outdoors or their aquatic habitats (Hakizimana et al. 2018).

Innovations in malaria vector control

Knowing that active data collection via experimental studies and field work is time-consuming, cost intensive and laborious (Hoel et al. 2015; Kampen et al. 2015; Kilama et al. 2014; Laguna-Aguilar et al. 2012; Matowo et al. 2016; Onyango et al. 2013; Silver 2008; Sukumaran et al. 2015), alternative approaches need to be investigated that can simplify data collection and that do not require the need for experts or scientists at a local level (Coleman & Hemingway 2008). ‘Citizen science’ is defined as the participation of the general public not only in the generation and sharing of data but also in raising participants’ awareness and empowering citizens. This type of community participation approach has been successfully implemented in several developed countries for the surveillance of native and invasive mosquito species (Braz et al. 2020; Caley et al. 2020; Jordan et al. 2017; Kampen et al. 2015; Palmer et al. 2017; Tyson et al. 2018). Several recent studies suggest that the application of citizen science for mosquito surveillance can complement vector control and can help in decision-making for vector-borne disease control including malaria (Braz et al. 2020; Jager et al. 2019; Jordan et al. 2017; Palmer et al. 2017; Tarter et al. 2019). Data sharing by citizens through an open web-based source can enable ‘passive’ monitoring of *Anopheles* malaria vectors, thereby providing information regarding their bionomics (Braz et al. 2020; Johnson et al. 2020; Muñoz et al. 2020; Vogels et al. 2015). Recently, a study conducted in rural Tanzania showed that a community participation approach applied in less well-resourced areas could predict outdoor biting malaria vector density and distribution (Mwangungulu et al. 2016). Applying citizen science in mosquito monitoring presents an interesting alternative for monitoring and control. It could provide a solution in tracking disease-carrying mosquitoes at low cost (Palmer et al. 2017). It expands opportunities for generating large amounts of distribution data, especially in locations where traditional sampling methods could not reach. Hence, it can provide spatial and temporal occurrence data of (invasive) mosquitoes carrying diseases for the benefit of community members. However, the engagement of citizens in mosquito collection is limited by their willingness to participate, which in turn largely depends on personal interests, awareness of the social responsibility, and strong partnerships

(Bartumeus et al. 2019). Additionally, the value of engaging citizens in mosquito collection is also determined by the ability of volunteers to use specific mosquito collection tools. Moreover, factors such as cost, ease of use, portability and the effectiveness of collection, especially the number of vector species caught, are crucial when deciding the approach or type of trap to be deployed (Bazin & Williams 2018). Citizen science for adult mosquito surveillance, specifically for malaria vectors in a rural context, has not been thoroughly investigated. For this purpose, mosquito collection schemes that citizens will use to capture adult mosquitoes need to be developed and validated in the field. Next to a suitable mosquito collection method, an infrastructure for data collection and analysis needs to be developed and evaluated. This should include mechanisms for providing feedback, as this is essential for the sustainability of any citizen science program (Braz et al. 2020; Rotman et al. 2014, 2012).

Progress in information and communication technologies (ICT), emerging digital technologies, and especially mobile devices has shown to be useful for improving public health in several contexts (Braz et al. 2020; Guilbaud & Guilbaud 2017; Hamer et al. 2018; Johnson et al. 2020; Kampen et al. 2015; Oltra et al. 2016; Palmer et al. 2018). In low income countries, digital technologies and mobile phones have been introduced to accelerate improvements in healthcare and to facilitate communication and to support data collection, management, and use in real-time during supervision and monitoring (Entsieh et al. 2015; Gibson et al. 2017; Kolff et al. 2018). Some studies showed that the use of mobile phones indeed contribute to the improvement of maternal and child health in developing countries (Barron et al. 2016; Entsieh et al. 2015; Ngabo et al. 2012). However, applications of mobile phone technology and ICT in malaria vector control are quite sparse and present challenges (Eskenazi et al. 2014; Mangam et al. 2016). Thus far, no studies have investigated how a citizen science approach in combination with emerging digital technologies can provide information on malaria vector bionomics and malaria transmission patterns in an African context. There is a scope for it to contribute to the monitoring of malaria vectors and malaria surveillance (Johnson et al. 2018; Jordan et al. 2017; Palmer et al. 2017; Walther & Kampen. 2017).

Besides investigating the ecological questions related to the implementation of a citizen science approach for malaria control, it will be critical to also address a series of social questions related to the adoption of novel technologies and approaches. Therefore, the results presented in this thesis are the result of an interdisciplinary project conducted in collaboration with a social component (PhD thesis Asingizwe 2020) that focused on the role of human behaviour in designing and adopting new intervention strategies for malaria control. The specific malaria project was conducted as part of a multidisciplinary programme entitled “Environmental Virtual Observatories for Connective Action (EVOCA)” which had the aim to develop virtual platforms and participatory monitoring programmes towards inducing connective action to address development challenges in crop, water, health and wildlife management (Cieslik et al. 2018).

Aim of this thesis

The aim of this thesis was to investigate the opportunities for citizen science in malaria vector surveillance in low resource settings in Rwanda, especially in areas where routine mosquito monitoring is not established. Ultimately, the aim is, with this approach, to contribute to the improvement of malaria vector control. Therefore, this thesis focuses on mosquitoes as key players in the transmission of malaria, their spatial and temporal distribution, the determinants of their distribution using citizen science data, and the role of citizens in the design of a monitoring programme (see Figure 1). The work described in this thesis has the following specific objectives:

1. To identify factors that contributed to the upsurge of malaria in Rwanda and define the desired characteristics for a system that uses citizen science for malaria mosquito surveillance.
2. To define the link between perception of mosquito nuisance and actual vector abundance as an indicator of malaria vector hotspots.
3. To evaluate a co-design process for the sustained implementation of a citizen science programme for malaria mosquito surveillance.
4. To assess the efficiency of a handmade, carbon-dioxide baited trap for collecting adult malaria vectors for use in a citizen science programme for malaria mosquito surveillance.
5. To evaluate the value of a citizen science programme in providing insight into potential malaria vector hotspots and other malaria related information, and to determine predictors of malaria vector distribution.

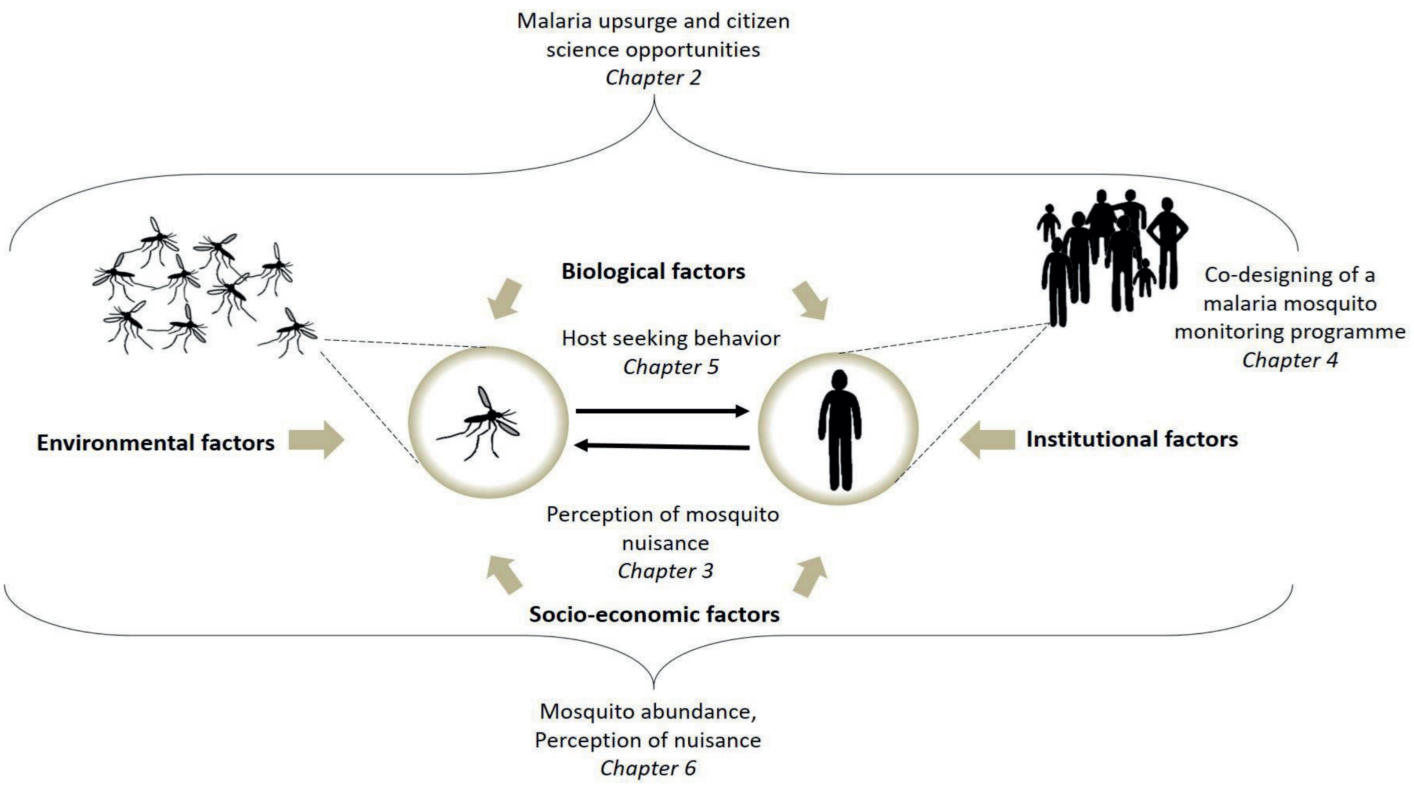


Figure 1. Overview of the topics of this thesis, described in the various chapters, and their link to vector-host interactions.

Outline of the thesis

For **Chapter 2**, I reviewed the literature that investigated the environmental, biological, socio-economic and institutional factors that have contributed to the resurgence of malaria in general, and in the Rwandan context specifically. In addition, the chapter examines the current challenges for the vector control surveillance system in Rwanda and explores the opportunity and feasibility of a citizen science approach as a new and alternative strategy to contribute to the improvement of malaria mosquito surveillance in low resource settings.

Chapter 3 assesses the species composition of malaria vectors in particular using a conventional standard mosquito collection tool through entomological surveys and determines the factors that explain malaria vector abundance collected through household surveys. Additionally, it investigates the correlation between perception of mosquito nuisance by citizens and actual malaria vector abundance as an indicator of malaria vectors hotspots for use in a citizen science programme.

In **Chapter 4,** the co-design process that was initiated in collaboration with communities in Ruhuha, Rwanda through participatory design workshops, was evaluated with the aim to develop and eventually implement a citizen science programme for sustained malaria mosquito surveillance. The chapter focuses on both the technical and social components of such a program.

In **Chapter 5,** I investigate the development and evaluation of a simple, handmade trap with different chemical and physical attractants for capturing malaria vectors. The trap was tested under both laboratory and field conditions with the intention to use it in a citizen science programme for malaria mosquito surveillance in rural Rwanda.

Chapter 6 evaluates the results of a one-year citizen science programme and focuses on hotspots of malaria vectors and other malaria related information gathered by volunteers in Ruhuha, Rwanda. In addition, the chapter describes the environmental predictors obtained using remotely sensed data for the presence of the malaria vector hotspots in Ruhuha, Rwanda.

For **Chapter 7,** I integrate the results of this thesis in a broader context by identifying the mosquito-related factors that are most important for malaria establishment. The results obtained from this thesis will be discussed in light of improving malaria vector surveillance and the opportunities for citizen science. Finally, recommendations for managing malaria in rural Rwanda will be put forward.

Acknowledgements

I thank Professor Willem Takken, Sander Koenraadt, Marijn Poortvliet and Arnold van Vliet for providing helpful comments on an earlier version of this chapter.

Chapter 2

A citizen science approach for malaria mosquito surveillance and control in Rwanda

Murindahabi M. Marilyn, Domina Asingizwe, P. Marijn Poortvliet, Arnold J.H. van Vliet,
Emmanuel Hakizimana, Leon Mutesa, Willem Takken, Constantianus J.M. Koenraadt

Published in *NJAS - Wageningen Journal of Life Sciences 86–87* (2018), 101–110

Abstract

Despite the implementation of a number of interventions aimed at controlling malaria, Rwanda is experiencing a countrywide resurgence of malaria cases over the past five years. To support malaria control, mosquito surveillance activities, such as systematic reporting of the distribution, the diversity and the infectivity rate of malaria vectors throughout the country, have been undertaken. However, mosquito monitoring programmes are not carried out to monitor the impact of all vector control interventions or to determine the distribution of mosquito species in all areas, especially in the remote regions of the country. With a target of reducing malaria mortality by 2020, implementation of mosquito surveillance in those regions is urgently needed as well. In this paper, a citizen science approach as a capacity resource for malaria vector monitoring for the Rwandan National Malaria Control Programme is presented. The ultimate aim is to complement existing mosquito surveillance currently in place by providing key information on the spatio-temporal distribution of mosquito nuisance and malaria vectors. This will contribute to an insight into the ecology of malaria vectors and thereby to a better understanding of malaria transmission patterns in Rwanda.

Introduction

Over the last decade, increased international investment coupled with the increase in malaria control interventions at country-level, have resulted in a substantial reduction of the global burden of malaria, particularly in Sub-Saharan Africa (SSA) (Chizema-Kawesha et al. 2010; Jaenisch et al. 2010; Mmbando et al. 2010; O'Meara et al. 2010). This reduction has been mostly attributed to the wide-scale deployment of Long-lasting insecticide treated bed nets (LLINs), indoor residual spraying (IRS) and artemisinin-based combination therapy (ACT) (WHO 2016b). However, malaria continues to cause significant morbidity and mortality and threatens the economic development of SSA in particular (WHO 2016b). In 2016, 216 million malaria cases were reported globally, and these had resulted in approximately 445,000 fatalities. SSA alone accounted for more than 90% of the malaria case mortalities (WHO 2017b). Importantly, after years of steady progress in malaria control, the World Health Organization (WHO) now reported that this progress had stalled.

Rwanda is one of the countries in SSA where malaria remains a major public health concern, putting the entire population at risk, including 1.8 million children under the age of five and 443,000 women (PMI 2017). The northern and the western regions, representing 60% of the country, are epidemic-prone. The remaining 40% of the country is malaria endemic with main foci in the eastern and south-eastern parts. In these regions the altitude is approximately below 1,500 meters above sea level and characterized by marshy plains, rice cultivation, and brick-making, all of which create suitable breeding sites for mosquitoes (PMI 2015).

Coordinated efforts to fight malaria began in the 1950s and continued until 1994, when the Rwandan genocide engulfed health research and further progress was not possible (Binagwaho et al. 2014). The government of Rwanda, as part of the 15 high burden countries in SSA, benefited from the financial support of the Global Health Initiative (GHI) to scale up malaria prevention and treatment interventions as well as reduce malaria-related mortality by 50%. The first comprehensive national malaria strategic plan of Rwanda for the years 2005 to 2010 received support from partners for scaling up vector control interventions. The primary donors were the Global Funds to fight HIV/AIDS, Tuberculosis and Malaria, and the United States President's Malaria Initiative (PMI) (Karema et al. 2012). After its launch, malaria dropped from being the leading cause of morbidity and mortality in children under five in 2005 to the third-leading cause in 2008 and subsequently the fourth-leading cause in 2012 (PMI 2017). Among children under five years, a reduction of more than 50% in malaria mortality and morbidity was observed (Otten et al. 2009). Further, a major decline of 86% in malaria incidence, 87% in malaria cases and 74 % of malaria deaths was reported between 2005 and 2011 (NISR, 2015; PMI, 2017). This reduction has been attributed to the scale-up of ACT, and to deployment of LLINs and IRS in high-risk districts of Rwanda (Karema et al. 2012).

During the last few years, malaria incidence has increased in some SSA regions, especially in the East African highland areas (Chaves et al. 2012; Hay et al. 2002; O'Meara et al. 2010; Wandiga et al. 2010). In Rwanda, malaria incidence increased again in 2009 due to logistic delays in the provision of new treated nets at a time when the effectiveness of the previously distributed LLINs was waning (Karema et al. 2012). The number of malaria cases declined again in 2010 following a massive LLIN distribution campaign in the country (Karema et al. 2012). According to the Rwanda Health Management Information System (HMIS), the country has experienced an increase of malaria morbidity since the end of 2012 to 2016. Rwanda has faced an increase from 1.1 million cases in 2013-2014 to more than 4.7 million of simple malaria cases in 2016. This increase was observed across all age groups. The 10 districts with the largest number of cases accounted for more than 62% of all malaria cases, mostly in the eastern and southern provinces (MOH 2017).

Malaria resurgence has been attributed to various interrelated factors that can be divided into four main groups including environmental, biological, socio-economic and institutional factors (Himeidan & Kweka 2012; Lindsay & Martens 1998; Protopopoff et al. 2009; Wijngaarden et al. 2012). To prevent and reduce malaria, there is a need to understand the system that underlies the interactions between the parasite, the mosquito vector and the human host (Figure 1). Malaria control relies on the surveillance anchored in this vector-parasite-host system. This requires data on spatial and temporal variability of malaria mosquito populations, parasite prevalence and malaria cases (Mnzava et al. 2014). Mosquito surveillance and a clear understanding of local transmission ecology of malaria vectors are central to reduce transmission and prevent re-establishment of the infection (Mnzava et al. 2014). Although mosquito surveillance is undertaken in Rwanda, the country has faced challenges, because frequently there is a sparsity of essential data on malaria vector bionomics (both the ecology of a mosquito species and its behaviour, e.g. host biting preferences) in remote regions. Moreover, the current methods used for mosquito monitoring, such as human landing catches, are constrained by costs and ethical concerns.

Several citizen science networks from developed countries provide large amounts of relevant data on mosquito bionomics and have proven their potential in mosquito monitoring (Bartumeus et al. 2018; Kampen et al. 2015; Tyson et al. 2018; Yujia 2017). We understand citizen science as the involvement of volunteer citizens in observation, classification, collection and/or the analysis of data (Kullenberg & Kasperowski 2016). The same term is also defined by others as engagement in scientific research with members of the public who join scientists in the collection and analysis of the large amount of data generated (Bonney et al. 2016). Among the myriad of definitions, the approach involves the participation of the public in the generation, sharing and possibly analysis of data. In many cases, volunteers are presented with websites and mobile or smart phone applications to report their observations which afterwards are analysed and visualised (Crain et al. 2014; Kampen et al. 2015). These innnovative tools give prospect to resolve connectivity and communication issues related to mosquito monitoring. In addition there is a prospect to be used as a data collection tool in rural areas as it has been demonstrated in some African countries where mobile phonses have been used for malaria surveillance activities (Zurovac et al. 2012).

Moreover, a citizen science approach can build capacity in mosquito monitoring especially where it is minimal or nonexistent (Mnzava et al. 2014). This, on its turn, may create more participation, involvement and awareness, resulting in more sustainable malaria control (Kampen et al. 2015; Yujia He 2017).

The question therefore is whether a citizen science approach can work similarly in Rwanda. Our case study project intends to set-up a citizen science approach for mosquito monitoring using a web-based reporting platform in Ruhuha sector, Rwanda. Ruhuha is one of the thirteen administrative sectors of Bugesera District in the Eastern province of Rwanda. Ruhuha is known as a high malaria transmission setting composed of five administrative cells (Kateera et al. 2015). This area has experienced an increase in malaria incidence in the last few years. Pre-existing collaborations among local partners and project members in malaria control studies guided the selection of Ruhuha (Ingabire et al. 2014; Ingabire, Hakizimana, et al. 2016; Ingabire, Kateera, et al. 2016; Kateera, Rulisa, et al. 2015; Kateera, Mens, et al. 2015).

The objectives of this chapter are i) to analyse how environmental, biological, socio-economic and institutional factors contribute to the resurgence of malaria; ii) to analyse the current surveillance and control systems/activities in Rwanda; iii) to determine the potential of a citizen science approach and the role of digital technology tools therein to support malaria vector surveillance and control; and iv) to describe opportunities and challenges that citizen science presents, including a possible workflow of a citizen science network in Rwanda for malaria mosquito monitoring. A companion paper focuses particularly on the factors that determine the adoption of the approach, as well as the mechanisms involved in the adoption process (Asingizwe et al. 2018). In this chapter, we emphasise the opportunities of citizen science in providing knowledge on biological factors, such as malaria vector species and population dynamics, in the context of environmental, socio-economic and institutional factors. This could lead to a better understanding of malaria transmission dynamics, and thus support decision making in malaria control.

Determinants of malaria transmission and their roles in resurgence

Environmental factors

Climatic and non-climatic environmental factors are main drivers of the trends in malaria incidence and possibly in malaria resurgence (Bhunu et al. 2016; Parham et al. 2015; Stern et al. 2011). Rainfall, temperature and humidity are important climatic determinants of vector abundance and distribution between low- and high-altitude areas (Omumbo et al. 2011; Stern et al. 2011). These factors influence directly and indirectly the availability of (breeding) habitats, growth and development, reproduction, survival of malaria vectors and hence the pathogen (Githeko et al. 2000). Breeding habitats of malaria mosquito species range from large, permanent bodies of water to small temporary pools and puddles (Fletcher et al. 1992; Himeidan & Kweka 2012; Kweka et al. 2013; Sattler et al. 2005).

Variability in humidity affects the pathogen of infectious disease such as malaria. Humidity was found to affect malaria parasite development in *Anopheles* mosquitoes (Patz et al. 2000).

In Rwanda, malaria transmission occurs year-round, often in two peaks associated with the two rainy seasons. One from March to June and another, shorter season from November to December, alternated with a long and a short dry season (MOH 2016; PMI 2015). The country has experienced unusual climate patterns including variability in rainfall frequency and intensity, extreme temperatures as well as increased temperature over the last thirty years (Loevinsohn 1994; Mutabazi 2010; Rwanyiziri & Rugema 2013). The analysis of trends in rainfall showed that rainy seasons have become shorter, with higher intensity of rainfall. Events such as droughts and floods associated with heavy rainfall and extreme temperatures were increasingly reported in the eastern and northern regions of Rwanda. While in the north floods were more frequent, the eastern part was affected by droughts and extreme temperatures (REMA 2006). Such changes in rain patterns directly affect the availability of breeding places. A study conducted by the Stockholm Environment Institute (SEI) in 2009 states that climate variability has and will have significant economic costs in Rwanda, and can result in a large increase in the health burden of malaria in the absence of adaptation in the coming years (SEI 2009).

Non-climatic environmental factors encompass factors responsible for proliferation of malaria vector breeding sites. The majority of the mosquito breeding sites in Africa are man-made (Chaki et al. 2009) and are the result of deforestation, land-use changes (irrigation in a riceland), construction of roads, water control systems (dams, canals, irrigation canals and reservoirs), commercial development and human settlement. All of these factors can create breeding sites for malaria mosquitoes (Keiser et al. 2005; Norris 2004; De Silva & Marshall 2012; Stern et al. 2011; Yewhalaw et al. 2009). In Western Africa, it has been found that after deforestation and irrigation, an increase in *P. falciparum* malaria transmission by *An. gambiae* occurred in villages in proximity to forest, by *An. funestus* in the savannah, and by *An. arabiensis* in urban and peri-urban areas (Patz et al. 2000). The development of agriculture in Rwanda has led to an important transformation of the ecological landscape, creating suitable habitats for malaria vectors. Evidence shows that an increase of rice cultivation in the south-eastern and western of the country was linked to an increase in malaria incidence in those regions (MOH 2017b). Studies suggest that the introduction of intensive irrigation increases malaria transmission. Human movement from high- to lowland areas has facilitated the distribution of parasites. Migration has been demonstrated as a key factor in the maintenance, or increase of malaria transmission, and hence its resurgence, as well as in the spread of antimalarial drug resistance (Lindsay & Martens 1998; Lynch et al. 2015). The influx of new populations from other regions is probably a side effect of deforestation and the initiation of agriculture, livestock keeping, mining and construction. Resettled populations lacking familiarity with the environment of the area may inadvertently engage in practices leading to infection and, in turn, become reservoirs of infection, leading rapidly to the increase of malaria incidence (Patz et al. 2000).

Biological factors

Several biological factors have been suggested as leading causes of malaria resurgence. These relate to the pathogen and health status of the human population, as well as to pyrethroid insecticide resistance, and feeding and biting behaviour changes of the malaria mosquito population. The health status of the population can be affected by malnutrition, a weakened immunity by HIV infection, spread of drug-resistance of the parasites and loss of immunity (Bates et al. 2004; Delenasaw & Kweka 2016; Protopopoff et al. 2009; Reddy et al. 2011; Russell et al. 2011). Acquired immunity to malaria protects millions of people routinely exposed to *Plasmodium falciparum* infection from severe disease and death in high malaria transmission areas. This acquired immunity is lost when the individual is no longer repeatedly exposed to the parasites, for example after migrating from an endemic area to a hypoendemic malaria zone (Doolan et al. 2009). A study conducted in Kabale, Uganda, showed that residents, who travelled to areas of higher transmission intensity than their home of origin characterized by a low transmission intensity, had a higher risk of malaria than those that had not travelled. They were more susceptible to malaria infection because they had lower or no immunity against malaria infection due to low or no exposure to malaria infection (Anderson et al. 2009; Lynch et al. 2015). Other factors such as pregnancy status, age, sex and genetic background influence the degree of immunity that an individual develops against malaria infection and thus are underlying risk factors for malaria (Bates et al. 2004).

In the nineties, parasites have been reported with resistance to the drug chloroquine in Rwanda (Loevinsohn 1994). In response to high rates of resistance to chloroquine recorded around the country, the Rwandan Ministry of Health (MOH) switched its first-line antimalarial treatment policy from chloroquine to amodiaquine (AQ) and sulphadoxine-pyrimethamine (SP, also called Fansidar®), implemented from 2002 up to 2005. In 2006, the country shifted from AQ-SP to an ACT, artemether-lumefantrine (Karema et al. 2012). Artemisinin and ACT are the currently used drugs to tackle malaria. Resistance to malaria drugs leads to treatment failure and increases the risk to maintain malaria infection and spread the disease among the population. Although resistance to ACT is emerging and spreading from the Greater Mekong Subregion in Asia, no case of resistance to the currently used ACT has been reported yet in Rwanda (WHO 2016a).

Extensive use of IRS and high coverage of LLINs impregnated with pyrethroids have contributed to the development of resistance in malaria mosquitoes against these compounds. High levels of pyrethroid insecticide resistance reduce the effectiveness of LLINs (Delenasaw & Kweka 2016). The use of LLINs and IRS may also cause shifts in mosquito behaviour in three ways: a shift in repellency, a shift in sibling species ratio and a shift in behaviour resistance. The shift in repellency is defined as the effect that some insecticide ingredients impregnated in nets or from IRS can exert on the mosquitoes (Bradley et al. 2015). According to Alipour et al. (2005), excito-repellency occurs in the vector population after an application of insecticides. Mosquitoes leave sprayed houses for non-sprayed houses or outdoor shelters before receiving a lethal dose of insecticide (Alipour et al. 2005).

This is less of a long-term threat to control because these changes are phenotypic and temporary. The shift in sibling species ratio describes a change in the relative abundance of species. This is the case for *Anopheles gambiae s.s.* and *An. arabiensis*. These species differ in their tendency to bite indoors, and therefore one species is more affected by indoor interventions than the other (Bradley et al. 2015). A shift in behavioural resistance is defined as genetically-determined behavioural resistant variants evolving within a species and becoming more common due to selection pressure caused by the insecticide (Bradley et al. 2015; Killeen 2014; Reddy et al. 2011; Russell et al. 2011). It has been demonstrated that the insecticide resistance trait in malaria vectors can also affect house entry behaviour (Asidi et al. 2005; Chandre et al. 2010), leading to an increase of exophilic biting behaviour. This results in malaria transmission outdoors where there is no coverage with adequate vector control interventions, thereby rendering malaria elimination infeasible (Killeen 2014).

Similar to other countries in Africa, vector control in Rwanda is highly dependent on the use of pyrethroids, the only class of insecticides currently recommended for LLIN treatment. A nationwide insecticide resistance survey conducted in 2011 and 2013 revealed a gradual increase of insecticide resistance to pyrethroids (lambda-cyhalothrin, deltametrin, permethrin) and organochlorines (DDT) (Hakizimana et al. 2016). This is a great concern because pyrethroids are the only class of insecticides currently recommended for LLINs (WHO 2015). In addition, changes in feeding and biting behaviour of malaria vectors have increased the concerns for the National Malaria Control programme as well. Entomological surveys conducted in 12 sentinel sites throughout the country showed an increase of entomological inoculation rate (EIR: number of infective bites per person per year) from an approximate of 24 in 2013 to 65 in 2015 (MOH 2016). The current vector surveillance information in the country shows that between 52% and 57% of *An. gambiae* and 52 % and 54% of *An. funestus* feed outdoors (MOH 2017b).

Socio-economic factors

A large number of socio-economic factors may influence malaria risk (Kienberger & Hagenlocher 2014). These include socio-economic status (poverty, level of education etc.), failure to use and misuse of treated bed nets, failure to sustain effective malaria control, and introduction of unsustainable control measures. Poverty puts some groups of people or individuals at high risk of malaria infection because they have limited capacity to respond to illness. Poverty coupled to level of education can also influence malaria incidence as it affects knowledge about malaria prevention (Bizimana et al. 2015). A study conducted in the southern highlands of Rwanda demonstrated that a low level of education was associated with increased risk of malaria infection and parasite load in children (Gahutu et al. 2011). Similarly, the interplay of poverty and other socio-economic variables intensified the vulnerability of communities to malaria infection in the highlands of the Lake Victoria basin (Wandiga et al. 2010). Socio-economic variables included food shortage, non-use of treated bed nets due to unavailability of sufficient bed nets per household etc.. Another study conducted in Rwanda found that maintaining universal net coverage is not enough to protect the population from malaria. In that study, factors such as poverty, education level, birth spacing and antenatal clinic attendance were risk factors linked to ITN use at individual, household and community level (Ruyange et al. 2016). In a study conducted in Ruhuha, Rwanda, it has been reported that bedbug infestation jeopardized the use of bed nets. Despite LLIN availability, seasonal use of nets, as well as non-use of nets by males were reported in Ruhuha. Both were linked to a lack of knowledge and perception of malaria as a non-severe infection. Participants in the survey underscored weather (e.g. high level of non-use of LLINs in the dry season) as one of the reasons for inconsistent ITN use (Ingabire et al. 2014, 2015; Kateera et al. 2015). Misuse of treated bed nets as fences for protection of vegetable gardens or as chicken pens, can put populations at risk of malaria, thus enhancing malaria resurgence (Alaii et al. 2003; Honjo et al. 2013; Mboera et al. 2013). However, other authors argue that many of these examples on misuse and non-use of bed nets are not evidence based and rather anecdotal (Eisele et al. 2011; Honjo et al. 2013; Minakawa et al. 2008).

Institutional factors

In addition to socio-economic, biological and environmental factors, institutional and political factors may ultimately also influence malaria risk. For example, failure to sustain previously effective malaria control measures due to limited resources, and the relaxation of vector control activities have both shown to contribute to malaria resurgence. This resurgence has been described as post-eradication epidemics (Najera et al. 1998). Cessation or interruption of control measures or ending of mosquito control such as spraying with Dichloro Diphenyl Trichloroethane (DDT), and disruption of antimalaria drug prophylaxis provision have played a role in the resurgence of malaria (Fisher 1985; Ijumba & Lindsay 2001; Mouchet 1998). Apparently, cessation of DDT was associated to a poor understanding of the parasite-host-environment-human system that required a continuous surveillance even if the containment of the disease was a success. Mouchet reported that IRS with DDT and distribution of chloroquine resulted in the elimination of malaria in Madagascar in 1960. Because of the success of the malaria programme, insecticide treatments were abolished, except in three suspected outbreaks sites until 1975. In 1986, a malaria epidemic broke out following the interruption of insecticide application since 1975 and cessation of chloroquine distribution since 1979 due to drug stock out. The cases increased exponentially from 1986 to 1988, and the disease persisted at a very high level until the end of 1993 (Mouchet 1998).

In Rwanda, institutional factors that have contributed to the resurgence of malaria also include the non-universal or drop in coverage of effective vector control interventions. This relates especially to the significant drop of LLIN, IRS coverage at community level and the deployment of bed nets with sub-optimal concentrations of insecticides (Binagwaho & Karema 2015; Hakizimana et al. 2014; Karema et al. 2012; MOH 2017b). Lack or delay in acquiring the funds to cover activities planned for malaria can also jeopardize malaria control (Head et al. 2017). In 2016, delays in acquiring funds affected timely IRS implementation, hence causing an increase of malaria cases in Bugesera district from November 2016 and April 2017 (MOH 2017b). In response to the resurgence of malaria, an Integrated Vector Management (IVM) programme was recently initiated in Rwanda as a framework for malaria interventions based on local ecology, disease epidemiology, socio-economic factors and inter-sectoral collaboration (PMI 2014; USAID 2012a, 2012b). The IVM approach encompasses environmental modifications through both infrastructural development and sanitation services to regulate the vectors with the final aim to improve public health (Karina et al. 2015). As IVM is at its initiation phase, collaboration among local and intersectoral institutions involved in malaria prevention and control is still low. Despite the efforts made in building local infrastructure and skills needed to sustain the coverage, technical capacity and funds necessary to cover the mosquito monitoring activities are still inadequate. Moreover, mosquito monitoring programmes are often inexistent in remote areas of the country (Mnzava et al. 2014; MOH 2017b).

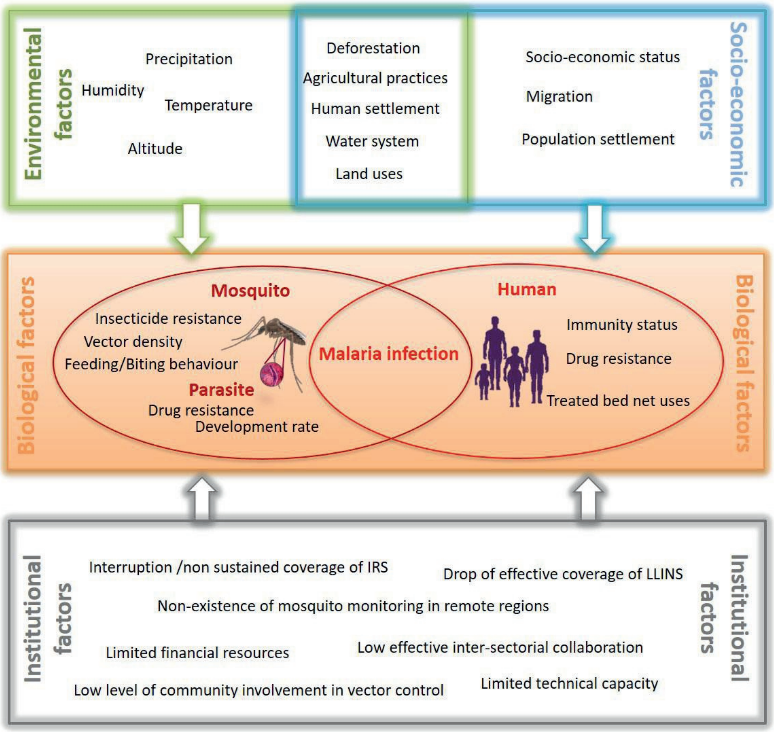


Figure 1. A conceptual model explaining Interrelated factors determining the resurgence of malaria in Rwanda. This figure is adapted from Lindsay & Martens (Lindsay et al. 1998), Lindsay et al. (2004) and Protopopoff et al. (2009). Factors are categorized into four main groups. The green label represents environmental factors, the orange label represents biological factors, the blue label represents socio-economic factors, and the grey label represents institutional factors.

Surveillance and control of malaria and mosquitoes in Rwanda

Surveillance and control of malaria

Any anti-malaria programme relies on a properly functioning information system. Rwanda has a malaria surveillance system in place through the Health Management Information System (HMIS) (Karema et al. 2012). A wide range of aggregate epidemiological or health data (e.g. malaria cases, but also demographic data reported from public health facilities) are reported into the HMIS and used for planning purposes and decision-making. HMIS is a reporting system that enables sharing of both qualitative and quantitative data. Qualitative data include patient profile and various demographic indicators from health surveys. Quantitative data include the number of malaria cases from health facilities, the proportion of malaria-attributed deaths among children under 5 years old and among all age groups at the health facility, as well as malaria Rapid Diagnostic Test (RDT) positivity rate in fever cases at community level and at the health facility. Within the HMIS for malaria control, Community Health Workers (CHWs) play a central role, as they are key in the diagnosis and treatment of malaria at the community level. On a monthly basis, CHWs report all data on malaria cases via the HMIS treated at the community level to the health centers. The data captured through the HMIS enables the Malaria and Other Parasitic Disease Division (MOPDD) of the Rwanda Biomedical Center to respond promptly to the community level needs (PMI 2017).

Surveillance and control of mosquitoes

Effective surveillance of malaria is critical for the distribution of appropriate vector control measures and resources to areas with a high malaria burden (Alonso et al. 2011; Feachem et al. 2010; Tanner & Savigny 2008). In line with WHO recommendations, vector control in Rwanda is guided by local epidemiological and entomological data, including vector behaviour and insecticide resistance. These data have been collected across the country in twelve sentinel sites since 2010 (Hakizimana et al. 2016). The National Malaria Control Programme (NMCP) is responsible for implementing the control measures countrywide. IRS and LLINs are the main measures used for vector control in Rwanda. In five districts with high malaria burden, IRS is applied (PMI 2017). Monitoring of insecticide resistance is carried out as well in these five high malaria burden districts (Hakizimana et al. 2016; Karema et al. 2012). Moreover, in response to resistance to insecticides, an insecticide resistance management plan has been developed and implemented since 2013 (Hakizimana et al. 2016).

For an effective malaria surveillance system, relevant entomological data, such as malaria vector bionomics (both the ecology of a mosquito species and its behaviour like host biting preferences), in combination with environmental data (e.g. temperature, rainfall) can help in the planning, implementation and management of vector control operations. Studies conducted in Rwanda showed the potential of using meteorological variables such as rainfall and temperature to explain patterns of malaria infection (Karema et al. 2012; Loevinsohn 1994). Quantitative spatial models have linked climatic factors with malaria occurrence, particularly in Africa (Kleinschmidt et al. 2000; Bhunu et al. 2016). Such disease risk mapping can be used to differentiate areas with epidemic or seasonal transmission from those with more stable transmission patterns where early detection is likely to be less useful (Kuhn et al. 2004). Geographic Information System-based surveys using satellites have identified probable vector breeding sites and mosquito abundance. Malaria risk is associated with the proximity of human hosts and mosquito breeding sites. Integration of remote sensing, epidemiological and climatological data have been used to predict malaria outbreaks (Ceccato et al. 2005). Based on such models, a malaria warning platform (map room) has been developed since 2014 through the Enhanced National Climate Services (ENACTS) project (Dinku et al. 2016). The platform provides national maps of malaria risk, based on historic climate data from weather station observations and malaria reported case data. Predictive maps and charts provide forecasts of malaria epidemics and outbreaks (ENACTS 2016).

Monitoring of malaria vectors, species composition, malaria vector abundance and dynamics, their feeding patterns and host finding strategies is part of an active malaria vector surveillance programme. This consists of deploying mosquito sampling techniques or strategies to determine feeding behaviour and the distribution of *Anopheles* species responsible for malaria transmission (Busula et al. 2015; Diabaté et al. 2013; Hiscox et al. 2014; Homan 2015; Kilama et al. 2014; Onyango et al. 2013; Sikaala et al. 2014). In Rwanda, Pyrethrum Spray Catches (PSC) and Human Landing Catches (HLC) are used as mosquito collection methods during entomological surveys (PMI 2017). HLC is the standard method used and targets adult mosquitoes both indoors as well as in the peri-domestic environment (Ferguson et al. 2010). HLC is used as the ‘golden standard’ method to estimate biting rates and malaria transmission by mosquitoes. However, deploying this technique on a large scale is costly, labour intensive and time consuming, may cause collector fatigue and has raised ethical concerns of exposing collectors to mosquito-borne parasites (Frederick et al. 2016; Govella et al. 2010; Onyango et al. 2013). Without proper prophylaxis, concerns about risk of malaria among HLC collectors can be an impediment to use HLC for research purposes (Gimnig et al. 2013).

Interestingly, there is no ‘Vector Management Information System’ yet at the central level in which entomological data such as vector density, resting behaviour, species composition, larval habitats, infection rate, blood feeding index and parity rate are uploaded. Currently, the entomological activities carried out on a monthly basis at sentinel sites are related to vector bionomics, vector resistance to insecticides and bioassays for quality control and status of residual efficacy of IRS and LLINs. Although such indicators are collected, the current system does not provide free access to information to improve our understanding of the parasite-host-environment-human system. Therefore, the development of a citizen science approach that implements the principles of community participation could serve as an addition to the existing system in place for malaria control. It may offer a novel way of data generation on malaria vectors in resource-constrained settings. This proposed approach would provide with mosquito nuisance indicator (level of biting nuisance experienced), a dimension currently missing from the information system on vector control.

Potential of citizen science and emerging information and communication technology to support malaria mosquito monitoring in Rwanda

Citizen science for mosquito surveillance and control

With regards to control of invasive mosquito species, data sharing on mosquito sightings by citizens through an open web-based platform can enable “passive” monitoring of several species of mosquitoes, thereby providing information regarding the bionomics of (invasive) mosquito species (Franzoni & Sauermann 2014; Kampen et al. 2015; Medlock et al. 2012; Vogels et al. 2015; Walther & Kampen 2017). Such approaches open up opportunities not only for monitoring invasive mosquito species, but also for malaria vector surveillance. A study conducted by Vogels et al. (2015), showed that a web-based reporting platform helped to identify *Culex pipiens* as responsible for indoor biting nuisance in the Netherlands and that a particular biotype of this species, *molestus*, remained active throughout the winter. In this case, Dutch citizens were requested to catch mosquitoes indoor and in their backyards. Prior to sending a mosquito specimen to the laboratory, a form was filled with a specific code via the mosquito radar web-platform that allowed linking the mosquito sample that was sent in with the accompanying data provided online. Statistical analysis based on these collected data provided spatial and temporal maps of vector species, and explained the biting nuisance that the population was experiencing (Vogels et al. 2015).

Another study conducted by Lozano-Fuentes *et* al. (2013), proposed a cell phone–based system (called Chaak) that deals with the collection of mosquitoes at the early stage (larvae) of dengue mosquitoes in artificial containers on individual premises. Numbers of immature stages of *Aedes aegypti* L. present per type of container (e.g. buckets, tyres, cisterns) and per house were uploaded via surveyors’ cell phones to the central repository of the Chaak system upon Wi-Fi connection. The study involved surveyors who were field workers using their own mobile phones. Maps representing the spatial distribution of the immature vectors were built based on data aggregated in the system. Recently, the Global Mosquito Alert was launched with the aim to stimulate citizen science approaches in tracking and controlling mosquitoes and mosquito-borne diseases. This global alert includes scientists and volunteers from different countries around the world (Yujia He 2017).

Another study conducted in rural Tanzania showed that community-based participation mapping could predict densities and distribution of disease-transmitting mosquitoes in remote rural areas. The study relied on local knowledge and experience of the community members to identify areas with the most abundant or least abundant mosquito biting or presence (Mwangungulu et al. 2016). This study revealed that scientific research could rely on community participation, knowledge, and experience although mobile phones were not used in this case. This makes such an approach a relevant option to explore for malaria control programmes.

Digital technology tools to support malaria mosquito monitoring

Participation in malaria surveillance and vector control in resource-limited settings in SSA malaria endemic countries has been stimulated using digital technology tools such as mobile phones. Short Message Service (SMS) text message has been used for malaria surveillance and control (Githinji et al. 2014; Kamanga et al. 2010; Zurovac et al. 2012). SMS text messaging has been explored in different settings for stock monitoring of ACT and RDT (Asiimwe et al. 2011; Hamainza et al. 2014; Kabanywanyi et al. 2010; Kamanga et al. 2010). In rural Uganda, an SMS-based reporting system supported by RapidSMS improved data reporting and return of key indicators, thereby supporting the management of RDT deployment by the National Malaria control programme. Frontline health workers sent an SMS form filled with malaria surveillance data on a weekly basis (e.g. number of malaria cases diagnosed, tested, and treated) to the peripheral health centers. Data reported via SMS were aggregated and analysed and the reports were shared with the health team involved in the study. The potential use of an SMS-based reporting system has been recognized for its immediate communication, ease of use and reduction in data transmission delays. Asiimwe et al. (2011) demonstrated the feasibility of reporting real-time epidemiological key data that are useful for malaria control managers.

A study conducted in five districts in rural Kenya showed the feasability of using an “SMS for life”, which is web-based and composed of an SMS management tool and a web-based reporting application. The system showed the feasability of using SMS text mesages in reporting the counts of four basic malaria surveilance indicators: number of outpatients recorded, number of outpatients tested for malaria, number of outpatients positive for malaria and number of outpatients treated with any anti-malarial treatment from peripheral health facilities. The website also provided current and historical data and summaries of testing rates, test positivity rates and appropiate treatment rates at each health facility and aggregated at the district level (Githinji et al. 2014). The same SMS text message has been used for disease surveillance in Madagascar, Zambia, and Ethiopia and for anti-malaria drug stock out monitoring in Tanzania. All SMS text messages were sent from health workers’ personal mobile phones in remote areas without additional software applications (Barrington et al. 2010; Davis et al. 2011; Randrianasolo et al. 2010; Yukich et al. 2014).

Apart from being used in reporting malaria surveillance data, text messaging has also been used for household mobilization in malaria prevention and control. Mangam et al. (2016) demonstrated that mobile Health (mhealth) was effective to mobilize households for executing an IRS campaign in Mali. Similarly, the South-African mSpray (a mobile phone project for IRS) provided timely and accurate data for monitoring of spray activities and locations for more targeted mosquito control (Eskenazi et al. 2014).

In Rwanda, several studies reported from health programmes pinpointed the opportunities that mobile phone technologies can present in Rwanda. Nsanzimana et al. (2012) demonstrated that mobile phone and internet-based reporting of key HIV Care and Treatment (HCT) indicators facilitated the rapid reporting of national Anti-Retroviral Treatment (ART) scale-up in the country. Another study by Ngabo et al. (2012) revealed that RapidSMS has contributed to the improvement of child and maternal death rates during pregnancy, thereby achieving one of the Millennium Development Goals. RapidSMS was used to develop an SMS-based system for improving Maternal and Child Health (MCH), which is a free and open-source software development framework. The RapidSMS-MCH system was customized to permit interactive communication among the Community Health Worker (CHW), who follows mother-child pairs in his/her community, a national centralized database, the health facility and in case of an emergency alert, the ambulance driver. The RapidSMS-MCH system was piloted in the Northern Province of Rwanda in Musanze district for a period of 12 months. RapidSMS highlights the opportunity that mobile phones can be used as a data reporting tool in rural areas and shows that a community participation approach can raise participants' awareness and empower the communities or citizens (Bucagu 2016; Munyaneza et al. 2014; Ngabo et al. 2012). However, no studies have reported the use of mobile phones as a collection and reporting tool for data on mosquito bionomics. We aim to fill this void by proposing an approach that will use an open-source toolkit that will be downloadable as an application. In order to realize this, minimal or no new infrastructure should be required, it should be locally maintained, and it should fit into the existing workflow of the health network system. Furthermore, it should be able to run in areas with limited connection and electricity.

Opportunities and challenges for citizen science: a framework for malaria mosquito monitoring in Rwanda

The approach is to implement a citizen science platform for malaria mosquito monitoring in Ruhuha by starting with using a paper-based form used by the community members. This will initially report entomological data, and other malaria related information, which later will be digitalized to feed a web-based platform. With these data, we will thus specifically collect information that can address the question related to malaria vectors. Below we also explain how this approach can address the concern on non-existence of mosquito monitoring in remote regions.

Before the conception and the design of the proposed platform, inclusion of key stakeholders involved in malaria prevention and control is an essential step for this study. The technical design and the implementation of such approach will be an iterative development process (Larman 2004) that relies on feedback from the different stakeholders engaged in malaria vector monitoring surveillance and control with the community as the end users (Asiimwe et al. 2011). As this will involve different actors involved in malaria surveillance and control, the citizen science approach will provide opportunities to strengthen the collaboration with the communities.

The importance of stakeholder involvement in malaria control in Rwanda was demonstrated by Ingabire et al. (2016), who used an intervention mapping approach including three main categories of stakeholders including a primary category (lay community), a secondary category (administrative and health institutions), and a tertiary category that are key stakeholders (policy makers and funders). The approach improved the adoption of the vector measures used in malaria control in the area (Ingabire et al. 2016). Such approach will enable participation of each stakeholder in problem definition, the design process of the platform, and the content of the platform proposed.

The approach is that, primarily, the open-based website platform will be fed with data filled in a paper form. The data will be reported to the CHWs who are also members of the earlier established Community Malaria Action Teams (CMATs) (Ingabire et al. 2014). Community members or citizens will collect information on mosquito nuisance that they experience indoor or in their immediate home environment. Data other than mosquito nuisance (e.g. on mosquito habitats, mosquito specimens collected and their bionomics etc.) will be reported and afterwards digitalized to feed the web-based platform. Malaria vector bionomics (nuisance and specimen collected) can be linked with available climate data from nearby weather stations, in order to understand the role of environmental factors on malaria mosquito population dynamics and biting nuisance experienced by the community.

After this initial stage, a mobile application (SMS or smartphone app) that also collects global positioning system (GPS) data, will be designed and used to capture the entomological data that were initially reported via the paper-based form. Linking the citizen science data with malaria incidence data collected at the health center level will provide knowledge on the malaria transmission patterns and the ecology of malaria vectors. This will complement the malaria vector control interventions that are implemented by the NMCP.

Rolling out a citizen science approach will require political commitment for the overall success of the project. This will also rely on the communication and information flow already in place. Therefore, CHWs will be important as they are members of the current workforce of the national health system in Rwanda and link up individuals, families and communities countrywide (Bucagu 2016; Condo et al. 2014).

Besides the need of stakeholder involvement, the implementation of citizen science requires expertise in programming, mobile telephone services for specific applications, considerable investment cost, mobile phone ownership and time associated with the setting up (design and implementation) of the system. Asiimwe et al. (2011) reported that the initial investment costed more than $50,000. The period from conception of the approach to the implementation of the system took three months and involved 147 health facilities. Although cost for the implementation of the RapidSMS system was not described in detail, Ngabo et al. (2012) reported that considerable investment was used for the implementation of the alert system piloted over a period of 12 months in Musanze District involving 432 CHWs, 13 health centers as well as one district hospital. In addition, the Government of Rwanda through the Ministry of Health covered the cost of SMS messaging although the SMS text messaging was free of charge for CHWs (Ngabo et al. 2012).

Poorly designed technology, lack of interest among decision makers, especially when it comes to the sustainability of the project, and lack of resources for implementation and running the project are factors that can impede the implementation of the citizen science project. Lack of time for participation, technological related problems, and local infrastructural barriers are the key factors to take into account when mobilizing communities (Rotman et al. 2014). Mobilizing, attracting and retaining participants in citizen science projects is a complex process because it relies on numerous cognitive, behavioural and social characteristics (Crain et al. 2014). However, if set-up properly, we argue that citizen science provides unique opportunities for involving malaria-affected communities in understanding and solving their health issues.

Finally, although internet and mobile phone ownership are increasing on the African continent, lack of steady electrical supply, poor network coverage and internet access as well as telephone maintenance remain challenges in some remote areas where CHWs live far from the health centers (Asiimwe et al. 2011; Ngabo et al. 2012). Guidelines and policies for health care data reporting were required for the RapidSMS based reporting tools adopted by the NMCP (Asiimwe et al. 2011; Ngabo et al. 2012). In addition, mobile phones used by the CHWs are not replaced in case of damage. Instead, CHWs are encouraged to purchase another mobile through a cooperative (Condo et al. 2014).

Conclusions

Malaria incidence is on the rise in many African countries, including Rwanda. Although malaria vector control is in place in Rwanda, current surveillance is costly and there is inadequate capacity to scale up malaria vector surveillance. CHWs are involved in many community-based interventions in Rwanda as “linkers” and “delivery channels” between communities and the highest ranked stakeholders such as the Ministry of Health. Much of the success of the health interventions has required community participation. However, limited infrastructure and the lack of comprehensive information systems for malaria vector distribution have made it difficult to monitor the indicators of malaria pre-elimination. We conclude that a citizen science approach for malaria mosquito monitoring can support malaria vector surveillance and control by providing insight in vector species distribution and malaria transmission patterns. We expect that the data generated via citizen science are relevant for the planning, implementation and evaluation of vector control activities by NMCP. Thus, the involvement of the local communities in the science of mosquito ecology will lead to more sustainable solutions for malaria control. Moreover, the citizen science approach presents opportunities to bring down institutional barriers, such as low level of community involvement in vector control, limited financial resources for mosquito surveillance and the current exclusion of more remote areas in mosquito monitoring.

Acknowledgements

This publication is part of the project funded by Interdisciplinary Research and Education Fund (INREF, Wageningen University, The Netherlands).

Chapter 3

Monitoring mosquito nuisance for the development of a citizen science approach for malaria vector surveillance in Rwanda

Marilyn Milumbu Murindahabi, Willem Takken, Xavier Misago, Elias Niyitima, Jackie Umupfasoni, Emmanuel Hakizimana, Arnold J.H. van Vliet, P. Marijn Poortvliet, Leon Mutesa, Nathalie Kayiramirwa Murindahabi and Constantianus J.M. Koenraadt

Submitted

Abstract

Many countries, including Rwanda, have mosquito monitoring programmes in place to support decision making in the fight against malaria. However, these programmes can be costly, and require technical (entomological) expertise. Involving citizens in data collection can greatly support such activities, but this has not yet been thoroughly investigated in a rural African context. Prior to the implementation of such a citizen science approach, we conducted a household entomological survey in October-November 2017 and repeated this one year later. The survey was conducted in Busoro and Ruhuha sectors, in southern and eastern province of Rwanda, respectively. The goal was to evaluate the perception of mosquito nuisance reported by citizens as a potential indicator for malaria vector hotspots. Firstly, we determined mosquito abundance and species composition using Centers for Disease Control and Prevention (CDC) light traps inside the houses. Secondly, we interviewed household members about malaria risk factors and their perceived level of mosquito nuisance. Tiled roofs, walls made of mud and wood, as well as the number of occupants in the house were predictors for the number of mosquitoes (Culicidae) in the houses, while the presence of eaves plus walls made of mud and wood were predictors for malaria vector abundance. Perception of mosquito nuisance reported indoors tended to be significantly correlated with the number of *Anopheles gambiae* s.l. and Culicidae collected indoors, but this varied across years and sectors. At the village level, nuisance also significantly correlated with *An. gambiae* s.l. and total mosquito density, but only in 2018 and not in 2017. We conclude that the perception of mosquito nuisance denoted in the questionnaire survey could be used as a global indicator of malaria vector hotspots. Hence, involving citizens in such activities can complement malaria mosquito surveillance and control.

Introduction

Malaria remains a public health concern in Rwanda despite the gains made in malaria reduction in the past decades (Karema et al. 2012). Since 2012, malaria has increased every year, thereby impeding the progress made up to 2011 (MOH 2017b). From 2012 to 2016, the country reported an eight-fold increase in malaria cases. Additionally, malaria–related deaths increased from 325 in 2012 to 663 in 2016. The eastern and southern parts of the country have been the most afflicted regions. The increase in malaria cases has been observed in all districts, including districts that were previously defined as being at the pre-elimination phase. This increase was observed across all age groups, suggesting the entire population is at risk of acquiring a malaria infection (MOH 2017b).

In Rwanda, malaria vector surveillance is carried out monthly in twelve sentinel sites across the country. It aims to monitor the key parameters of malaria vectors, and provides entomological data, such as the entomological inoculation rate (EIR), to guide the planning of vector control interventions (Hakizimana et al. 2018; Tusting et al. 2014). At present, the monitoring of malaria mosquito density is combined with malaria incidence and helps to determine the spatio-temporal spread of infections. However, active mosquito surveillance is conducted in only 3% (12/416) of the sectors of the Rwandan territory, while in the remaining 97%, mosquito monitoring is not implemented (MOH 2013). It is challenging to implement the current mosquito surveillance in all areas of the country due to the inadequate local capacity in entomology and the high costs related to extending vector surveillance countrywide, hence alternative approaches are desired (Hakizimana et al. 2018).

Passive surveillance through citizen science is a tool to track mosquito presence and spread, such as of *Aedes albopictus,* a potential vector of dengue, Zika and chikungunya viruses (Heym et al. 2017; Palmer et al. 2017). Studies carried out in the Netherlands and Spain showed that citizens provided scientifically valuable information through questionnaires and sending mosquito samples to the laboratories in charge of mosquito identification. This can ease cost constraints for mosquito surveillance (Palmer et al. 2017; Vogels et al. 2015). Mosquito nuisance reported by citizens via a questionnaire in combination with actual mosquito samples collected by the citizens revealed the presence or absence of the two biotypes of *Culex pipiens* and their hybrids, which can be important vectors of West Nile virus (Vogels et al. 2015).

In the current study, prior to the implementation of a wider citizen science programme for malaria mosquito surveillance in Ruhuha, Rwanda (Chapters 4 and 6), we aimed to determine mosquito species composition using a conventional mosquito trapping method in two rural sectors of Rwanda by means of two cross-sectional surveys performed in 2017 and 2018. In addition, we aimed to analyze the factors that could explain the observed spatial distribution of mosquito species collected. Lastly, we investigated whether perceived mosquito nuisance reported by the participants could provide an indication of potential (malaria) mosquito hotspots, especially in areas where malaria mosquito surveillance is not implemented.

Materials and methods

Study site

Household and entomological surveys were carried out in Ruhuha and Busoro sectors, located respectively in Bugesera district (eastern province), and in Nyanza district (southern province) in Rwanda (Figure 1). The choice of the study sites was based on the large number of malaria cases reported in Ruhuha since 2012, and the long-term working relationship with the local health center. Ruhuha sector covers 54 km^2 and is sub-divided into 35 villages (Ingabire et al. 2014). An estimated 24,000 people are living in more than 5,100 households (HHs) (Kateera et al. 2015). The area is a higher malaria endemic zone (Rulisa et al. 2013). Busoro is a sector covering 74 km^2 and is subdivided into 41 villages. It has a total population of approximately 34,000 people living in 8,000 households (HHs) (Asingizwe et al. 2019). Irrigated rice fields are the main type of land use in both sectors. Both sectors differ from each other by the area of irrigated rice fields which is relatively 40% larger in Busoro (178 hectares or 2.4% of the land surface) in comparison with Ruhuha (93 hectares or 1.7% of the land surface) (Hakizimana 2019; Musabe 2012). Additionally, Ruhuha is located near the shores of Lake Cyohoha South (Rwanyiziri & Rugema 2013). The wetlands potentially serve as favourable places for mosquito breeding.

Study design

Household and entomological surveys were conducted in Ruhuha and Busoro for three weeks in October-November 2017 and were repeated in a modified form in the same period in 2018. Data from the household survey were coupled with entomological data collected for both sectors and years. In addition to the demographic characteristics, we defined factors that could explain the variation in mosquito abundance (Figure 2). Additionally, we assessed the relationship between perceived mosquito nuisance experienced by the citizens in their house and mosquitoes collected using CDC light traps in Busoro and Ruhuha.

Household selection

The studied households were selected among those that were part of a larger household survey (Asingizwe et al. 2019). Six villages per sector were selected by simple random sampling. In Busoro sector, the villages of Kireranyana, Gikombe, Karambi, Muhindo, Rucyamo, and Runazi were selected while in Ruhuha sector, Kagasera, Kamweru, Kibaza, Kiyovu, Mubano, and Rusenyi villages were selected for the study (Figure 1). At the village level, lists of households were provided by the village leaders, and a systematic random sampling was used to draw a sample of households to be visited. As a result, 30 to 31 households were selected for each village for the large household survey. From these households 6-8 households were selected for the household survey. In 2017, 82 households were thus selected randomly (42 from Busoro and 40 from Ruhuha) and considered for our study.

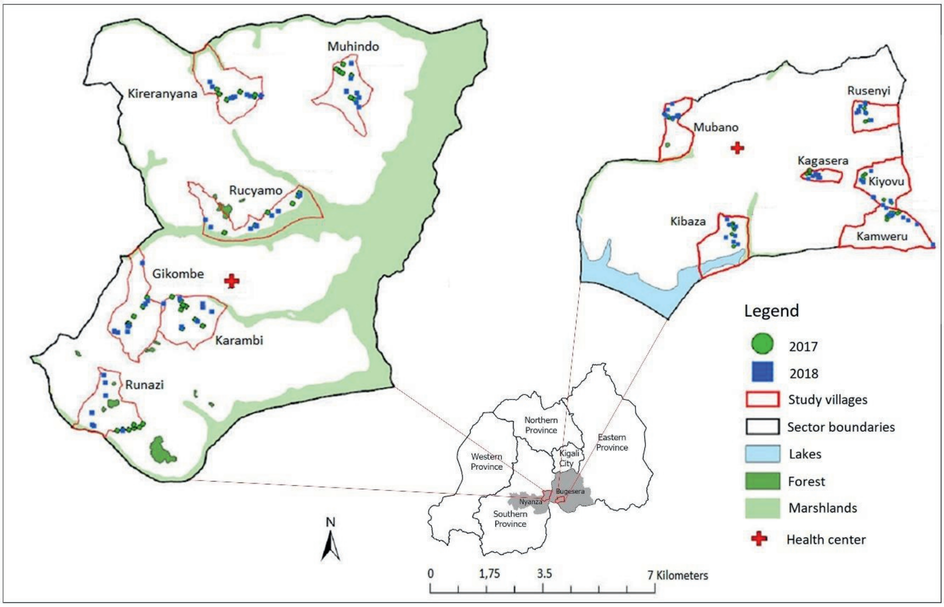


Figure 1. Maps of Busoro and Ruhuha sectors, located in Bugesera and Nyanza districts (in grey) showing houses randomly selected (blue and green dots for the different years of study) from six villages that were selected for the household survey and mosquito survey using Centers for Disease Control and Prevention miniature light traps (CDC-LT).

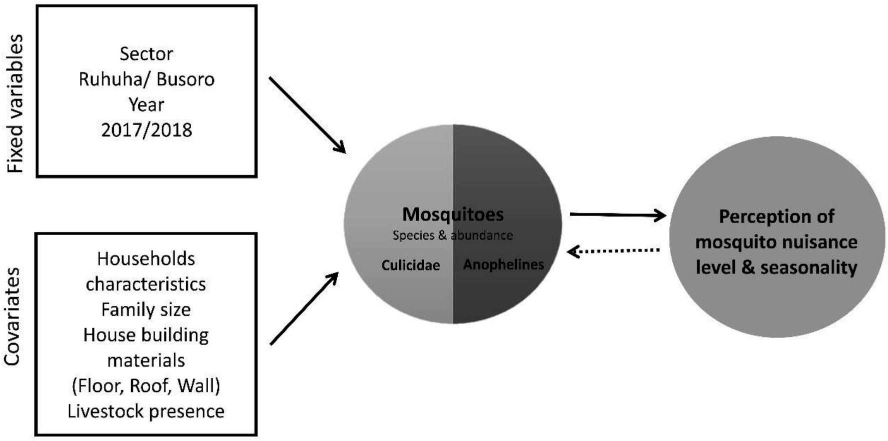


Figure 2. Overview of different variables analysed in the present study.

In 2018, the same survey was repeated by selecting randomly and directly from the list, excluding households sampled during the previous survey in 2017. In other words, households interviewed in 2017 were different from those from 2018. In 2018, 84 households were selected, 42 from Busoro and 42 from Ruhuha. If household members were absent at the time of the interview, the interviewers progressed to the next selected house. Only residents above 18 years of age and who consented to participate in the study were interviewed. The house occupied by the interviewed resident was selected for the mosquito collection after obtaining informed consent.

Data collection

Household survey

For the household survey conducted in 2017 and 2018, we assessed perception of mosquito nuisance experienced indoor and per season by the citizens in Busoro and Ruhuha. Six to eight household surveys were carried out per day. The average interview time was 45 minutes up to one hour per household. The questions were originally written in English and translated into the local language (Kinyarwanda), and prior to the survey back translated into English. The questionnaire was pre-tested in a pilot study of 10 households selected randomly in the neighbouring Mareba sector, Eastern province, for its consistency, and then revised accordingly. Field data collectors were trained in addressing the questionnaire before conducting the survey. Only participants who consented were interviewed in the local language (Kinyarwanda). All translations were made by professional translators including members of the project team and cross-checked by native speakers. The translators were asked to review and cross check the items and identify any problems in wording, terminology, ability to understand and relevance.

Questionnaire data were collected in electronic forms using Open Data Kit (ODK) Collect setup (Asingizwe et al. 2019; Raja et al. 2014). The questionnaire encompassed questions with closed- and open-ended questions. The questionnaire contained different sections with questions related to demographic data of the participants, perception of mosquito nuisance and its seasonality, knowledge on malaria, and to vector and malaria control practices. For statistical analysis, we used the data on demographic characteristics, household characteristics and mosquito nuisance perceived indoor by the respondents in this study (Figure 2).

Demographic characteristics

Demographic data included gender, age, marital status, education level, occupation, Ubudehe category (a community-based social categorisation of the household and its dependents into different groups based on their incomes) (Fenny et al. 2018), size of the household, bed net ownership, and mobile phone ownership of the participants. Other characteristics such as the house features (type of wall (mud/clay or wood), type of floor (cement or mud), type of roof (iron or tile sheets), presence of eaves, and livestock ownership (species owned and location where they were kept)) were also included in the questionnaire.

Perception of mosquito nuisance

Respondents were asked to answer the question whether they experienced mosquito nuisance in their environment. Participants who experienced mosquito nuisance were requested to scale the level of nuisance experienced in the house on a 5-point Likert scale (1 = very little; 5 = very much). They were also requested to indicate when (rainy or dry seasons) they experienced mosquito nuisance.

Mosquito collection by CDC light traps

Mosquitoes were collected in 2017 and 2018 in 166 selected houses in Ruhuha (82) and Busoro (84) among the interviewed participants using a miniature CDC light trap. The CDC light traps were set up in the bedroom and hung at the foot end of the bed, with the shield of the trap at 1.5 meters from the floor (Mboera et al. 1998). The traps were set up at 6:00 pm, and the owner of the room was instructed to put off the light of the trap and tie the bag connected to the collecting cup at 6:00 am the next morning to avoid mosquitoes escaping from the traps. After their collection from the traps, mosquitoes were stored in labelled petri dishes before morphological identification in the laboratory.

Mosquito identification

Mosquitoes were identified using standard morphological identification keys for anophelines and culicines (Gillies & Coetzee 1987; Service 1993). Mosquitoes were scored as unfed or blood fed. The mosquitoes were then stored under silica gel in labelled Eppendorf tubes with the codes of the respondent interviewed and kept in an envelope with the name of the village under cool conditions at the central Laboratory of Entomology for identification of *Plasmodium falciparum* infection and further molecular species identification.

Laboratory processing

Blood meal identification. For samples collected in 2017, each mosquito abdomen was ground in 100 microliter (µl) of phosphate-buffered saline (PBS), and then filled up to 1 ml PBS. Blood meals were identified by direct ELISA using antihost (IgG) conjugate against human and cow proteins in a single-step assay (Beier et al. 1988). The non-reacting samples were then tested subsequently using goat IgG. ELISA results were read visually (Beier & Koros 1991). The anthropophilic rate was determined as the proportion of mosquitoes that fed exclusively on human blood among all fed mosquitoes.

Sporozoite rates. The head and thorax of all female *An. gambiae* s.l. mosquitoes collected in 2017 using CDC light traps were used to test for the presence of circumsporozoite protein (CSP) of *P. falciparum* using Enzyme-Linked Immunosorbent Assay (ELISA). A sample with an

Optical Density (OD) value above the cut-off (cut-off = 2 x mean OD of 7 negative samples) was considered positive (Dotson et al. 2011). The sporozoite rate was calculated as the number of mosquitoes infected with *P. falciparum* sporozoites divided by the total number of mosquitoes processed.

Molecular species identification. For molecular identification of the sibling species of *An. gambiae* s.l., a random sample of 9% (n=233/2,514) of the total number of *An. gambiae* s.l. collected during the two years and from both sectors was identified using the rDNA-Polymerase Chain Reaction (PCR) assay (Scott et al. 1993). If the initial PCR testing failed to amplify a sample, then the PCR analysis was repeated once or twice until successful amplification was achieved. If a sample could not be identified after three rounds of PCR, it was scored as unknown (Minakawa et al. 1999).

Data analysis

Household survey data were imported from ODK software into Microsoft Excel (2016) and checked for consistency in the values and answers. They were electronically loaded onto a central server for backup, translated, and coupled with data from the entomological survey. Statistical analysis was undertaken in SPSS 23.0 (SPSS Inc, Chicago, IL, USA), and included the calculation of frequencies and Chi-squares statistics. Bivariate analysis of correlation between the dependent variable (number of *An. gambiae* s.l. or Culicidae) and the independent variables (number of members of the household, house structural features (floor, wall and roof), species of animal kept in the house and presence of eaves) was performed to determine predictors that could explain the mosquito abundance indoor. Only predictors that had a screening significance lower than 0.10 were then considered for our final models. For this purpose, Generalized Linear Models (GLM, negative binomial with log link) were used. Besides house structural features, household size and livestock ownership, other factors included in the GLM were sector (Ruhuha/Busoro) and the year of study (2017/2018) (see Figure 2). All data on mosquito collections were entered into Excel to calculate the sporozoite rate and human blood index and the various mosquito species identified were summarised as proportions. Furthermore, Spearman correlation analysis was used to analyse the relationship between mosquito nuisance reports by the respondents and number of mosquitoes and species collected in the respondents' houses (Figure 2).

Ethics statement

Permission from the Rwanda Institutional Review Board of the Rwandan Biomedical Center, and Bugesera District office were obtained prior to the conduct of the study. A Memorandum of Understanding was signed between University of Rwanda and CARITAS – a religion-based organization that provides supervisory leadership to health centers – which allow working with the health center. Informed consent was obtained from adult respondents before the questionnaire was addressed and the entomological survey carried out. All information was evaluated anonymously.

Results

Demographic characteristics

One hundred and sixty-six respondents were enrolled both in the household survey and mosquito collection, out of 167 (n = 166/167, 99%) requested respondents to participate. Table 1 provides an overview of the demographic characteristics of the participants. Overall, both sectors were similar in their demographics. In both sectors more than half of the respondents were female (64%). The average age of the respondents in Busoro was 44 years (n = 84), and 41 years (n = 82) in Ruhuha. Almost 76% of the respondents were schooled (no category) while the remainder (24%) was unschooled. Respondents from Busoro were more highly educated (81%) than those from Ruhuha (71%). Most of the respondents were farmers (93%), followed by self-employed (1%), private officer (1%), student (1%), and unemployed (4%). However, more respondents in Busoro than in Ruhuha were farmers (98% versus 89%). In latter sector, more individuals were unemployed. There was no significant difference when comparing Ubudehe categories between the two sectors. Most of the participants owned a bed net (74%), but there was a significant difference in bed net ownership between Busoro and Ruhuha (81% versus 66%). Almost half of the participants owned a mobile phone (46%). There was no significant difference in mobile phone ownership when comparing both sectors.

Table 1. Demographic and household characteristics of 166 respondents in Busoro and Ruhuha sector, Rwanda. *P*-values are based on chi-square analysis of the proportions between the two sectors.

Variables		**Busoro n (%)**	**Ruhuha n (%)**	**Total**	***P***
Gender					
	Male	38 (45)	22 (26)	60 (36)	0.014
	Female	46 (55)	60 (73)	106 (64)	
Age					
	19-24	7 (8)	8 (10)	15 (9)	
	25-44	31 (37)	42 (51)	73 (44)	
	45-59	35 (42)	23 (28)	58 (35)	0.222
	> 60	11 (13)	9 (10)	12 (12)	
Marital Status					
	Never married	4 (5)	7 (9)	11 (7)	
	Married	40 (48)	36 (44)	76 (46)	
	Living together	16 (19)	19 (23)	35 (21)	0.444
	Separated (Divorce)	11 (13)	5 (6)	16 (10)	
	Widow	13 (16)	15 (18)	28 (17)	
Education					
	None	16 (19)	24 (29)	40 (24)	
	Incomplete Primary	43 (51)	31 (38)	74 (45)	
	Primary	15 (18)	19 (23)	34 (21)	0.342
	I complete secondary	5 (6)	3 (3)	8 (5)	
	Secondary	5 (6)	5 (5)	10 (5)	
Occupation					
	Farmer	82 (98)	73 (89)	155 (93)	
	Self-employed	0 (0)	2 (2)	2 (1)	
	Private officer	1 (1)	0 (0)	1 (1)	0.088
	Student	0 (0)	1 (1)	1 (1)	
	Unemployed	1 (1)	6 (4)	7 (4)	
Ubudehe category					
	Category 1	12 (14)	16 (20)	28 (17)	
	Category 2	37 (44)	34 (42)	71 (43)	0.666
	Category 3	35 (42)	32 (39)	67 (36)	
Size of the household					
	1 to 2	9 (11)	9 (11)	18 (11)	
	3 to 5	47 (56)	48 (58)	95 (57)	0.925
	>= 6	28 (33)	25 (31)	53 (32)	
Bed net ownership					
	No	16 (19)	28 (34)	44 (27)	0.028
	Yes	68 (81)	54 (66)	122 (74)	
Mobile phone ownership					
	No	45 (54)	45 (55)	90 (54)	0.866
	Yes	39 (46)	37 (45)	76 (46)	

House features

Most of the participants had mud floor houses (83%) with mud walls (59%), closed eaves (65%) and iron sheet roofing (69%) (Table 2). There were no significant differences between both sectors for house features (eaves presence, floor, wall features), except for iron sheeting (100% in Ruhuha versus 38% in Busoro) (Table 2).

Table 2. Houses features of the 166 respondents in Busoro and Ruhuha sector, Rwanda. *P*-values are based on Chi-square statistical comparisons.

Variables		**Busoro n (%)**	**Ruhuha n (%)**	**Total**	***P***
House features					
Eaves					
	No	55 (65)	53 (64)	108 (65)	0.909
	Yes	29 (35)	29 (36)	58 (35)	
Floor					
	Cement	13 (15)	16 (19)	29 (18)	0.494
	Mud/Clay	71 (85)	66 (81)	137 (83)	
Roof					
	Iron sheets	32 (38)	82 (100)	114 (69)	
	Tile sheets	52 (62)	0 (0)	52 (31)	< 0.0001
Wall					
	Mud/clay	47 (56)	51 (62)	98 (59)	
	Wood & Mud	37 (44)	31 (38)	68 (41)	0.414

Livestock ownership

Overall, there was a significant difference in livestock ownership when comparing both sectors (*P* = 0.029). Of the respondents, 72% owned at least one species of livestock (cows, pigs, poultry, rabbits, goats or sheep; Table 3). However, participants in Busoro owned more livestock than participants in Ruhuha (80% versus 65%). Goats were the most frequently owned livestock. Poultry ownership differed significantly between Busoro and Ruhuha (*P* = 0.003): respondents in Busoro owned more poultry indoor than Ruhuha.

Mosquito species composition by CDC light traps

From the mosquitoes collected in 2017 and 2018, 74% (n = 7,370) were collected in 2017 (Table 4) and 26% (n = 2,595) in 2018 (Table 5). Of all mosquitoes, 74.2% were morphologically identified as culicines and 25.2% as anophelines. Among female mosquitoes collected, 25.6% (n = 2,210) were fed and 77.8% (n = 7,755) were unfed. Of the total anophelines collected (n = 2,514), 94.2% was *An. gambiae* s.l. Giles. Other *Anopheles* species collected included *An. brohieri* (0.2%), *An. funestus* Giles (1.2%), *An. maculipalpis* Giles (1.1%), *An. pharoensis* Theobald (0.6%), *An. rufipes* Gough (0.5%), and *An. ziemanni* Grunberg (2.2%). Busoro recorded 72.8% of the total *An. gambiae* s.l. collected, the remaining 27.2% was collected in Ruhuha. *An. funestus* (n = 31), *An. pharoensis* Theobald (n = 14), and *An. ziemanni* Grunberg (n=56) were the most frequently encountered other human-biting *Anophele*s species identified in Busoro, while *An. brohieri* (n = 2),) *An. rufipes* (n = 13), and *An. maculipalpis* Giles (n = 5) were *Anopheles* species known as non-human biting. In Ruhuha, *An. pharoensis* Theobald (n=1) was another human biting malaria vector, and *An. brohieri* (n = 2), *An. maculipalpis* Giles (n = 22) were the non-human biting anophelines. The malaria mosquito *An. funestus* was not collected in Ruhuha.

From 7,451 culicines, 99.7% (n = 7,425) consisted of *Culex (Cx)* spp. and 0.3% (n = 26) of *Mansonia* spp. The highest proportion of culicines identified was *Cx. quinquefasciatus* (99.6%), with 89.2% (n = 6,647) from Busoro and 10.4% (n = 778) from Ruhuha, respectively.

Table 3. Proportion of households keeping each species of livestock indoor and/or outdoor in Busoro and Ruhuha sector, Rwanda. *P*-values are based on Chi-square statistical comparisons.

Species of livestock	**Busoro**			**Ruhuha**			
	Indoor n (%)	**Outdoor n (%)**	**Household without livestock**	**Indoor n (%)**	**Outdoor n (%)**	**Household without livestock**	***P***
Cow	0 (0)	33 (49)	34 (51)	0 (0)	28 (53)	25 (47)	0.697
Pig	0 (0)	6 (9)	61 (91)	0 (0)	5 (9)	48 (91)	0.928
Poultry	25 (37)	5 (8)	37 (55)	9 (17)	0 (0)	44 (83)	0.003
Rabbit	2 (3)	1 (2)	64 (96)	2 (4)	0 (0)	51 (96)	0.654
Goat	44 (66)	10 (15)	13 (19)	30 (57)	11(21)	12 (23)	0.572
Sheep	0 (0)	0 (0)	67 (100)	1(2)	0 (0)	52 (99)	0.259

Blood-feeding behaviour

Of 1,046 *Anopheles* spp. collected, 100 were selected randomly. Ninety-eight specimens were *An. gambiae* s.l., while the other two were *An. maculipalpis* and *An. rufipes*. Of all 100 blood fed specimens, 82 (82%) had fed on a single host (human, goat or bovine), while 5 (5%) had fed on mixed hosts and 13 were unspecified for the antigens assayed suggesting that these *An. gambiae* s.l. had fed on other hosts than humans, goats, or cattle (Table 6). For the remaining 87 *Anopheles*, 58% (n = 58) of the *An. gambiae* s.l. were engorged with human blood and 1% (n=1) *An. gambiae* s.l. was engorged with blood of both human and goat origin.

Plasmodium falciparum infection rates

Of the 1,046 mosquitoes tested by ELISA, *P. falciparum* CSP antigen was detected in 14 out of 1,013 mosquitoes, 11 out of 971 tested (1.1%) *An. gambiae* s.l. and 3 out of 42 tested *An.*

ziemanni (7.1%). The overall sporozoite rate of anopheline species was 1.3%. The infection rate was 0.5% (3/573) in Busoro and 2.3% (11/473) in Ruhuha.

Table 4. Mosquito species collected using CDC light traps in selected villages in Busoro and Ruhuha sector, Rwanda (2017).

Mosquito species collected	**Busoro**						**Ruhuha**						**TOTAL**	**Species composition %**
	Gikombe	Karambi	Kireranyana	Muhindo	Rucyamo	Runazi	Kagasera	Kamweru	Kibaza	Kiyovu	Mubano	Rusenyi		
An. gambiae s.l.	45	11	84	47	34	290	3	49	261	17	35	95	971	13.2
An. maculipalpis	0	0	0	0	1	0	0	3	4	0	0	5	13	0.2
An. pharoensis	0	0	0	0	6	0	0	0	1	0	0	0	7	0.1
An. rufipes	0	11	0	0	1	1	0	0	0	0	0	0	13	0.2
An. ziemanni	0	0	0	0	42	0	0	0	0	0	0	0	42	0.6
Total *Anopheles* spp	45	22	84	47	84	291	3	52	266	17	35	100	1046	14.2
Culex spp	68	213	469	149	4678	129	9	40	410	11	110	28	6314	85.7
Mansonia spp	0	1	0	0	1	1	0	7	0	0	0	0	10	0.1
Total Culicinae spp	68	214	469	149	4679	130	9	47	410	11	110	28	6324	85.8
Total Culicidae spp	113	236	553	196	4763	421	12	99	676	28	145	128	7370	100.0
% *Anopheles* spp	39.8	9.3	15.2	24.0	1.8	69.1	25.0	52.5	39.3	60.7	24.1	78.1	14.2	

Table 5. Mosquito species collected using CDC light traps in selected villages in Busoro and Ruhuha sector, Rwanda (2018).

Mosquito species collected	**Busoro**						**Ruhuha**						**TOTAL**	**Species composition %**
	Gikombe	Karambi	Kireranyana	Muhindo	Rucyamo	Runazi	Kagasera	Kamweru	Kibaza	Kiyovu	Mubano	Rusenyi		
An. brohieri	0	0	0	0	1	1	0	0	0	1	0	1	4	0.2
An. funestus	0	0	0	0	31	0	0	0	0	0	0	0	31	1.2
An. gambiae s.l.	72	93	105	78	250	600	0	27	94	7	14	57	1397	53.8
An. maculipalpis	0	0	0	1	2	1	0	0	5	0	1	4	14	0.5
An. pharoensis	0	0	0	1	7	0	0	0	0	0	0	0	8	0.3
An. ziemanni	0	0	0	0	14	0	0	0	0	0	0	0	14	0.5
Total *Anopheles* spp	72	93	105	80	305	602	0	27	99	8	15	62	1468	56.6
Culex spp	24	127	141	165	449	35	13	20	70	7	14	46	1111	42.8
Mansonia spp	0	3	2	0	11	0	0	0	0	0	0	0	16	0.6
Total Culicinae spp	24	130	143	165	460	35	13	20	70	7	14	46	1127	43.4
Total Culicidae	96	223	248	245	765	637	13	47	169	15	29	108	2595	100.0
% *Anopheles* spp	75.0	41.7	42.3	32.7	39.9	94.5	0.0	57.4	58.6	53.3	51.7	57.4	56.6	

Table 6. Host blood antigen detected in three mosquito species collected from Busoro and Ruhuha sector, Rwanda (October–November 2017)

Host blood	***An. gambiae* s.l.**	***An. maculipalpis***	***An. rufipes***	***Total***
	n (%)	n (%)	n (%)	
Human	58 (58)	0 (0)	0 (0)	58
Goat	2 (2)	1 (1)	0 (0)	3
Bovine	20 (20)	0 (0)	1 (1)	21
Goat and Bovine	4 (4)	0 (0)	0 (0)	4
Human and goat	1 (1)	0 (0)	0 (0)	1
Unspecified	13 (13)	0 (0)	0 (0)	13
Total	98 (98)	1 (1)	1 (1)	100

Molecular identification of members of the An. gambiae complex

Of the 9.4% (236/2,514) of the *An. gambiae* s.l. selected for sibling species identification from 2017 and 2018, 145 (61.4%) were identified as *An. gambiae* s.s. Giles and 74 (31.4%) as *An. arabiensis* Patton (Table 7). Sixteen (6.8%) were not amplified and one sample was contaminated (0.4%).

Table 7. Members of the *An. gambiae* complex found among samples of *An. gambiae* s.l. tested from Ruhuha and Busoro sector, Ruhuha

Sibling species	**2017**						**2018**						**TOTAL**	
	Busoro		Ruhuha		*S/total*		Busoro		Ruhuha		*S/total*			
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
An. arabiensis	31	36.0	3	3.5	34	39.5	25	16.7	15	10.0	40	26.7	74	31.4
An. gambiae s.s.	13	15.1	31	36.0	44	51.2	96	64.0	5	3.3	101	67.3	145	61.4
Not amplified	4	4.7	3	3.5	7	8.1	6	4.0	3	2.0	9	6.0	16	6.8
Contaminated	1	1.2	0	0.0	1	1.2	0	0.0	0	0.0	0	0.0	1	0.4
TOTAL	49	57.0	37	43.0	86	100.0	127	84.7	23	15.3	150	100.0	236	100.0

Factors explaining mosquito abundance

After bivariate analysis, different livestock species kept in the houses such as poultry, rabbit, goat and sheep (Table 3) did not show a statistically significant correlation ($P > 0.1$) with the dependent variables. Therefore, they were not selected for GLM analysis. The house features that included the materials used for the construction of the roof, and the wall, and the number of occupants in the house were predictors for the number of mosquitoes (Culicidae) in the houses. The predictors roof, wall, and size of the household had a statistically significant effect on the number of mosquitoes indoor, while the floor composition did not contribute statistically to a difference (Table 8).

There were significantly more Culicidae in Busoro than in Ruhuha, and the incidence rate ratio for 2017 was 2.5 times that of 2018 ($P < 0.001$; Table 8). Houses with tiled roofs were more exposed to mosquitoes than houses with an iron roof ($P = 0.002$; Table 8). Likewise houses with walls made of mud and wood had a larger number of mosquitoes (Culicidae) than houses with walls made with mud ($P < 0.001$; Table 8).

Table 8. Determinants of mosquito and malaria vector abundance.

			Culicidae		
Variables		β	Incidence rate ratio	95% CI	*P*
Sector	Busoro	1.079	2.941	1.807 -4.787	<0.001
	Ruhuha	*			
Year of the study	2017	0.928	2.528	1.742 - 3.668	<0.001
	2018	*			
Floor	Cemented	-0.309	0.734	0.421 - 1.280	0.276
	Earthed	*			
Roof	Iron sheets	-0.846	0.429	0.253 - 0.729	0.002
	Tile sheets	*			
Wall	Mud / clay	-0.913	0.401	0.273 - 0.591	<0.001
	Wood-Mud	0a			
Family size	Family size	0.153	1.166	1.058 -1.284	0.002
			An. gambiae **s.l.**		
Variables		β	Odd ratio	95% CI	*P*
Sector	Busoro	1.223	3.399	1.913 - 6.038	<0.001
	Ruhuha	*			
Year of the study	2017	0.110	1.117	0.706 - 1.766	0.637
	2018	*			
Eaves	No eaves	-0.560	0.571	0.361 - 0.904	0.017
	Eaves	*			
Floor	Cemented	-0.393	0.675	0.359 - 1.271	0.223
	Earthed	*			
Roof	Iron sheets	0.425	1.529	0.844 - 2.771	0.162
	Tiles sheets	*			
Wall	Mud / clay	-0.838	0.433	0.278 - 0.673	0.000
	Mud & wood	*			

*Reference category

Similarly, for the total number of only *An. gambiae* s.l., there were more females collected in Busoro than in Ruhuha. However, the year effect was not significant (Table 8). Houses with closed eaves and mud walls were more likely to not have malaria vectors resting inside the house than houses with open eaves or walls made with wood and mud.

Perceived mosquito nuisance per sector

In total, 96% (n = 159, both 2017 and 2018) of the respondents reported to have experienced at least some mosquito nuisance. There was a significant difference between both sectors in the proportion of respondents that reported mosquito nuisance ($P = 0.050$) (Table 9).

Table 9. Perceived mosquito nuisance reported by 166 respondents in Busoro and Ruhuha sector, Rwanda. *P*-value is based on a Chi-square statistical comparison between sectors.

Variables		**Busoro** n (%)	**Ruhuha** n (%)	**Total**	***P***
Nuisance	No	1 (1)	6 (7)	7 (4)	
	Yes	83 (99)	76 (92)	159 (96)	0.05
Total		84	82		

Of those that did perceive nuisance, 6% (n = 10) experienced "very little", 25% (n = 43) experienced "little", 17% experienced "some", 32% experienced "much" (n = 52, 32%) and 16% reported "very much" nuisance. There was no significant difference between the two sectors when comparing the level of mosquito nuisance experienced indoor ($P = 0.177$; Table 10).

Table 10. Perceived mosquito nuisance indoors for 2017 and 2018 in the Busoro and Ruhuha sector, Rwanda. *P*-value is based on a Chi-square statistical comparison.

Nuisance scale	**Busoro n (%)**	**Ruhuha n (%)**	**Total n (%)**	***P***
No nuisance	1 (1)	6 (7)	7 (4)	
Very little	3 (4)	7 (9)	10 (6)	
Little	23 (27)	20 (24)	43 (25)	
Somewhat	14 (17)	13 (16)	27 (17)	0.177
Much	31 (37)	21 (26)	52 (32)	
Very much	12 (14)	15 (18)	27 (16)	
Total	84	82	166	

Temporal variation in perceived mosquito nuisance

When asked in what season respondents perceive mosquito nuisance, most respondents reported to experience "very much" nuisance during the long rainy season (March – May; Figure 3B). Interestingly, more respondents in Busoro (n = 73) perceived mosquito nuisance in this season than in Ruhuha (n = 54). Respondents perceived "little" to "very much" nuisance during the short rainy season (September until November; Figure 3-A). Respondents mostly perceived "some" to "little" nuisance during the small rainy season (December-February; Figure 3-C) and mostly "very little" to "little" in the big dry season (June until August; Figure 3-D).

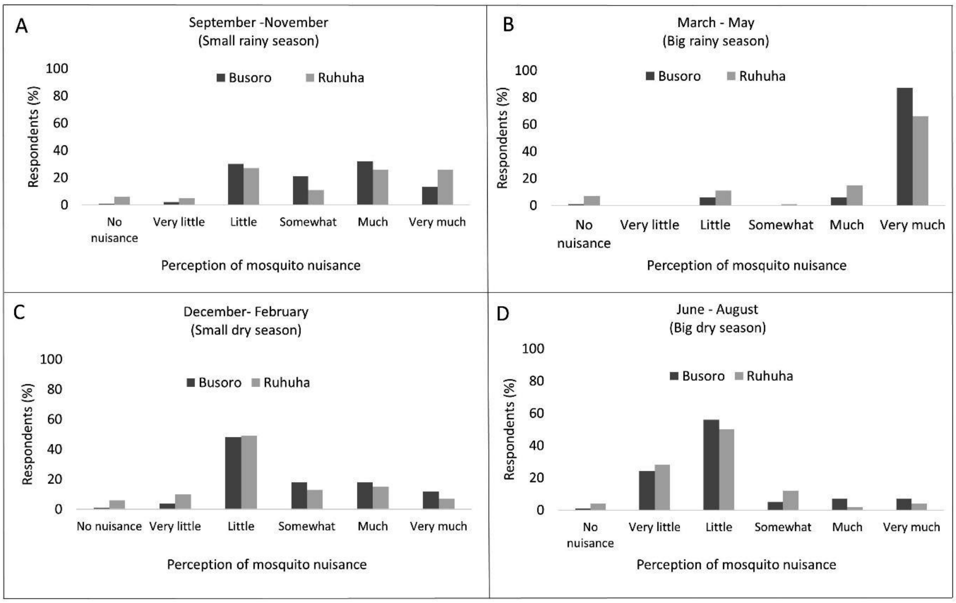


Figure 3. Mosquito nuisance as experienced during the four seasons reported by 166 respondents from Busoro and Ruhuha sector, Rwanda.

Spatial variation in perceived mosquito nuisance

Within each sector, there was substantial spatial variation at village level in the degree of mosquito nuisance perceived indoor (Figure 4). In Busoro, the highest perceived mosquito nuisance was from Runazi ($\bar{x} = 3.9$), Rucyamo ($\bar{x} = 3.8$), and Kireranyana ($\bar{x} = 3.6$), followed by Gikombe ($\bar{x} = 3.1$), Muhindo ($\bar{x} = 2.9$) and Karambi ($\bar{x} = 2.5$) (Figure 4). In Ruhuha, households from Kamweru ($\bar{x} = 3.5$) and Rusenyi ($\bar{x} = 3.6$) reported to experience much nuisance in their houses while the remaining participants from Kibaza ($\bar{x} = 2.9$), Kiyovu ($\bar{x} = 2.9$), and Mubano ($\bar{x} = 3.1$) reported having experienced some nuisance. Interestingly, respondents from Kagasera reported having experienced little mosquito nuisance ($\bar{x} =1.8$) during the period of study.

Relationship between mosquito abundance and perceived indoor mosquito nuisance

First, a Spearman correlation coefficient was computed to assess the relationship between the number of mosquitoes (total Culicidae and total *An. gambiae* s.l.) collected and the perceived nuisance level experienced in the houses by the participants. When data from both years and sites were aggregated, there was a moderate, but significant correlation between the total Culicidae and perceived nuisance indoors ($r_s = 0.316$, $n = 166$, $P < 0.001$). Similarly, there was a moderate, but significant correlation between the number of *An. gambiae* s.l. collected indoors and the perceived mosquito nuisance ($r_s = 0.281$, n = 166, $P < 0.001$).

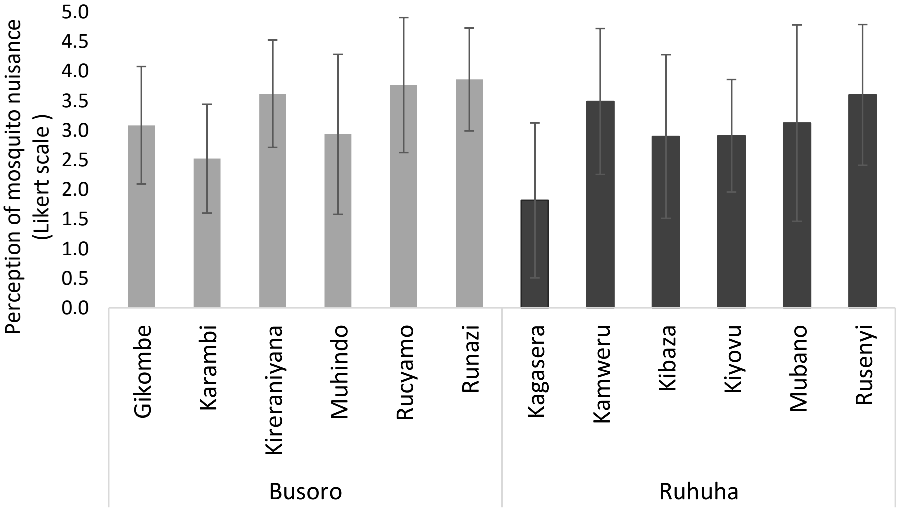


Figure 4. Perceived mosquito nuisance in twelve villages in Busoro and Ruhuha sector, Rwanda.

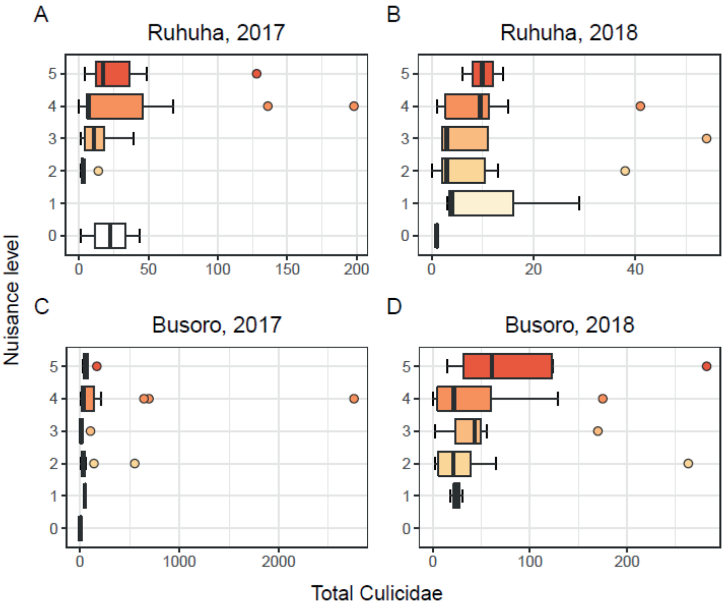


Figure 5. Boxplots showing the distribution of total mosquito (Culicidae) densities for each nuisance level reported per household in the two years (2017 and 2018) and two sectors (Ruhuha and Busoro) of study. Note the different scales of the x-axes for each panel.

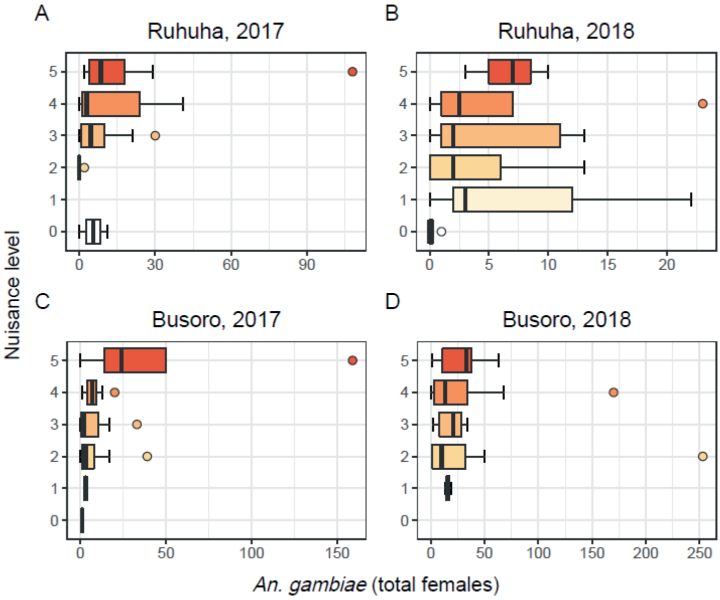


Figure 6. Boxplots showing the distribution of *An. gambiae* densities for each nuisance level reported per household in the two years (2017 and 2018) and two sectors (Ruhuha and Busoro) of study. Note the different scales of the x-axes for each panel.

When the data were analysed per year and per sector for Culicidae, there was a strong spatial difference. No significant correlation was observed between total mosquito numbers and perceived nuisance indoor in Busoro for both years (2017: r_s = 0.055 n = 42, *P* = 0.731 and 2018: r_s = 0.200 n = 42, *P* = 0.204; Figure 5-C and D), whereas there were moderate, but significant correlations between the total mosquito numbers and perceived mosquito nuisance in Ruhuha for both years of study (2017: r_s = 0.389, n =40, *P* = 0.013 and 2018: r_s = 0.305, n = 42, *P* = 0.049; Figure 5-A and B)(Table 11).

Table 11. Spearman correlation coefficients between perceived level of mosquito nuisance and number of mosquitoes (*An. gambiae* only, or total Culicidae). Significant *P*-values are indicated in bold.

Site	**Year**	**Mosquito group**	**r_s**	**n**	***P***
Busoro	2017	Culicidae	0.055	42	0.731
Busoro	2018	Culicidae	0.200	42	0.204
Ruhuha	2017	Culicidae	0.389	40	**0.013**
Ruhuha	2018	Culicidae	0.305	42	**0.049**
Busoro	2017	*An. gambiae* s.l.	0.370	42	**0.016**
Busoro	2018	*An. gambiae* s.l.	0.119	42	0.452
Ruhuha	2017	*An. gambiae* s.l.	0.450	40	**0.004**
Ruhuha	2018	*An. gambiae* s.l.	0.251	40	0.109

For *An. gambiae* s.l. per sector and year, there was a strong temporal difference. There were significant correlations in both sectors in 2017 (Busoro: r_s = 0.37, n = 42, *P* = 0.016; Ruhuha: r_s =0.45, n = 40, *P* = 0.004; Figure 6-A and C). However, in 2018, these significant correlations between perceived nuisance and *An. gambiae* s.l. were absent for both sectors (Busoro: r_s = 0.119, n = 42, *P* = 0.452; Ruhuha: r_s = 0.251, n = 40, *P* = 0.109; Figure 6-B and D) (Table 11).

When data were analysed one spatial level lower, i.e. by village, there were significant correlations between average nuisance level reported and *An. gambiae* s.l. (r_s = 0.607, *P* = 0.002, Figure 7-A), as well as between average nuisance level and total mosquitoes (r_s = 0.528, *P* = 0.008, Figure 7-B). When analysed per year separately, the correlations were strong and significant for 2018 (black dots, Figure 7), but not significant for the data from 2017 (grey dots, Figure 7).

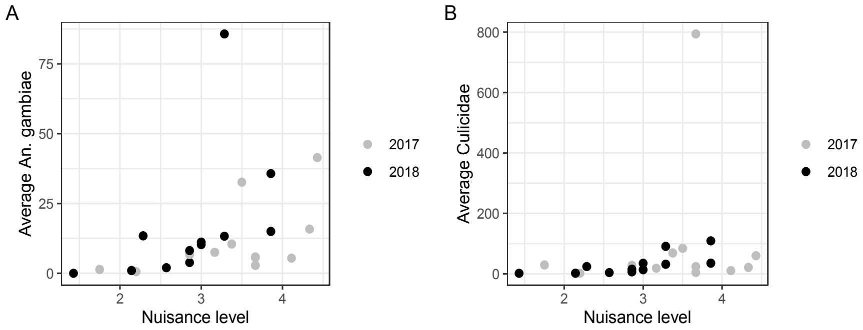


Figure 7. Scatterplots showing the correlation between average level of mosquito nuisance reported and average number of mosquitoes at village level (*An. gambiae* s.l., panel A; Total Culicidae, panel B). Each dot represents the average for one village consisting of 6-8 sampled households (2017: grey dots, 2018: black dots)

Discussion

The mosquito nuisance derived from the questionnaires, revealed a significant nuisance caused by mosquitoes, notably from the Culicidae. Our findings show that there was a high mosquito density including *Culex* species and mainly *An. gambiae* s.l. for both years in both sectors. A higher density of mosquitoes and malaria vectors were partially explained by the construction materials for roof and walls, as well as by the presence of eaves and number of occupants in the house. Hence, these factors also contribute to a high level of perceived mosquito nuisance, although correlations were space (sector) and time (year) dependent. The presence of *P. falciparum* infected mosquitoes in the houses, as shown for the 2017 data, contributes to the risk of contracting malaria. Our results are supported by several other findings that show that individuals who live in rural areas in poorly constructed houses are exposed to more mosquito bites, and hence to an increased risk of malaria transmission (Charlwood et al. 2003; Jatta et al. 2015; Kaindoa et al. 2018; Kateera et al. 2015; Snyman et al. 2015; Tuyishimire 2013). In a study conducted in Kenya, houses made of wood and mud exhibited a significant effect on mosquito abundance in the houses (Zhou et al. 2007a).

Factors such as closed eaves reduced rates of house entry by anopheline mosquitoes compared to fully open eaves as was also demonstrated in Tanzania, the Gambia, and Kenya (Mburu et al. 2018; Menger et al. 2016; Njie et al. 2009; Ogoma et al. 2010; Ondiba et al. 2018).

We found spatio-temporal variations in mosquito abundance between Busoro and Ruhuha sectors. In Busoro, mosquitoes, including numbers of *An. gambiae* s.l., were collected in significantly higher numbers in comparison with Ruhuha. This spatial difference may be explained by the fact that although both sectors have wetlands, Busoro is characterized by a larger area (178ha) than Ruhuha (93ha) that is dedicated to rice irrigation (Hakizimana 2019). The difference was strongly influenced by collections from one of the villages (Rucyamo), which is the village closest to the irrigated fields (Figure 1) and which contributed to 65% of all mosquitoes collected. This village was also the village with the one, but highest level of mosquito nuisance reported. Living near the rice field, the chance to have more mosquitoes and experience more nuisance was higher, because the wetlands are a good mosquito breeding habitat (Sanford et al. 2014; Tabue et al. 2014).

In the present study, the predominant sibling species of the *An. gambiae* complex was *An. gambiae s.s.* for both years and sectors combined (66%). This dominance of *An. gambiae s.s.* is similar to a study conducted in one site near Kigali City in 2007 by PMI-Rwanda, in which it was reported that *An. gambiae s.s*. accounted for 93.6% of the total of 157 *An. gambiae* s.l. examined by PCR while *An. arabiensis* accounted for the remaining 6.4% (Lansana 2008). However, our finding was contrary to the study where the characterization of *An. gambiae* s.l. from 10 sentinel sites revealed that the predominant sibling species was *An. arabiensis* (83%) (Hakizimana et al. 2016). Although *An. funestus* was recognized as dominant *Anopheles* species in previous studies from Rwanda (Loevinsohn 1994; Munyantore 1989), this species was collected only in Rucyamo, the village closest to the more permanently inundated wetlands and rice fields, which are ideal habitats for this vector species.

In our study, for 2017, the *P. falciparum* infection rate in Busoro was higher compared to that in Ruhuha. Other anopheline species collected in low numbers should not be neglected in the strategies for malaria control and elimination, because they can transmit other mosquito borne diseases such as the Babanki virus (BBKV) that is transmitted by *An. brohieri* as it was found in Senegal (Diagne et al. 1994). Albeit in low numbers, both *An. pharoensis* and *An. ziemanni* can transmit *P. falciparum* as observed in other studies from Cameroon, Ethiopia, Guinea Bissau, Tchad and Kenya, respectively (Hinzoumbé et al. 2009; Kamau et al. 2006; Kibret et al. 2012; Sanford et al. 2014; Tabue et al. 2014). Although *Culex* spp. have not been incriminated as vectors of disease in Rwanda, the species caused a high burden of nuisance in Cameroon (Nchoutpouen et al. 2019). Considering this group of species as potential vector for other vector-borne disease will be important in the framework of Integrated Vector Management. Reducing their numbers would substantially reduce mosquito nuisance experienced, and thus enhance community involvement in uptake of vector control for malaria prevention (Ingabire et al. 2015).

The respondents experienced mosquitoes as a significant problem in their daily life, especially during the rainy season that lasts from March to May. The increase in perception of mosquito nuisance over the seasons corresponds to the increase of vector density at the start of the long rainy season which is mainly due to an increase in wetland area which provides suitable habitats for larval development of mosquitoes. The percentage of participants who reported much nuisance was higher in Busoro (87%) than in Ruhuha (67%). Living in the vicinity of marshlands increased the chance to experience higher mosquito abundance and hence higher nuisance level as observed from participants from Busoro and Ruhuha (Hakizimana 2019; Musabe 2012). In addition, we also noted the presence of blocked ditches produced by of the exploitation of sand for house constructions. Ditches are known as artificial mosquito breeding sites (Castro et al. 2010; De Silva & Marshall 2012).

The correlations that we found between nuisance and number of mosquitoes can be explained as larger numbers of mosquitoes collected indoors will result in more biting activity and, hence a higher level of mosquito nuisance. Our results thus suggest that levels of perceived mosquito nuisance are in some way indicative of mosquito densities indoors. Consequently, one could argue that perceived mosquito nuisance in the peridomestic area can be used as a global indicator for malaria transmission risk by filling out a questionnaire indicating the level of nuisance expressed on a Likert scale. A study conducted in Algeria demonstrated that perception of citizens can help to identify occurrence of *Ae. albopictus* in a residential neighbourhood in Bir-Khadem (Benallal et al. 2019). This helped to put in place vector control measures that could prevent the propagation of *Ae. albopictus* to other areas and to avoid the massive use of insecticides for vector control which could ultimately lead to insecticide resistance (Benallal et al. 2019). It should be noted that, when considering each sector separately, perceived mosquito nuisance was significantly correlated to the numbers of *An. gambiae* when data from both years were added together, while for Culicidae perceived mosquito nuisance was correlated to the number of Culicidae for Ruhuha, but not for Busoro, even not after adding datasets of both study years together. The reasons why there was no correlation for Busoro remains unclear. We hypothesize that this may be explained by the fact that in Busoro, mosquito densities were more extreme than in Ruhuha and that variation in nuisance by these high densities could no longer be caught in the level of perceived mosquito nuisance.

Conclusions

Our findings demonstrated that poor housing construction significantly led to increased malaria vector density and thus possibly malaria risk in rural Rwanda. This suggests that good house construction needs to be considered as one of the vector control strategies that can be provided for poor populations. At the largest scale in this study, i.e. if data for years and sectors are combined, the relationships between the level of perceived mosquito nuisance and mosquito density at family and species level were clearly shown. Perception of mosquito nuisance denoted in a questionnaire survey could be used as an indicator of mosquito abundance and, hence, for *An. gambiae* s.l. occurrence. Therefore, involving citizens in reporting the level of mosquito nuisance can contribute to improved mosquito surveillance and control.

Acknowledgements

The authors sincerely thank the Mayor of Bugesera district, Ruhuha Health Centre, Ruhuha administrative sector. We thank all the research assistants for their support in data collection. We thank Hans Smid, Julien Rougeot and Jeanine Loonen for their assistance and for the technical support. Finally, we also thank the leaders in Ruhuha and Busoro of the study sites, the lay community members for their willingness to participate in the study. Interdisciplinary Research and Education Fund (INREF, Wageningen University, The Netherlands), and the University of Rwanda generously supported the study. We also thank the laboratory of Entomology from The Malaria and Other Parasitic Diseases Division and Wageningen University and Research for the technical support.

Chapter 4

Co-designing a citizen science program for malaria control in Rwanda

Domina Asingizwe[*], Marilyn Milumbu Murindahabi [*], Constantianus J.M. Koenraadt, P. Marijn Poortvliet, Arnold J.H. van Vliet, Chantal M. Ingabire, Emmanuel Hakizimana, Leon Mutesa, Willem Takken and Cees Leeuwis

[*]: shared first authorship

Published in *Sustainability (*2019), 11(24): 1-17

Abstract

Good health and human wellbeing is one of the sustainable development goals. To achieve this goal, many efforts are required to control infectious diseases including malaria which remains a major public health concern in Rwanda. Surveillance of mosquitoes is critical to control the disease, but surveillance rarely includes the participation of citizens. A citizen science approach (CSA) has been applied for mosquito surveillance in developed countries, but it is unknown whether it is feasible in rural African contexts. In this chapter, the technical and social components of such a program are described. Participatory design workshops were conducted in Ruhuha, Rwanda. Community members can decide on the technical tools for collecting and reporting mosquito species, mosquito nuisance, and confirmed malaria cases. Community members set up a social structure to gather observations by nominating representatives to collect the reports and send them to the researchers. These results demonstrate that co-designing a citizen science programme (CSP) with citizens allows for decision on what to use in reporting observations. The decisions that the citizens took demonstrated that they have context-specific knowledge and skills and showed that implementing a CSP in a rural area is feasible.

Introduction

Malaria remains a major public health concern in many sub-Saharan African countries, including Rwanda (Karema et al. 2012; PMI 2018). In Rwanda, a significant reduction in malaria has been achieved through the use of control measures including long lasting insecticidal nets (LLINs), indoor residual spraying (IRS), and artemisinin-based combination therapy (ACT) (Karema et al. 2012). However, from 2012 to 2016, Rwanda experienced an upsurge of malaria cases that was reported across the country, especially in the eastern and southern regions. This increase put the entire population at risk and children under five years old and pregnant women were the most exposed to malaria infection (MOH 2017b, 2017a).

The increase of malaria in Sub-Saharan African countries urged the global community to improve the disease and vector control response because human wellbeing is one of the United Nations' sustainable development goals (WHO 2017a). Since the level of investment in malaria control across the world remains inadequate (WHO 2017a, 2018), the World Health Organization supports the development of effective, locally adapted and sustainable vector control (WHO 2017a). The latter includes mosquito surveillance, which consists of regular reporting of the density and the pathogen prevalence rate of vectors in a specific region. This helps to identify how vectors spread the infections to hosts and to determine appropriate interventions to reduce the risk of infection (Wu et al. 2016). In Rwanda, in addition to active surveillance of malaria cases, mosquito surveillance is carried out in 12 sentinel sites established across the country (PMI 2019). Trained entomology technicians and officers, and some trained local community members are employed on a monthly basis to undertake mosquito surveillance in their assigned areas. Hence, this requires stable financial resources for staff payment. In addition, mosquito surveillance is based on the systematic reporting of the distribution, diversity, and density of malaria vectors using pyrethrum spray and human landing catches (HLC) as mosquito collection methods. Another indicator that is being reported is the entomological inoculation rate (EIR), expressed as the number of infectious bites per person per year (Hakizimana et al. 2018). The entomologists submit a compiled monthly report with entomological indicators mentioned above to the person in charge of the vector control unit of Rwanda Biomedical Center for compilation, and further analysis (Murindahabi et al. 2018) to guide the planning of interventions.

Despite this program, there are several gaps in the surveillance system. For example, beyond the 12 sentinel sites there are still many regions where mosquito surveillance is not established because of limited funds or lack of trained entomologists. Consequently, it hinders the progress in malaria reduction, and limits community awareness on malaria vectors. A possible solution to complement the current malaria mosquito surveillance is to involve the public via a citizen science-based program (CSP). Citizen science as a tool for mosquito surveillance requires an understanding of who is going to collect or report what, how, and when. This chapter outlines how such a surveillance program could be designed, put in place, and what preferences exist in local communities with regard to the technical and social components of such an approach. A description of what activities are required to implement such a program are also described. We focus on several aspects including (1) the process of recruiting volunteers, (2) technical tools for collecting and reporting observations, (3) frequency of collection and reporting observations, and (4) feedback generation. The following section provides the conceptual background which elaborates on existing CSPs in mosquito surveillance and explains how the co-design concept was used to develop the CSP.

Conceptual background

Citizen science as a tool for mosquito surveillance

Citizen science can be described as a collaboration between scientists and volunteers, particularly to expand opportunities for scientific data collection and to provide access to scientific information for society (Shirk et al. 2012, 2009). With acknowledgement of participatory action research (PAR) and other community-based interventions (CBI) that have been conducted in the last decades to improve health literacy and ability to make decisions related to malaria prevention and control (Ingabire et al. 2016; Rickard et al. 2011; Yukich et al. 2014), citizen science has been used to actively engage people in the collection, and/or in the analysis and the interpretation of data. This approach has been explored for monitoring invasive and endemic mosquito species in developed countries (Kampen et al. 2015; Vogels et al. 2015). In these countries, citizen science has provided large amounts of relevant mosquito data, hence, citizen science proved its potential in the monitoring of (invasive and endemic) mosquito species (Kampen et al. 2015; Vogels et al. 2015).

These projects involved volunteers that participated in different ways such as collecting and mapping *Culex pipiens* biotypes, or assessing mosquito nuisance experienced by citizens in the Netherlands (e.g., Muggenradar) (Vogels et al. 2015). Additionally, volunteers also participate in detecting country-wide changes in mosquito fauna (e.g., Mückenatlas in Germany), and adding real-time information for daily mosquito management such as the Asian tiger mosquito (*Aedes albopictus*) (e.g., Mosquito Alert in Spain) (Hecker et al. 2018; Palmer et al. 2017; Walther & Kampen 2017).

CSPs have become more interactive because of the availability of the internet, and most of the projects are now online-based. However, in absence of the internet and with limited access to electricity, for example in rural areas of Rwanda (NISR 2017), traditional methods and strategies of reporting mosquito observations can be used instead (e.g., paper forms) (Chaki et al 2011; Kiware et al. 2016).

In addition to providing a valuable extension of the professional surveillance networks, CSPs can have other important functions in the strategies to reduce malaria. These include the increase of public awareness and engagement in the topic (for example about mosquito-borne diseases) (Eritja et al. 2019). In addition, participation in citizen science creates new opportunities for connections between various stakeholders such as researchers, citizens, policy makers, funding agencies, and decision-makers, thereby extending their own social network (Asingizwe et al. 2018), and it can strengthen community-based management of residual foci of malaria transmission (Ingabire et al. 2016).

CSPs consists of technical and social components. The technical component defines the citizen science infrastructure like the physical kit, and the technology assets. The physical kit may consist of various equipment that could be, for example, mosquito traps, microscopes, buildings, etc. The technology assets are the information technology-based platforms/tools and services used to collect, store, manage, process, share, visualize, and analyse information (data and metadata) which is produced by citizen science (Hecker et al. 2018). However, the technology asset is not a required component for running a CSP (Jordan et al. 2017). The social component includes the organizers of the projects or researchers, the citizens, and the social networks of connected individuals (Hecker et al. 2018).

Co-designing a CSP

The development of a robust and context-specific CSP for mosquito surveillance requires the inclusion of people in the design process and integration of a diverse range of experiences, interests, and knowledge (Bartumeus et al. 2019; Bonney et al. 2009; Campos et al. 2017). Co-designing a malaria mosquito surveillance system includes defining the social structure of the program, and defining the infrastructures and the tools, sampling and feedback strategies to be used (Figure 1).

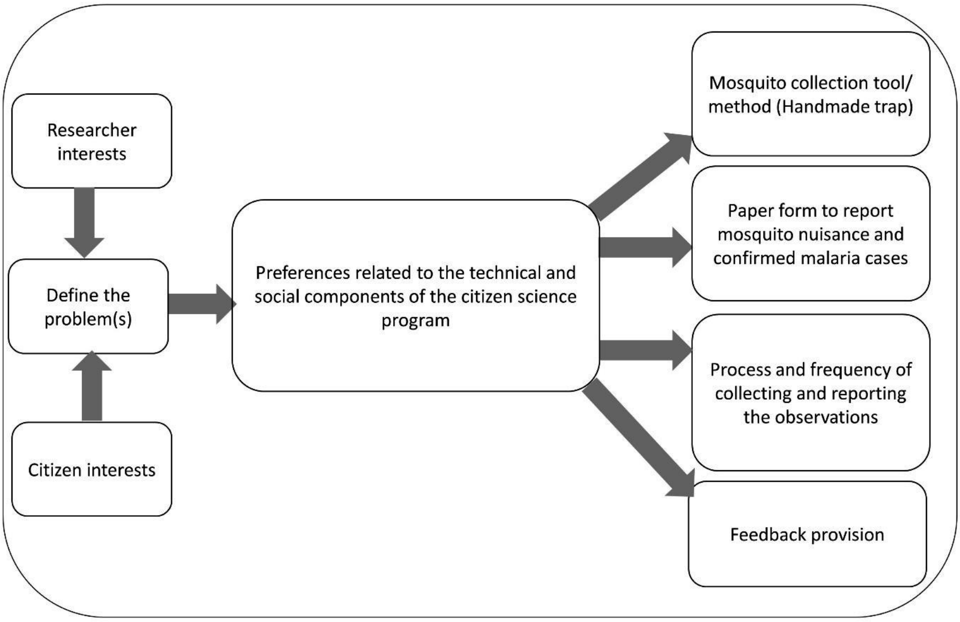


Figure 1. The framework indicating the co-designing process of the citizen science program for malaria control.

Participation in the design of the technical and social components of a malaria mosquito surveillance programme provides opportunities for the citizens to express their preferences with regard to relevant design choices and give feedback on proposed design components. As a result, this may foster ownership of the program (Hecker et al. 2018). Additionally, citizen participation and engagement in citizen science projects increases resource capacity for mosquito surveillance, and also promotes the acceptability of, and adherence to malaria control strategies among the community members (Asingizwe et al. 2019). Strong partnerships with communities in vector control are required for sustainable innovation such as through citizen science (Bartumeus et al. 2019). The success also involves the provision of feedback that is essential in every CSP to keep people engaged in the research (Campos et al. 2017; Jordan et al. 2016).

Methods

Study area

The study was carried out in the Ruhuha sector of Bugesera district, located in the eastern province of Rwanda. Ruhuha covers an area of 54 square kilometers with a population of more than 24,000 (over 5,500 households; 2017 data). The Ruhuha sector encompasses five cells with 35 villages. Ruhuha is bordered by Lake Cyohoha in the south and is characterized by its many water streams and marshlands classifying the region as a historical malaria endemic zone. Rice cultivation and wetland agriculture are the main economic activities. The Ruhuha sector was selected because of the high number of malaria cases reported since 2012. One village per cell was randomly selected for inclusion in the study. These five villages were Busasamana, Kagasera, Kibaza, Kiyovu, and Mubano.

Study design, population, and sampling

Six workshops (including one for the pilot) were carried out in 2018 prior to the implementation of a CSP for malaria mosquito surveillance in selected villages in the Ruhuha sector. Participatory design workshops (PDWs) were used. A PDW is defined as a workshop through which all stakeholders including users (citizens in this case) that are affected by the upsurge of malaria in their environment, are invited to collectively define the problem that affects them, and to set up mechanisms to solve the problem while anticipating their needs (Sanoff 2007). It is therefore a user-centered design method in which the focus is on the active role of the users. The first workshop was a pilot conducted in March 2018 and aimed to discuss the malaria upsurge and to explore whether participants were willing to participate in malaria control by being enrolled in the CSP and how they could participate in such a program. In addition, the pilot workshop aimed to inform the process of the main PDWs through testing the content and steps of the PDW. The pilot study was conducted in one of the ten villages in which a baseline study was conducted (Asingizwe et al. 2019) and was randomly selected. With the results from this pilot workshop, five follow-up workshops were organized and conducted in August 2018. Each workshop lasted around 6 hours and aimed to establish a citizen network that was willing to actively participate in the CSP.

Recruitment process

Based on ten villages selected in the baseline survey (Asingizwe et al. 2019), six of these (one for pilot and five for the main PDWs) were selected for the implementation of the CSP. Generally, each village has approximately 150 households and we targeted a third (45 community members) of the total number of the households. In each village, the households are grouped in *isibo* (cluster of 15 neighbouring households) thus each village has approximately 10 *isibos*. Therefore, three community members in each *isibo* and the *isibo* leaders were targeted to participate which results in a total of 40 participants per village. In addition, each village has three community health workers (CHWs) and one village leader. Consequently, these were also added to the 40 selected community members. Lastly, an executive of the respective cell was also expected to attend the workshop. Hence, in total 45 people were supposed to attend in each of the five selected villages for the PDWs. This number was the same in the pilot workshop. The village leaders selected the community members and we were careful that these community members were not from the same household or were relatives.

To ensure this, the village leader announced this workshop during a village meeting and those who showed interest were invited to participate. At the beginning of each workshop, the researchers (two first authors) verified whether the criteria had been fulfilled through requesting people from each *isibo* to stand up and asking them whether they are from different households. Although this verification was done, people were not informed whether researchers were cross checking. In few cases, it was obvious that a husband and wife could attend when one of them was a village leader and another was a CHW; this was inevitable. As shown in Table 1, in some villages community members did not attend in sufficient numbers. The main reason for this low turn up in some villages was that these villages (Busasamana and Kibaza) are located further away from the health center where the workshops were conducted. In addition, the day we conducted a workshop for Busasamana it was raining, and some community members decided to go to their farms for field work instead of attending the workshop.

Co-design processes

Pilot workshop

During the pilot workshop, the following guiding question was asked to the participants: “As a community member, how are you going to be engaged in malaria control?” The main reason of this question was that, “citizen engagement in malaria prevention and control activities” was one of the three strategies to improve consistent use and acceptance of malaria control measures mentioned by the participants from the baseline survey in the study area (Asingizwe et al. 2019). This clearly indicated that the community members were willing to participate. After the guiding question, each participant was requested to write (maximum) three ways of engagement in malaria control. These notes were then collected by the researchers, who in turn grouped those which were similar to the themes. Among the themes listed, control of mosquito breeding sites stood out. Furthermore, participation in community mobilization was also listed. Participants were divided into small groups in order to discuss the themes, and after discussion, participants presented the outcomes of the discussions. In addition, participants were requested to fill out a small questionnaire to indicate whether (1) they have ever experienced mosquito nuisance, (2) they were willing to participate in the collection of mosquitoes, and if so, (3) to describe how they think they can collect mosquitoes.

Five participatory design workshops

The PDWs were structured in three main steps (Figure 2). As informed by the pilot workshop, the guiding questions had to be modified based on the outstanding theme. The first two authors facilitated all PDWs and the sixth co-author also joined one of the PDW.

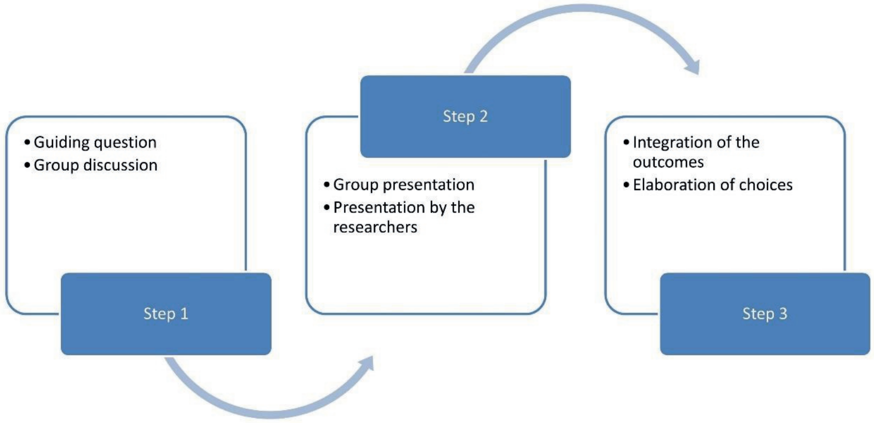


Figure 2. Steps followed during the participatory design workshops.

Step 1: Part 1. Guiding questions

At the beginning of each PDW, guiding questions were announced. The first set of guiding questions focused on understanding of mosquito nuisance, as well as reporting of mosquito nuisance and collecting mosquito specimens: “How do you interpret the term mosquito nuisance? If you are asked to provide observations such as mosquito nuisance experienced and mosquitoes (mosquito species), how can you report it and /or collect it? Do you think it is feasible? Why would you (not) do that? How frequently can you do that”? The second guiding question focused on ways or means for providing feedback: “If you report observations, what and how would you like to get feedback”? A handout of the guiding questions was given to the groups to facilitate the discussion. It was observed that providing these guiding questions could help the participants to reflect more on their ways of engagement in malaria control rather than giving them one broad guiding question as was done during the pilot workshop.

Step 1: Part 2. Group discussion

Following the guiding questions, three different homogeneous groups were formed including only men, only women, and only youth (between 18–25 years). These homogenous groups were created to ensure that all participants had equal opportunities to share their views. This was also a result of the pilot workshop, in which we observed that some participants could not express themselves in heterogeneous groups. During the focus group discussion, which lasted for 1 hour, members of the group elected a group leader and a reporter who presented the outcomes of the discussions. The different groups were requested to write down the answers of all the guiding questions on flip charts which further helped them during the presentation of the outcomes of the discussion.

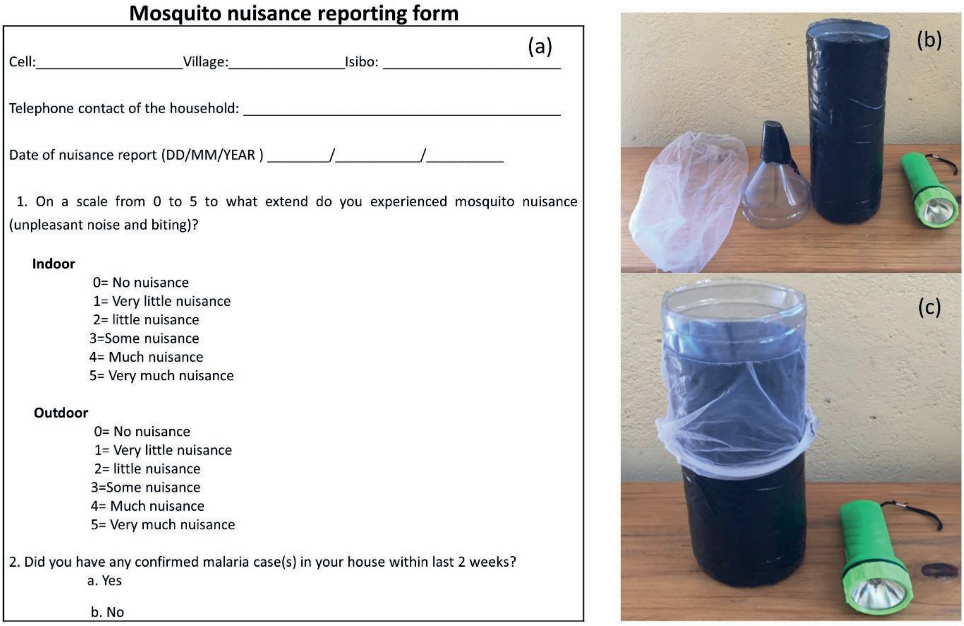
Step 2: Part 1: Group presentation

After group discussions on the guiding questions, a representative from each group shared their outcomes of the discussion and participants from other groups were allowed to ask questions or provide comments. After the group presentation by the different focus groups, a summary of the outcomes of each group was presented by the researchers and all participants were able to add inputs into the summary. This was done to ensure harmonization of ideas and to facilitate the integration session (Step 3).

Step 2: Part 2. Presentation from the researchers

The researchers' presentation explained how to report mosquito nuisance and malaria cases by filling out a paper-based form (Figure 3a) and on how to catch and report mosquitoes (Figures 3b and 3c). The reporting form included two questions: (1) To what extent do you experience mosquito nuisance (unpleasant noise and biting; to be assessed on a scale from zero (no nuisance) to five (very much nuisance))? and (2) did you have any confirmed malaria case(s) within the last two weeks in your household?

Additionally, participants also gave feedback on the perceived efficacy of a carbon dioxide-baited trap in collecting mosquitoes following the results presented by the researchers. This trap was prototyped and tested in the laboratory and in the field. The trap was proposed as recommended by the participants of the pilot workshop, as the majority requested tools to collect mosquitoes. Thus, researchers followed up on this by validating a low-cost, easy to use "handmade carbon dioxide-baited trap" (Figure 3c, Chapter 5). This trap uses carbon dioxide as a stimulus which is produced by mixing yeast, sugar and water (Smallegange et al. 2010) and light. The trap was designed taking into account the cost and the practicability of the existing mosquito collection method in rural Rwanda. Prior to its application for the CSP, different trap designs were evaluated under laboratory conditions at Wageningen University and Research, and afterward in the field in Kibaza village (Chapter 5). During the workshops, researchers presented the results from the lab and field evaluations. Furthermore, researchers presented different means of feedback provision after collecting and reporting data by the volunteers.



Mosquito nuisance reporting form

Cell:__________________Village:_______________Isibo: ______________________

Telephone contact of the household: ____

Date of nuisance report (DD/MM/YEAR) ________/__________/__________

1. On a scale from 0 to 5 to what extend do you experienced mosquito nuisance (unpleasant noise and biting)?

Indoor

0= No nuisance
1= Very little nuisance
2= little nuisance
3=Some nuisance
4= Much nuisance
5= Very much nuisance

Outdoor

0= No nuisance
1= Very little nuisance
2= little nuisance
3=Some nuisance
4= Much nuisance
5= Very much nuisance

2. Did you have any confirmed malaria case(s) in your house within last 2 weeks?
a. Yes

b. No

Figure 3. (**a**) The paper-based form for reporting observations, (**b**) the handmade carbon dioxide-baited trap showing the different elements that are part of the trap, and (**c**) the trap after assembly. The torch was suggested by volunteers and added afterwards.

Step 3: Part 1. Integration of outcomes

After a presentation by the researchers on the proposed tools, participants returned to their groups to discuss which proposed tools were preferred and why. Additionally, participants indicated the reporting scheme of the observations, process of reporting, preferred frequency of reporting, and ways to provide feedback. A handout of the proposed methods together with flip charts were given to the participants to facilitate the discussion and help them write down the answers for further presentation. This integration part was added to explore the different reasons to why some methods were preferred over the others and observe whether participants could be able to compare and criticize different methods including those listed by other groups. All flip charts were kept for further transcription and analysis.

Step 3: Part 2. Elaboration of choices

All results from the group discussions were presented and discussed among all participants to ensure the validity of the information provided during the focus group discussions. Hence, the researchers made a summary of the choices presented by each group and then shared it with the participants for final agreement and approval.

Data analysis

The results from flip charts were transcribed and analysed. The summaries from the large group discussion taken by the researchers were also used to facilitate and inform the transcription process in case some hand writings were not readable; and complement the transcripts in case the flip charts did not include the details because some questions for clarifications were asked during the group presentations. All transcripts were classified under different categories that emerged from the guiding questions and each group was coded independently to avoid duplication of the results. In reference to the guiding questions, different categories that included reporting mosquito nuisance, collecting mosquito specimens, recruitment of volunteers to be enrolled in citizen science, process of collecting and reporting the information, the frequency of reporting observations, and feedback generation were defined. Finally, after the completion of the data analysis, the categories were divided into two themes (technical and social) according to the co-design framework of CSP (Figure 1). The presentation of the results followed this framework as well.

Ethical approval

Ethical approval was granted to the study (Approval Notice: No 414/CMHS/IRB/2017) by the Institutional Review Board of the College of Medicine and Health Sciences, University of Rwanda.

Results

The results are organized in two main sections. The first section presents the outcomes of the pilot workshop which indicate the responses about mosquito nuisance experienced and willingness to collect mosquitoes. The second section elaborates the results of the five PDWs which are divided into two main themes: (a) The technical component that includes tools to collect and report the observations, and (b) a social component that consists of (1) recruitment of volunteers, and collection of information, (2) strategies for collecting and or reporting the observations, and (3) mechanisms of feedback to the community members about the outcomes of the shared observations.

Pilot workshop

Forty-four participants attended the pilot workshop. These included 29 community members, ten *isibo* leaders, three CHWs, one village leader, and one Kindama cell representative.

Mosquito nuisance experienced

All participants (100%) answered that they had experienced different levels of mosquito nuisance. Locations reported where participants experienced more mosquito nuisance are in the bush (22 times; 61%), the rice field (24 times; 69%), and near the pond (21 times; 72%).

Willingness to participate in mosquito collections

Among the participants, 11 (26%) reported willingness to collect the mosquitoes. Among these, four (36%) answered the question related to which tool/materials to be used and three (27%) were not able to mention any method. In relation to the materials to be used, one participant indicated catching mosquitoes by hand, two reported that they would need materials from researchers, and one reported using a light from a torch to "hypnotize" the mosquito and catch it afterward.

The participatory design workshops

Characteristics of the participants who attended the five PDWs

One hundred and eighty-five participants (82%) out of 225 that were expected, attended the workshops (57% women; Table 1).

Table 1. Characteristics of the participants attended the Participatory Design Workshops (PDWs).

Village	Expected Participants	No. of Participants Attended PDWs	Male	Female
		Frequency (%)	**Frequency (%)**	**Frequency (%)**
Busasamana	45	17 (38%)	10 (59%)	7 (41%)
Kagasera	45	45 (100%)	12 (27%)	33 (73%)
Kibaza	45	33 (73%)	17 (51%)	16 (49%)
Kiyovu	45	43 (95%)	17 (40%)	26 (60%)
Mubano	45	47 (104%)	24 (51%)	23 (49%)
Total	225	185 (82%)	80 (43%)	105 (57%)

Technical component of the CSP

In general, not many changes were made to the technical components (tools to be used to collect and report the observations) of the design as the participants already expressed that they could not report the information if the materials are not given. Below these technical tools are presented in detail.

Technical tool to report mosquito nuisance and confirmed malaria cases

All groups indicated that it was feasible to estimate and report the level of mosquito nuisance experienced as well as reporting confirmed malaria cases. Participants described mosquito nuisance as the biting and the sound that the mosquito produces when flying. They all highlighted that nuisance does not necessarily relate to number of mosquitoes, because even one mosquito can bite or make noise. Among the different ways proposed by the participants to communicate the results on mosquito nuisance and confirmed malaria cases, phone calls, Short Message Service (SMS) text, and *isibo* or village meetings were mentioned. Both paper forms and SMS were viewed as a possible means to report mosquito nuisance and confirmed malaria cases. However, weighing the constraints that these two methods pose, some participants indicated that using a paper form is more preferable because it does not require much costs, while airtime credits may hinder the usage of SMS text. Therefore, the paper form was opted for to report mosquito nuisance and confirmed malaria case instead of an SMS text.

Another group of participants reported potential delays in reporting and loss of paper forms as a drawback of using paper forms. Therefore, these participants proposed to use both paper forms and SMS text or mobile phone calls as a means for reporting mosquito nuisance to the researchers. When discussing the advantages and disadvantages of using the two proposed methods, the majority of participants eventually preferred the paper form as it is less costly.

Technical tool for collecting mosquitoes

Participants indicated that it was feasible to collect mosquitoes if proper mosquito sampling tools were provided. To anticipate on this and based on the results from a pilot workshop conducted in March 2018 during which the majority of the participants reported not to be able to collect mosquitoes, the researchers proposed a handmade carbon dioxide-baited trap (Figure 3c). Based on the results presented from the trap test trial (conducted in the lab), participants indicated that using the carbon-dioxide-baited trap was feasible. However, some constraints with regard to the trap's practicability were mentioned. These included the difficulty to find the different components used to assemble the trap, such as the net and the 1.5 litres plastic bottle.

Other constraints mentioned by the participants were related to the costs (in case the volunteers have to buy these themselves) of the ingredients such as the yeast and the sugar used for the production of carbon dioxide to attract the mosquitoes in the trap. Participants indicated another constraint in relation to a new law that will ban the use of plastic bottles. The government of Rwanda is planning to remove plastic bottles from the market. The law on banning single-use plastics is still under evaluation in the Rwandan parliament. From the group discussions, one of the male groups indicated that catching mosquitoes using hands was also a possible option, as it does not require money.

In terms of quantity, the buckets or bottles proposed were preferred as means for collecting mosquitoes, as they seem to catch more mosquitoes than using hands and are also less sensitive to collector bias. Some of the participants reported that a bucket is easy to find, and it does not require sugar or yeast such as when using a bottle. On the other hand, if the proposed handmade carbon dioxide-baited trap and the ingredients used as odour attractant are provided, the proposed handmade-trap using plastic bottle was the preferred tool to use by the participants to collect mosquitoes.

Social component

Initially, the research team assumed that the data could be collected by the CHWs, or the *isibo* leaders and can be reported on biweekly basis. Additionally, CHWs would submit the collected data to the researchers at the health center on biweekly basis as well. Alternatively, the researchers could gather the collected data at the household level in the studied villages. As indicated below, most of these initial design options changed after the discussion with the community members.

Recruitment of volunteers for the CSP and the collection of the observations

There were no predefined rules on how to select volunteers and who should collect what information as all participants were eligible to participate in the program. However, as participation was on a voluntary basis, participants were invited to write their names and their telephone contacts on a list if they were willing to participate in the proposed CSP. It was made clear that they could either report mosquito nuisance and confirmed malaria cases only using the paper form, collect mosquitoes only using the proposed carbon dioxide-baited trap, or do both. It was also explained that there would be no monetary incentives for participation. Among 185 community members who attended the workshops, 116 volunteers (63%) wrote their names on the list as potential participants. Of these 116, 19 were willing to report mosquito nuisance only, 42 were willing to collect mosquitoes only, and 55 were willing to participate in both.

There was a general agreement among the participants on why it would be useful to participate in the collection and reporting of the observations such as mosquito nuisance, confirmed malaria cases, and mosquitoes. All groups highlighted that collecting mosquitoes was a way of contributing to malaria control as malaria affects many people.

Participants believed that through collecting and reporting of observations, their awareness related to mosquitoes and malaria can be enhanced. Consequently, the increased awareness may play a role in reducing malaria incidence. In addition, one of the male groups indicated that collecting mosquitoes could improve their knowledge on different mosquitoes' parts and species. However, collecting mosquitoes requires some skills and appropriate tools to do so. For this reason, one of the female groups voiced that they may not be able to collect mosquitoes because of the lack of knowledge and appropriate materials. The same concern was also reported in another mixed-gender group.

Strategies for collecting and reporting the observations

The initial idea to use the CHWs, *isibo* leaders, or collecting the observation at the household level by the research team was changed because of (1) high number of *isibo* leaders (approximately 50 for the five selected villages), (2) high workload attributed for the CHWs in the community, and (3) logistic issues in case the researcher collects the data at the household level. Hence, each group of volunteers in five selected villages nominated an *isibo* representative. The organizational structure of the reporting and collecting system was defined (Figure 4).

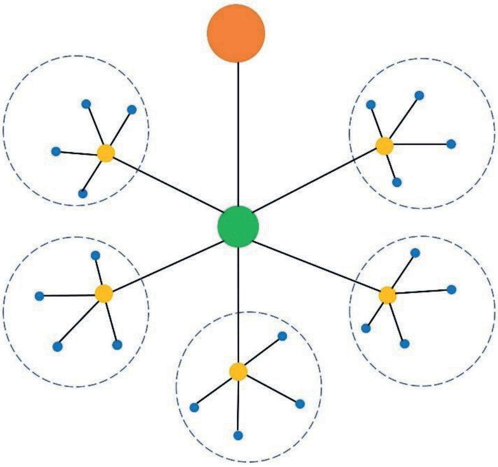


Figure 4. Fundamental architecture of the citizen science program (CSP) for malaria control. The blue dots represent the volunteers; the yellow dots represent the *isibo* representatives; the blue dashed circles represent villages, the green dot represents the researchers, the orange dot is the vector unit of the Malaria and Other Parasitic Disease Division (MOPDD). The lines represent the two-way communication between the different stakeholders involved in the CSP for malaria control. Specifically, the black lines show that these *isibo* representatives are directly connected to researchers and to the volunteers because (1) they are the ones submitting the observations every last Friday of the month, and (2) they collect the reports from volunteers, receive the feedback and share it with the volunteers in their respective villages.

These *isibo* representatives were tasked to distribute the tools (paper form and mosquito collection traps), and to request and report the data on a monthly basis. They were also tasked to inform and remind the volunteers about when to fill out the forms and set up the traps. In addition, volunteers who indicated the preferred period for them to collect and report the observation in collaboration with the researchers shared a proposed schedule. The schedule indicated when to submit the collected data at the Ruhuha health center where the research team for this study was located. Hence, *isibo* representatives gathered the observations from all volunteers in their respective villages and submitted the observations to the researchers every last Friday of the month. *Isibo* representatives received feedback sent by the researchers, which in turn they shared and discussed with the volunteers among the five selected villages. The process of reporting observations starts with distributing the tools/materials for collecting the citizen science data to *isibo* representatives and ends when volunteers meet and discussed about the feedback from the data reported the previous month. In addition, they also discussed about the actions or measures to be taken.

Frequency of collecting and reporting observations

Initially, the researchers expected that volunteers could report the observations on a bi-weekly basis. However, there was an extensive discussion on the frequency of collecting and reporting the observations because some participants assumed that collection and reporting of observations would take much time (Table 2). Hence, the general consensus was to report the observations once a month. They decided on this based on the fact that *isibo* members meet once a month.

Table 2. Frequency of reporting and collecting mosquitoes suggested by the different discussion groups during the workshops.

Village	**Groups**		
	Men	**Women**	**Youth**
Busasamana village	Once a month		
Kagasera village	Once a month	Once in two weeks	Once a week
Kibaza village	Once a month	Once a month	
Kiyovu village	Once a month	Once a month	Once a month
Mubano village	Once a month	Once a month	Once in two weeks

Note: Because of a limited number of participants for Busasamana, one mixed group was made.

Feedback generation from the reported observations

Participants mentioned that getting feedback on their submitted observations was a precondition for knowing what to improve. In relation to why that feedback should be generated, participants indicated that by getting the results from what was reported, participants could be motivated to consistently use malaria preventive measures. Participants mentioned that this feedback could be given in various ways such as flyers, public talks, workshops, home visits, and SMS text messages. Participants highlighted that once submitting the mosquitoes to the researchers, they will need to know whether the collected mosquitoes were malaria vectors or not. As chosen by the participants, SMS texts could be sent to the i*sibo* and the results could be shared during the *isibo* meetings with the volunteers and during the workshop every three or four months, and via the flyers. Flyers could be distributed during the quarterly workshop as proposed by the participants.

The technical and social choices made based on the citizens' preferences are summarized in Table 3.

Table 3. Key technical and social choices made based on citizens' preferences.

Components	**Preferences/Choices**
Technical Component	
Reporting mosquito nuisance	-Write down the mosquito nuisance level indoor, outdoor, and in general on paper forms and this is done every last Wednesday of the month by the volunteer. -Weighing the costs of using a paper-based form and mobile phone, paper-based form does not cost much. Hence it was preferred for reporting mosquito nuisance.
Collecting mosquitoes	-Mosquitoes are caught with a handmade trap that consists of a plastic bottle filled with yeast and sugar, a torch, and this is done every last Wednesday of the month. -Provision of materials to collect mosquitoes (yeast, sugar, torch, and the trap).
Social component	
Volunteer recruitment	-Everybody that attended the participatory workshop was eligible to be a volunteer. Those who were willing to participate were invited to write their names on the provided sheet.
Who should collect what?	-Volunteers could choose whether to report mosquito nuisance only, collect mosquitoes only, or do both.
Reporting the information	-Volunteers selected the *isibo* representatives who are responsible for gathering the collected information and submit them to the research team. -Volunteers hand in the forms indicating the mosquito nuisance experienced and the mosquitoes caught to the *isibo* representatives during the monthly meeting that takes place in the last week of the month. The *isibo* representatives then have to submit the observations to the researchers at the health center where the researchers are based every last Friday of the month.
Frequency of collecting and reporting the information	-Once a month during the *isibo* meeting.
Feedback generation	-Once a month, researchers provide feedback to the *isibo* representative via SMS. He/she then communicates the feedback to the volunteers when volunteers collect the materials for the next round. Volunteers discuss the feedback and may take measures based on it. For example, if some malaria cases were reported, they discussed why those cases appeared and aimed to reduce the number of cases reported in the next round of reporting by a more consistent use of malaria control measures.

Discussion

Our results indicate that a CSP for malaria control in a rural context in Rwanda is likely to work best if the inputs and insights from citizens are included in the selection of the technical and social components. By using PDWs, this study presents the design process to follow for implementation of a CSP with much attention to co-design principles as key for better implementation of such a programme.

Involving citizens in the co-design process

Involving citizens in the design process of a CSP is of particular importance as it may stimulate other beneficial effects (for example: new knowledge) (Mahajan et al. 2019). This involvement helps participants in making the decision whether to participate or not. Some CSPs that do not engage citizens in the co-design process, have to incentivize participants once they submit the data (Jordan et al. 2017), and these incentives (money in most of the cases) can be a key motivation for them to participate.

The co-design process that was employed in our program provided added advantages beyond normal trainings, because apart from acquiring new knowledge, the participants felt part of the design and were motivated to contribute to both scientific research as well as malaria control with no monetary incentives. The engagement of citizens in the design process may influence the recruitment rate and level of participation in CSPs (Jordan et al. 2017; Worthington et al. 2012). Hence the participatory design workshops proved an important step for the community members to be able to decide whether to participate in a CSP.

When the research team started, it was optimistic about the use of mobile phones as one of the communication channels and for reporting observations. It was clear that the participants did not prefer it as this option presented more challenges than solutions to the problem. Similarly, Beza et al. (2018) also revealed that the price of sending SMS can affect the decision to participate in a project that requires the use of a mobile phone. Consequently, the mobile phones were only used in this study to provide feedback to volunteers via SMS on a monthly basis and this has no cost implications for the receiver of this SMS.

It was clear that when volunteers have different options (for example different mosquito collection tools), they can critically reflect, discuss, and decide what works better for them. Including citizens in the design process promotes critical discussion which may foster further community actions to tackle the problem under study (Mahajan et al. 2019). Communities are different, have different backgrounds, ideas, and they may learn from each other. While some groups indicated that catching mosquitoes is not possible unless collection tools are provided, others proposed some materials including buckets and the use of hands to catch and submit mosquitoes to the research team. This created a new learning synergy needed to implement a CSP.

Why providing feedback to volunteers?

Communicating the key messages that result from what people report is crucial in any CSP (West & Pateman 2016). Keeping volunteers updated about the progress of the project is an important aspect as it increases the interaction between volunteers and researchers, and volunteers can provide feedback on how to improve the project (West & Pateman 2016). In turn, this feedback can retain participants, and hence sustain the project (Rambonnet et al. 2019). As volunteers contribute their time without financial or any other direct benefits, giving feedback is one of the non-monetary incentives that motivates participants (Rambonnet et al. 2019). Different forms of feedback, including automated SMS text to individual volunteers, newsletters and websites, have been used in CSPs (Nov et al. 2014; Wal et al. 2016; West & Pateman 2016). When regular feedback is provided, it enhances opportunities for learning and development for the participants. This, in turn, may strengthen the network among participants and may improve collective practices (Ryan et al. 2018). As reported in this study, feedback provision was also considered during the design process. Participants indicated a wish to have monthly feedback from the reports in a form of SMS text. Additionally, quarterly workshops in order to meet with other volunteers and learn from others, as well as develop leaflets indicating and comparing reports from different villages were also added.

Study limitations and future research

We realize that additional components may be needed, such as well-documented rules and regulations for guiding volunteers and preventing them using other than agreed tools. However, if tight rules are put in place, the withdrawal rate may be higher as volunteers may think that it is too difficult for them if they have to obey too many rules for voluntary work. On the other hand, not putting these rules and regulations in place may lead to misuse or non-use of the agreed trap. Future research will explore the use of the trap, by assessing the quality of the reports that the citizens are submitting.

At the start, the research team was interested in the collection of mosquitoes and reporting of mosquito nuisance, and this limited the generation of information about community preferences regarding what should be observed in the first place. However, researcher motivation is in most cases an important reason to start a CSP (Rambonnet et al. 2019). Still, in our case there was also a clear societal reason for why to start this CSP, i.e., addressing the burden of malaria. The selection of gathering observations of nuisance and mosquitoes was based on the best available knowledge and successful experiences elsewhere (Ingabire et al. 2016; Kampen et al. 2015). In addition, the research team wanted to see whether participants would come up with their own mosquito collection method. Unfortunately, this was not the case. Therefore, the team designed and proposed a trap that could be easily used in low resource settings. The paper-based form also was designed and proposed based on the results of the baseline survey in 2017. In this survey, only 45% of the people in the study area owned a mobile phone, and among these only a small proportion (18%) mentioned that they also use their mobile phone for SMS activities (sending and/or receiving any message). To this end, proposing a paper-based form was a way to overcome this technology-related barrier.

Besides the information gathered by the current CSP, the program may provide other information which may help to design more targeted interventions such as spraying and larval source management. Follow-up studies will determine the spatio-temporal distribution and population dynamics of the collected malaria mosquitoes in relation to malaria transmission risk (Chapter 6) and assess how this will support the current government-led mosquito surveillance programme.

The perceived initial motivations to participate in citizen science may be subject to change over time, and participants presented different motivational factors including a desire to contribute to malaria control, and to gain knowledge and awareness about mosquito species. Thus, future studies should remain exploring ongoing motivations as this is a key determinant for the retention of the participants and thus the sustainability of the program. Although in this study volunteers indicated interest to acquire new knowledge and skills, for further collective decision making it is important to evaluate the CSP by assessing throughout the participation process what people gain while participating, and whether there are some individual and collective actions (for example collective management of mosquito breeding sites) that may result (Asingizwe et al. 2018; Leeuwis et al. 2018).

Conclusion

Considering the possibilities and preferences of citizens prior to the implementation of a CSP for mosquito surveillance is essential for its success. Some technical as well as social changes were made together with volunteers following their preferences and choices. Following involvement of citizens in a co-design process, we arrived at different decisions that we did not always foresee beforehand. For example, involving volunteers in organizing the process of collecting and reporting observations facilitated the decision about how and who should gather the observations, and submit them to the research team. Deciding on the tools to use while reporting data is of importance, because participants know what works for them. Furthermore, this may positively influence the level of participation. The findings also revealed that providing feedback from what people reported is crucial in a CSP. Thus, this study revealed a number of technical and social components that are relevant to making a CSP applicable and feasible in rural areas and/or in locations where internet connectivity is limited. Additionally, a CSP can build capacity and increase knowledge which in turn, may lead to further individual and collective actions for malaria prevention and control.

Acknowledgments

Special thanks to the citizen science volunteers for their collaboration and involvement in this project. We also thank the Ruhuha sector leadership and Ruhuha health center for their regular support and collaboration.

Chapter 5

A handmade trap for malaria mosquito surveillance by citizens in Rwanda

Marilyn Milumbu Murindahabi, Willem Takken, P. Marijn Poortvliet, Arnold J.H. van Vliet,
Emmanuel Hakizimana, Leon Mutesa, Constantianus J.M. Koenraadt

To be submitted

Abstract

For effective sampling of mosquitoes in malaria surveillance programmes, it is essential to include attractive cues in traps. With the aim of implementing a citizen science project on malaria vectors in rural Rwanda, a handmade plastic bottle trap was designed and tested in the laboratory and in the field to determine its effectiveness in capturing adult *Anopheles gambiae,* the main malaria vector*,* and other mosquito species.

Carbon dioxide (CO_2) and light were used as attractive cues. Carbon dioxide was produced by inoculating sugar or molasses with yeast. The light from the torch was emitted from light-emitting diodes (LEDs). Under field conditions in rural Rwanda, three handmade trap designs were compared to CDC light traps in houses.

In the laboratory, no significant differences between traps baited with sugar plus yeast and molasses plus yeast were observed. Adding heat to the trap reduced capture rates of malaria mosquitoes. in the field, the trap baited with yeast-sugar produced CO_2 and light caught the highest number of mosquitoes compared to the traps baited with light alone or CO_2 alone. The number of *An. gambiae* s.l. in the handmade trap with light and CO_2 was about 8-10% of the number caught with a CDC light trap. This suggests that about 10 volunteers with a handmade trap could capture a similar-sized sample of *An. gambiae* as one CDC light trap would collect. In other words, if sufficient volunteers can be recruited in a citizen science project, this could replace the need for instalment of CDC light traps. Based on these findings, the handmade plastic bottle trap baited with sugar fermenting yeast and light represents an option for inclusion in mosquito surveillance activities in a citizen science context in rural areas.

Introduction

Malaria remains a public health concern in many Sub-Saharan African countries including Rwanda. The disease is transmitted by mosquitoes of the genus *Anopheles*. In Rwanda, the most important vectors are *An. gambiae s.s.* and *An. arabiensis*. The country achieved a significant reduction in the burden of malaria through the implementation and scale-up of malaria control interventions from 2005 to 2011 (Karema et al. 2012). However, from 2012 to 2016, the country experienced an eight-fold increase in reported malaria cases (MOH 2017). The increase in malaria incidence has been observed in all 30 districts of the country, thereby putting the entire population at risk of the disease (MOH 2017b; PMI 2018).

Vector control currently forms the most effective way to reduce the spread of mosquito-borne diseases such as malaria. These control programmes include mosquito monitoring with the aim to provide information on mosquito abundance and species composition (WHO 2017a). It thereby enables the assessment of malaria disease risk, and guides vector control in reducing disease transmission and preventing infection (Hakizimana et al. 2018). In Rwanda, mosquito monitoring programmes have been established in twelve sentinel sites across the country (MOH 2017c). However, not all regions of the country benefit from these mosquito monitoring programmes. Factors such as limited funds, limited number of trained entomologists and inaccessibility of some regions, hinder the progress towards malaria elimination. In Rwanda, the main methods used for mosquito collection are pyrethrum spray collection (PSC) and human landing catches (HLC) (Hakizimana et al. 2018; Hoel et al. 2014; Sikaala et al. 2014; Wong et al. 2013). HLC is a collection method based on the use of human volunteers as baits where volunteers collect mosquitoes landing on their exposed legs and feet (Tusting et al. 2014). As such, the method remains ethically disputed (Maliti et al. 2015), although it remains the most effective estimator of biting intensity currently in use (Hakizimana et al. 2018; Hoel et al. 2014; Sikaala et al. 2014; Wong et al. 2013). Therefore, other collection methods are desired, but the feasibility, the cost and the practicability should be considered.

In 2017, the World Health Organization launched the Global Vector Control Response (GVCR) and encouraged countries to employ science and innovation with the aim to bring tangible changes in current vector control programmes (WHO 2017a). The GVCR sets out the guidance needed to make vector control programmes effective, acceptable and sustainable (Rabinovich et al. 2017; WHO 2017a; Wirth et al. 2017). Among innovative approaches, citizen science can provide benefits in terms of capacity building and tracking mosquito populations, as evidenced by a number of recent publications (Bartumeus et al. 2019, 2018; Jordan et al. 2017; Kampen et al. 2015; Palmer et al. 2017; Tyson et al. 2018, Chapter 2). These initiatives engage volunteer citizens, for example in adult mosquito collections using different trapping techniques (Bazin & Williams 2018). These techniques include the capturing of mosquitoes with hands or with containers against walls (Kampen et al. 2015; Walther & Kampen 2017), or include the submission and identification of adult mosquito pictures or even of mosquito sounds recorded by volunteers (Kampen et al. 2015; Mukundarajan et al. 2017; Palmer et al.

2017). Mosquito traps such as BG sentinel or BG Gravid *Aedes* traps have also been used in a citizen science project to collect *Aedes* species (Bazin& Williams. 2018).

Interestingly, citizen science approaches for adult mosquito surveillance, and malaria vectors in particular, have hardly been studied in a rural African context. Factors such as cost, ease of use, portability and the effectiveness of the mosquito collection method are crucial when deciding about the sampling approach to employ in a citizen science project, especially in low resource settings (Bartumeus et al. 2019; Bazin & Williams 2018; Asingizwe et al. 2019). Results of a recent survey based on a participatory approach showed the necessity to provide a simple mosquito sampling tool (Asingizwe et al. 2019, Chapter 4) to capture *Anopheles* mosquitoes in a citizen science context.

Many mosquito sampling tools have been designed on the principle of attraction of mosquitoes towards their hosts. Blood-seeking mosquitoes rely on olfaction, visual and thermal cues to locate and identify their vertebrate hosts on which they feed (Takken & Knols 1999, 2010; Zwiebel & Takken 2004; Raji & DeGennaro 2018; Hawkes et al. 2017; Moon et al. 2015). Odours and light as stimuli have been incorporated in many mosquito sampling tools and used to monitor mosquito populations (Hoel et al. 2014; Lima et al. 2014). In addition, visual stimuli such as dark contrast are used by blood-seeking mosquitoes to spot a host (Raji & DeGennaro 2018; Hawkes et al. 2017; Moon et al. 2015), and thermal sensory information to detect body heat (Hawkes et al. 2017). Carbon dioxide (CO_2) is one of the main olfactory stimuli involved in the orientation of mosquitoes and other insects that feed blood from their hosts (Gillies 1980). All vertebrates produce CO_2 through respiration, and these elevated levels of CO_2 make mosquitoes more responsive towards volatile host odours. The synergistic combination of CO_2 and artificial blends of host odours has been extensively studied for deployment in mosquito traps (Maliti et al. 2015; Mboera et al. 1997; Smallegange et al. 2010; Spitzen et al. 2008; Takken & Knols 1999, Takken & Koenraadt 2013). For field sampling purposes, CO_2 can be produced by fermenting sugar and yeast in water. Hence, sugar-fermenting yeast in a bottle trap has been suggested as a potential cheap and efficient tool for sampling *Anopheles* and other human-biting mosquito species in rural settings (Smallegange et al. 2010).

The objective of the present study was to design and evaluate a low cost, easy-to-use mosquito trap to capture adult mosquitoes, including *An. gambiae* s.l., for mosquito surveillance in rural Rwanda. The goal was to employ this trap in a larger one-year citizen science programme that was co-developed with the local population (Chapter 4) and which ran from November 2018 to October 2019 (Chapter 6). In the laboratory, we first evaluated different designs of the trap that produced CO_2 with different carbon sources (brown sugar and molasses) in combination with baker’s yeast, water and a physical attractant, in this case heat. Later, visual stimuli were added, and trap designs were evaluated under field conditions by comparing them to CDC light traps, which are considered the gold standard sampling method (Asale et al. 2019; Wong et al. 2013).

Materials and methods

The study consisted of a first phase that aimed to determine the most effective CO_2 source for capturing adult *Anopheles* species in the laboratory. The second phase of the experiment was to test the trap with the optimum capture rate under African field conditions in comparison with CDC light traps as the standard method. Prior to its prototyping and testing in the laboratory and in the field, citizens' observations of the trap usability were made during participatory workshops in Ruhuha (Asingizwe et al. 2019, Chapter 4), the site where the trap was deployed for mosquito monitoring using citizen science.

Laboratory experiments

The first series of experiments was conducted at the Laboratory of Entomology of Wageningen University & Research, the Netherlands. A handmade carbon dioxide-baited mosquito trap was made from a 1.5 litre transparent plastic bottle (Figure 1). The top was cut off at about three-quarter height and inverted into the remaining part. The opening was elongated with a piece of black paper as a funnel to prevent mosquitoes from escaping from the trap (Figure 1). One hundred non-fed female *An. coluzzii* were obtained as described by Smallegange et al. (2010) and placed in small release cages. The age of the female mosquitoes used was 8-10 days. Mosquitoes were hydrated with water-moisturized cotton wool on top of the release cage 16 h before the experiments were started.

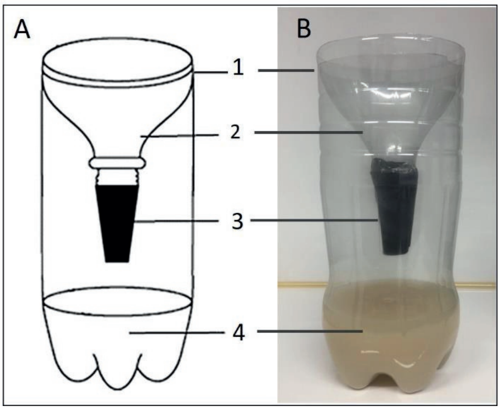


Figure 1. The handmade trap made from a 1.5 L plastic bottle. Panel A: Drawing showing (1) the plastic bottle, (2) funnel part, (3) black paper folded into a funnel which was added to narrow the exit/entry to avoid mosquitoes escaping once inside the trap, and (4) the mixture composed of yeast, sugar and water; Panel B shows a picture of the trap.

Experiments were carried out to determine the attractiveness of traps baited with CO_2 produced by (1) yeast and sugar, (2) yeast, sugar and heat, or (3) yeast and molasses (Table 1). For experiments (1) and (2) the CO_2 was obtained by mixing 6 grams of dry yeast (Dr.

Oetker, The Netherlands), 75 gr of brown sugar (Van Gilse Kristalsuiker, Suiker Unie, The Netherlands) and 750 ml of tap water. For experiment (2), a heat cable was wrapped around the bottle and set at 37 °C to mimic human body temperature to attract the mosquitoes to the trap. For experiment (3), 4.4 gr of dry yeast (Dr. Oetker, The Netherlands) were mixed with 62.5 gr of molasses (Golden Molasses Unsulfured, Sweet Harvested, Natural American Foods™) and 500 ml of tap water (Mweresa et al. 2014). Mixing of sugar or molasses with water and yeast took place 1-1.5 h before mosquitoes were released. No additional stirring was done during the experiments. A Xentra 4100 CO_2 analyzer (Servomex, the Netherlands) was used to check the production of carbon dioxide in each experiment. After measuring the flow rate of CO_2, the trap was placed at the center of a gauze cage (330 X 250 X 233 cm; Howitec Netting BV, The Netherlands) which was situated inside a larger climate-controlled room (22.2 ± 1.6 °C and 52.6 ± 7.8% RH). The experiments were conducted in the last four hours of the dark phase when *An. coluzzii* is normally searching for a host (Haddow & Ssenkubuge 1973; Killeen & Chitnis 2004; Maxwell et al. 1998). The experiments were alternated to rule out day effects. Each experiment was replicated three times (see table 1) except for molasses the treatment was replicated twice. For each replicate, the 100 mosquitoes were released from their release cage placed in the right corner of the screen cage and left overnight (24 h). Afterwards, mosquitoes remaining in the release cage and in the trap were counted.

Table 1. Experiments conducted in the laboratory phase

Experiment	Chemical attractant	Physical attractant
1	Yeast + sugar	-
2	Yeast + sugar	Heat (37 ^{0}C)
3	Yeast + molasses	-

Field experiments

Field experiments were conducted from 24 September to 4 October and from 29 October to 1 November 2018 in Kibaza, Bugesera district, Eastern province, Rwanda. Twelve houses from three village clusters called “isibo” (a cluster of a maximum of 15 households) were selected for mosquito collection (Figure 2). Kibaza village is situated at 1°52'18.0"S and 30°16'11.0"E. The area is a rural, agricultural setting with a traditionally high malaria transmission (Kateera et al. 2015). The study site is characterized by two rainy seasons (March-May and October-December) which alternate with two dry seasons (January-February and June-September). Kibaza village is bordered by one irrigated rice scheme with two annual rice growing cycles and the selected houses were mainly made of mud walls with iron sheet roofs.

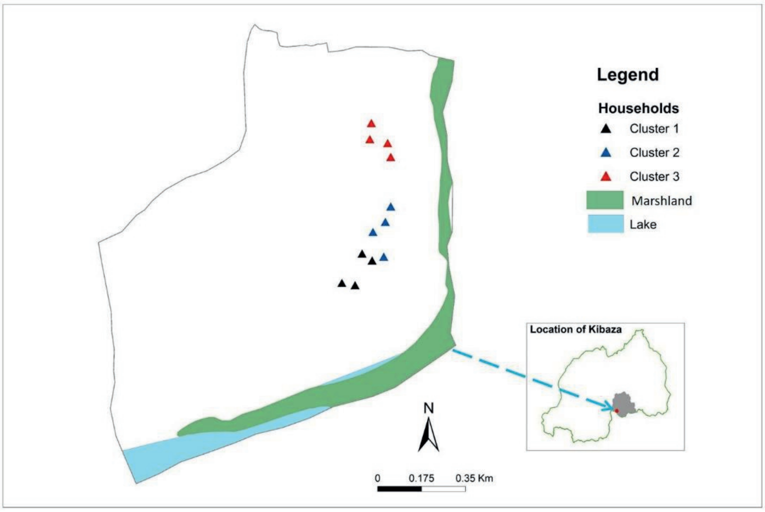


Figure 2. Map of Kibaza showing the three groups of selected houses (in black, blue and red) included in the study. Inset shows Rwanda in green with Bugesera district in grey and the location of Kibaza village indicated with a red dot.

At each house, two handmade carbon dioxide-baited traps were placed. One indoors next to a human sleeping under a bed net, and one outside of the selected house, preferably near the main entrance of the house and positioned against the wall. The traps were placed on the ground, where the opening of the trap was at 9 cm from the ground (Figure 3). For the field experiments, CO_2 was provided by preparing a mixture of 25 g brown sugar, two grams of yeast and 250 mL of water (Laguna-Aguilar et al. 2012; Smallegange et al. 2010)**.** The same design of the trap used in the laboratory experiments was used for the field experiment with the difference that a gauze net was inserted to prevent the mosquitoes from entering the fermenting solution. In addition, the bottle was wrapped with black scotch tape (Figure 3) (Hawkes et al. 2017; Maria et al. 2012; Rosanti et al. 2017). The mixture was prepared at 9:00 am, and the traps were set up in the bedroom of the community members at the foot end of the bed. At least two occupants slept in the bedroom, protected by an ITN. To examine the effect of light on trap catches, light was provided from a torch (Super bright DH-168 light-emitting diodes (LEDs) powered by 2 X Tiger Head R6S AA UM3 1.5 volts batteries (last for ± 12hours). In the experiment with light the torch was suspended 5 cm above the trap entrance. The torch and the trap were operated from 6:00 pm till 6:00 am.

Three experiments using the bottle trap with either only CO_2 produced by sugar-yeast fermentation, with CO_2 in combination with light, or with light alone were evaluated (Table 2). To compare the effectiveness of the handmade trap, CDC light traps were used as a reference. For each house, a CDC light trap was set either indoor or outdoor. Indoor, CDC light traps were set up in the bedroom and hung at the foot end of the bed, with the shield of the trap at 150 cm from the floor (Mboera et al. 1998). Outdoor traps were positioned outside the house in the peridomestic area (outside against the wall at the house entrance). To control for night and location effects, a 4 x 4 Latin square design was used for comparative studies of mosquito traps at each of the three sites (Table 3). All three experiments were carried out over a period of 12 trapping nights (Table 3).

Table 2: Experiments carried out during the field phase

Experiment	Chemical attractant	Physical attractant
A	Yeast + sugar	-
B	-	Light
C	Yeast + sugar	Light

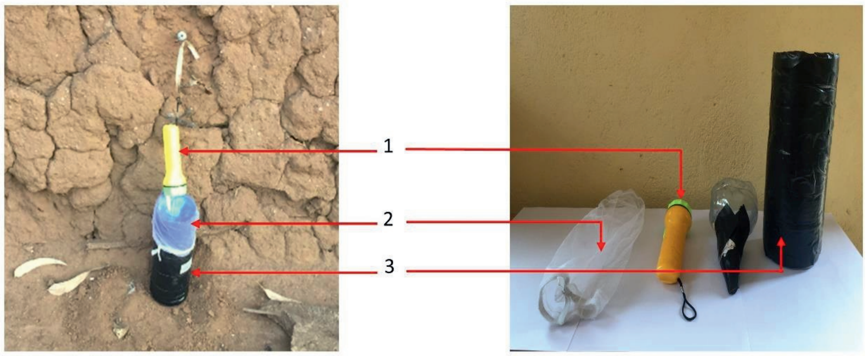


Figure 3. The handmade trap evaluated in the field: (1) torch suspended at 5 centimeters above the trap entrance, (2) gauze net, (3) a ¾ cut plastic bottle wrapped with black scotch.

The traps were set at 6:00 pm and the owner of the room was instructed to switch off the light of the CO_2 or CDC trap and tie the bag connected to the collection cup or the net of the CO_2 traps at 6:00 am the next morning to avoid mosquitoes escaping from the traps. After their collection from the traps, mosquitoes were stored in labelled petri dishes before morphological identification at the laboratory of entomology in Kigali for further analysis.

Mosquito species identification

Mosquitoes collected per trap per house per night from each experiment were kept separately in labelled Petri dishes and were sorted by genus and sex. All female *Anopheles* mosquitoes were morphologically identified to species level using standard morphological identification keys (Gillies & Coetzee 1987) at the central laboratory. Mosquitoes from each house and trap were then pooled in 1.5 ml labelled vials with silica gel and kept for molecular species identification. A random sample of 254 *An. gambiae* s.l. caught indoors and outdoors using CDC light traps and handmade traps were used for DNA extraction and characterization using the Polymerase Chain Reaction method (PCR) (Scott et al. 1993). DNA was extracted only from the heads and thoraxes of the mosquitoes for amplification. For samples that did not show a product on the electrophoresis gel, legs and wings were tested.

Table 3. Mosquito collection scheme for one experimental treatment following a 4x4 Latin square design

Nights	***1***	***2***	***3***	***4***
House 1	Handmade trap indoor	Handmade trap outdoor	CDC light trap indoor	CDC light trap outdoor
House 2	Handmade trap outdoor	Handmade trap indoor	CDC light trap outdoor	CDC light trap indoor
House 3	CDC light trap indoor	CDC light trap outdoor	Handmade trap indoor	Handmade trap outdoor
House 4	CDC light trap outdoor	CDC light trap indoor	Handmade trap outdoor	Handmade trap indoor
Night	**5**	**6**	**7**	**8**
House 5	Handmade trap indoor	Handmade trap outdoor	CDC light trap indoor	CDC light trap outdoor
House 6	Handmade trap outdoor	Handmade trap indoor	CDC light trap outdoor	CDC light trap indoor
House 7	CDC light trap indoor	CDC light trap outdoor	Handmade trap outdoor	Handmade trap indoor
House 8	CDC light trap outdoor	CDC light trap indoor	Handmade trap indoor	Handmade trap outdoor
Night	**9**	**10**	**11**	**12**
House 9	Handmade trap indoor	Handmade trap outdoor	CDC light trap indoor	CDC light trap outdoor
House 10	Handmade trap outdoor	Handmade trap indoor	CDC light trap outdoor	CDC light trap indoor
House 11	CDC light trap indoor	CDC light trap outdoor	Handmade trap indoor	Handmade trap outdoor
House 12	CDC light trap outdoor	CDC light trap indoor	Handmade trap outdoor	Handmade trap indoor

Statistical analysis

For the laboratory experiments, the data collected were analysed using Generalized Linear Model (GLM, binomial, logit link function, dispersion estimated) to test for the differences in proportion of *An. coluzzii* caught for each of the three treatments. The covariates associated with the experimental design (temperature, humidity and CO_2 release rate) were included, but removed from the model when not significant ($P > 0.05$). The post-hoc test that was performed is the Least Significant Differences test (LSD) to identify if any treatment was significantly different from the other treatments.

For the field experiments, the numbers of collected female mosquitoes were analysed using different Generalized Linear Models (GLM with negative binomial with log function, dispersion estimated) to test the differences in capturing effectiveness of different odour baits in combination with or without visual cue (light) using the handmade trap versus the CDC light traps. The main effects tested were the trap type and location (indoor/outdoor). Covariates associated with the experimental design (house, date) were included the model. The model, including (significant) covariates, was used to calculate the incidence rate ratios and their 95% confidence intervals. Catches from traps baited with light and with yeast and sugar were not included in the statistical analyses as the number of mosquitoes was too low. A Chi-square test was computed to compare proportions of *An. arabiensis* and *An. gambiae* s.s. collected with CDC and traps baited with yeast, sugar and light. All statistical analyses were performed using SPSS (Version 25.0, IBM Corporation, New York, USA).

Ethical approval

Written informed consent for the study was obtained from the head of the selected houses for the experiment. The Medical Research Centre of the Rwanda Biomedical Centre approved the research project and the Institutional Review Board (IRB) of the College of Medicine and Health Sciences granted ethical clearance (408/CMHS IRB/2016).

Results

Laboratory experiments

Of the 778 *An. coluzzii* released for all the screen-cage experiments, 123 (15.8%) *An. coluzzii* were caught over a 24-hr period. The mean trap entry response for a trap baited with sugar and yeast was 0.18 ± 0.057, 0.06 ± 0.026 for a trap baited with yeast, sugar and heat, and 0.19 ± 0.053 for a trap baited with molasses and yeast (Figure 4). There was a significant difference among treatments on catches of *An. coluzzii* (GLM; df = 2; *P* = 0.028). The effect of CO_2 release was included as a covariate in the final model, and although not significant (GLM; df = 1; P = 0.192), this gave the model the lowest AIC value and best fit. Our LSD post hoc test showed that the trap baited with molasses and yeast caught significantly higher proportions of *An. coluzzii* than the trap baited with yeast, sugar and heat (*P* = 0.015) (Figure 4). All other comparisons were not significant. For practical purposes (sugar being more readily available than molasses) for further evaluations in the field, our choice favoured the yeast and sugar treatment above the other two treatments. Heat was not included as an attractive cue in the field experiments.

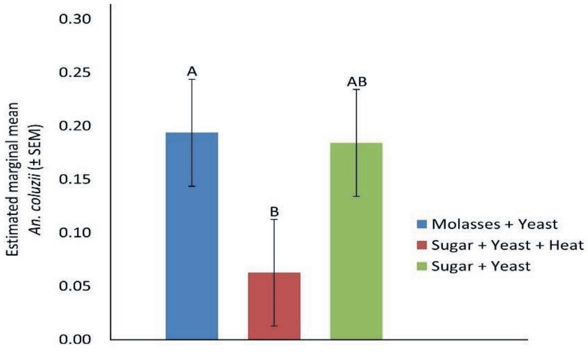


Figure 4. Proportion of *An. coluzzii* caught per treatment, consisting of combinations of yeast, sugar/molasses and heat. Different letters indicate significant differences among treatments (LSD post-hoc test, $P < 0.05$)

Field experiments

Three mosquito genera were identified from collected mosquitoes (Table 4). Almost two-thirds (n=2,196, 69.3%) were *Culex* spp, followed by *Anopheles* (n=855, 27%), and *Mansonia* (n=116, 3.7%). The species collected from the genus *Anopheles* included *An. gambiae* sensu lato (n=742, 86.7%), *An. ziemanni* (n=69, 8%), *An. maculipalpis* (n=39, 4.6%), *An. brohieri* (n= 3, 0.4%), *An. pharoensis* (n=1, 0.12%) and *An. rufipes* (n=1, 0.12%).

Table 4. Numbers of mosquitoes per genus collected in Kibaza. A, B and C in the Trap type column indicate the three different experiments carried out in the field study which covered 12 trapping nights per experiment.

Trap type	**Location**	***Anopheles* spp**	***Culex* spp**	***Mansonia* spp**	**Total**
Sugar-yeast-baited trap (A)	Indoors	1	1	0	2
CDC-LT (A)	Indoors	105	65	10	180
Sugar-Yeast-baited trap (A)	Outdoors	0	1	0	1
CDC-LT (A)	Outdoors	124	143	25	292
Light-baited trap (B)	Indoors	0	1	7	8
CDC-LT (B)	Indoors	67	61	19	147
Light-baited trap (B)	Outdoors	1	2	0	3
CDC-LT (B)	Outdoors	114	401	26	541
Sugar-Yeast-Light-baited trap (C)	Indoors	16	59	3	78
CDC-LT (C)	Indoors	168	189	8	365
Sugar-Yeast-Light-baited trap (C)	Outdoors	22	69	2	93
CDC-LT (C)	Outdoors	237	1204	16	1457
	TOTAL	855	2196	116	3167
	Species composition (%)	27.0	69.3	3.7	

Of the handmade traps, the trap baited with yeast-sugar produced CO_2 and light (Experiment C) had the highest catch (n=171) and collected all three genera. This was followed by the light-baited trap (n=11, experiment B). The trap baited with CO_2 produced by the yeast-sugar mixture (Experiment A) only collected three mosquitoes over 12 collection nights in total (Table 4).

Comparison between handmade and CDC light traps

CDC light traps collected significantly higher numbers of female mosquitoes (Culicidae) and *An. gambiae* s.l. (GLM, $P < 0.001$; Table 5) compared to all three handmade traps baited with sugar and yeast alone (Figure 5), light alone (Figure 6), or with the combination of sugar, yeast and light (Figure 7).

The number of mosquitoes (Culicidae) caught indoors was significantly lower than those collected outdoors for the trials in which we tested light only (GLM, $P = 0.050$), or the combination of sugar, yeast and light (GLM, $P = 0.005$). This was not the case for numbers of *An. gambiae* or for the experiment with sugar and yeast only (Table 5).

All models reported in Table 5 included the random factors house location and collection date. In experiments B and C, these random factors were not significant, but for trial A, collection date was significant in the model for all mosquitoes (GLM, Wald chi-square = 4.320, $P = 0.038$), while house location was significant in the model for *An. gambiae* (GLM, Wald chi-square = 4.477, $P = 0.034$).

When comparing the overlap in 95% confidence intervals of the incidence rate ratio's, we can deduce that traps baited with the combination of sugar, yeast and light performed significantly better in catching mosquitoes (Culicidae) as well as in catching *An. gambiae* s.l. than traps baited with sugar and yeast only or light only (Figure 8).

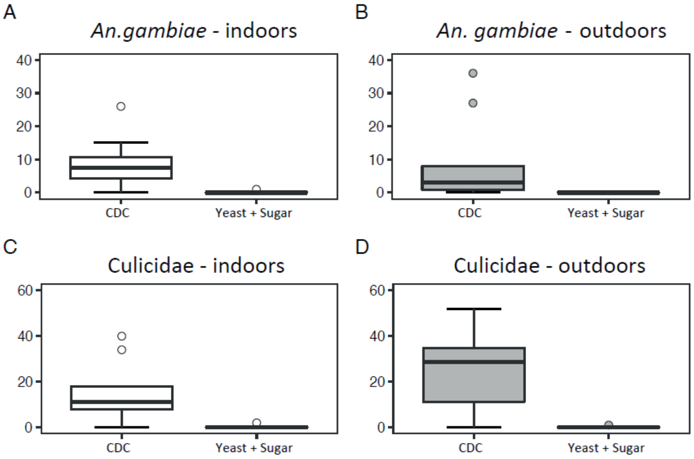


Figure 5. Boxplots showing the number of *Anopheles gambiae* s.l. (A and B) and Culicidae (C and D) collected using CDC light trap (12 trapping nights per treatment) or a trap baited with sugar and yeast (12 trapping nights per treatments) in Ruhuha sector, Rwanda.

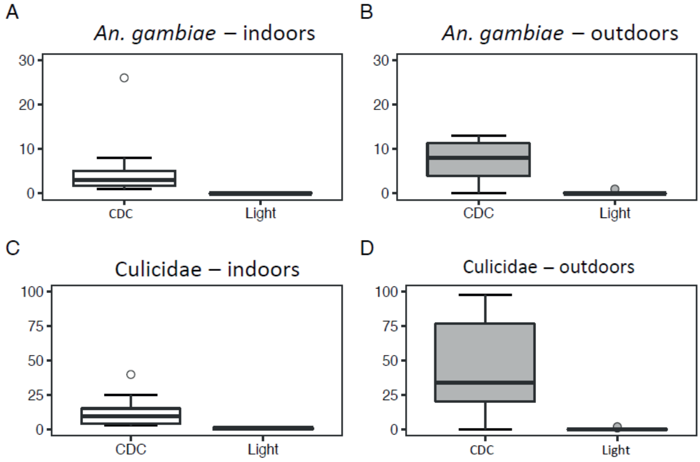


Figure 6. Boxplots showing the number of *Anopheles gambiae* s.l. (A and B) and Culicidae (C and D) collected using CDC light trap (12 trapping nights per treatment) or a trap baited with light (12 trapping nights per treatments) in Ruhuha sector, Rwanda.

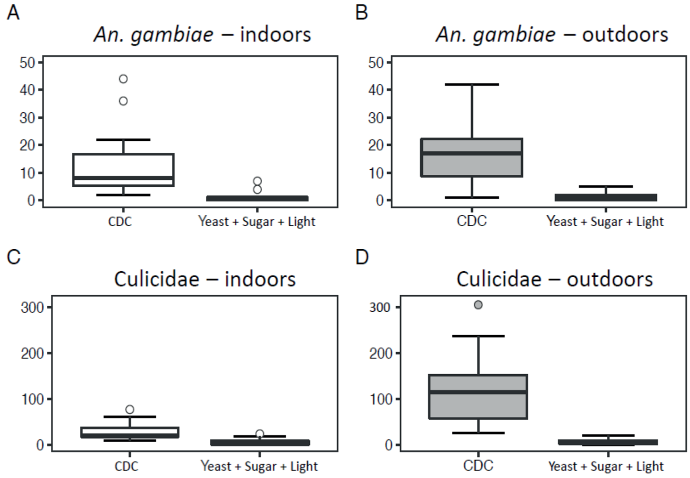


Figure 7. Boxplots showing the number of *Anopheles gambiae* s.l. (A and B) and Culicidae (C and D) collected using CDC light trap (12 trapping nights per treatment) or a trap baited with the combination of yeast, sugar and light (12 trapping nights per treatments) in Ruhuha sector, Rwanda.

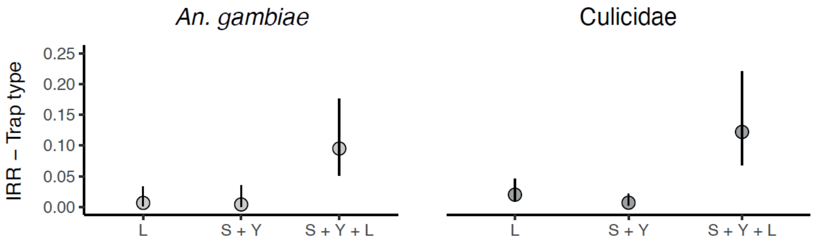


Figure 8. Estimated incidence rate ratio's (IRR, Table 5) and their 95% confidence intervals for the main effect of trap type (handmade trap versus CDC light trap) for the numbers of female *An. gambiae* (left panel) and Culicidae (right panel). L: handmade trap baited with light only, S + Y: handmade trap baited with sugar and yeast, S + Y + L: handmade trap baited with sugar, yeast and light.

Table 5. Mosquito groups as caught per different type of trap and per location.

Experiment	Mosquito group	Trap type and Location	Beta	Exp(B)	95% CI	P
A	Culicidae	Sugar+Yeast	-5,107	0,006	0.002 - 0.020	< 0.001
		CDC-LT	*			
		Indoors	-0,361	0,697	0.340 - 1.429	0,325
		Outdoors	*			
	An. gambiae s.l.	Sugar+Yeast	-5,486	0,004	0.0005 - 0.035	< 0.001
		CDC-LT	*			
		Indoors	0,303	1,354	0.559 - 3.280	0,502
		Outdoors	*			
B	Culicidae	Light	-3,964	0,019	0.008 - 0.044	< 0.001
		CDC-LT	*			
		Indoors	-0,729	0,483	0.233 - 1.001	0,050
		Outdoors	*			
	An. gambiae s.l.	Light	-5,008	0,007	0.001 - 0.033	< 0.001
		CDC-LT	*			
		Indoors	-0,409	0,665	0.338 - 1.307	0,236
		Outdoors	*			
C	Culicidae	Sugar+Yeast+Light	-2,135	0,118	0.066 - 0.213	< 0.001
		CDC-LT	*			
		Indoors	-0,836	0,434	0.241 -0.779	0,005
		Outdoors	*			
	An. gambiae s.l.	Sugar+Yeast+Light	-2,357	0,095	0.051 - 0.176	< 0.001
		CDC-LT	*			
		Indoors	-0,137	0,872	0.479 - 1.587	0,653
		Outdoors	*			

Sibling species identification

Of a random sample of 250 female *An. gambiae* s.l. identified to sibling species by PCR, 4% (11/250) were *An. gambiae s.s.* and 96% (239/250) were *An. arabiensis*. There was no significant difference between the relative proportions of both species collected in CDC traps and in traps baited with yeast, sugar and light (Chi-square = 0.206, *P* = 0.650). Similarly, the relative proportion of both sibling species in indoor and outdoor collections was the same (Chi-square = 2.134, *P* = 0.144).

Discussion

This study showed that CO_2 produced by fermenting baker's yeast with brown sugar alone or when adding thermal and/or visual cues attracts mosquitoes both in a laboratory and/or in a field environment. However, CO_2 alone, without adding any additional cue, caught much fewer mosquitoes in the field. Many studies have demonstrated that sugar-fermenting yeast is a practical source of CO_2 in traps, especially in the field, because industrial CO_2 is not always available and is costly (Hoel et al. 2015; Laguna-Aguilar et al. 2012; Smallegange et al. 2010; Zhou et al. 2018). However, its potential for use in simple, handmade traps for mosquito surveillance in a citizen science context had not been fully explored.

The current study showed that, in the laboratory, adding heat to yeast-produced CO_2 via a heating cable caught surprisingly fewer mosquitoes in the trap. This suggests that heat to mimic human body temperature did not act synergistically with CO_2. These findings disagree with previous research which demonstrated that adding heat to CDC traps baited with CO_2 and octenol resulted in increased trap collections of *Ae. taeniorhynchus*, *An. atropos*, and *Cx. nigripalpus* (Lemire 1995; Zhou et al. 2018). In another study, heat combined with human odour caused mosquitoes to fly in significant numbers to the trap (Spitzen et al. 2013). It is important to note that attraction of a mosquito does not necessarily mean that it will land and be caught in a trap. In our study, the trap baited with molasses (as an alternative sugar source) and yeast captured a similar number of mosquitoes as yeast and sugar. This is in contrast with a study that demonstrated that yeast and brown sugar caught more mosquitoes than dark brown sugar (molasses) (Abdon-liwanag & Tansengco 2015). For this reason and for practical purposes (sugar being more readily available than molasses), the trap baited with yeast and sugar, without the heat, was selected for further evaluation under field conditions. To make the comparison with CDC light traps, light was also included as a stimulus in the field experiments.

Overall, handmade traps baited with yeast, sugar and light, or yeast and sugar, or with light alone caught very few mosquitoes compared to CDC light traps. Although this study investigated the effects of CO_2 produced by the fermentation of brown sugar and dry yeast in capturing adult mosquitoes, cues such as light added to the trap and the visual contrast (black tape around the trap) seemed to play a role in capturing mosquitoes. When comparing the three handmade traps among each other, the trap baited with yeast, sugar and light captured more mosquitoes and more *An. gambiae* s.l.. The same was true for other species such as *Cx. quinquefasciatus*, *Cx. annulioris*, *Mansonia africana* and *M. uniformis.* Thus, malaria vectors and other mosquitoes seem to make use of visual cues in host-seeking despite their nocturnal habit (Hawkes et al. 2017), but here only in combination with CO_2. Interestingly, traps baited with yeast, sugar and light caught significantly more mosquitoes outdoors than indoors, which agrees with previous research (Rosanti et al. 2017). However, traps baited with yeast and sugar did not catch more mosquitoes outdoors and these findings therefore disagree with other studies conducted previously (Abdon-liwanag et al. 2015; Rosanti et al. 2017).

It is clear that CDC traps caught the highest number of mosquitoes, as well as a higher diversity of species. It should be noted that the CDC trap requires a powered fan, whereas the handmade trap is a non-mechanical, passive trap without an active suction mechanism. Interestingly, both indoors and outdoors, the number of *An. gambiae* s.l. in the handmade trap with light and CO_2 was about 8-10% of the number caught with a CDC light trap (16 out of 168 indoor, and 16 out of 197 outdoor; experiment C). This suggests that 10 bottle traps distributed over 10 houses would capture as many mosquitoes including *An. gambiae* as one CDC light trap placed in one house. In other words, if sufficient volunteers can be recruited in a citizen science project using a bottle trap, this could replace the need for instalment of a CDC light trap. In the present study, use of 10 bottle traps required USD $ 25 for one night while operating one CDC light trap was more expensive (more than USD$ 150).

If such citizen science reporting can be linked with digital technology (e.g. reporting observations through a mobile app), rapid assessments of malaria risk can be made with high spatial coverage. Chapter 6 will report on the results of a one-year study in which 116 volunteers participated in the collection of mosquito data.

Of course, the traps can be further developed by evaluating the effectiveness of the handmade trap for *Anopheles* mosquitoes in other malaria endemic areas in Rwanda. It will also be important to standardize the trap by replacing the plastic bottles by a glass bottle, because in the long-term plastics are expected to be banned by the government of Rwanda.

Conclusion

A handmade trap that produced CO_2 by yeast-sugar fermentation attracted and caught mosquitoes, including malaria vectors, in both the laboratory and in the field in Rwanda. Additional visual cues such as a light source and a dark colour increased the attractiveness of the trap for mosquitoes. Although there are limitations with using the handmade trap, which are related to the duration of CO_2 production, the trap presents a cheap option for inclusion in mosquito surveillance activities in a citizen science context in rural areas. The longevity of the batteries and, durability of the torch, and human foot odour collected on socks as an attractant are options to explore and possibly will improve the capture of mosquitoes.

Acknowledgements

The authors sincerely thank the Ruhuha Health Centre, Ruhuha administrative sector, the entomology technicians as well the study participants for their collaboration. We also thank the laboratory of Entomology from The Malaria and Other Parasitic Diseases Division and Wageningen University, The Netherlands for the technical support. The warm appreciation is addressed to Pieter Rouweler, Frans van Aggelen, and Andre J. Gidding for mosquito rearing. Finally, we thank Hans Smid, Julien Rougeot and Jeanine Loonen for their assistance and for the technical support. This work was supported by Interdisciplinary Research and Education Fund (INREF, Wageningen University, The Netherlands).

Chapter 6

Citizen science to evaluate the spatial and temporal dynamics of malaria vectors in relation to environmental risk factors in Ruhuha, Rwanda

Marilyn Milumbu Murindahabi, Arash Hoseni, Corné Vreugdenhil, Arnold J.H. van Vliet, Jackie Umupfasoni, Alphonse Mutabazi, Emmanuel Hakizimana, P. Marijn Poortvliet, Leon Mutesa, Willem Takken, and Constantianus J.M. Koenraadt

To be submitted

Abstract

Malaria remains a public health problem in Rwanda. As part of malaria prevention and control, the distribution and density of malaria mosquitoes requires continuous monitoring. Resources for long-term surveillance of malaria vectors, however, are limited. A one-year citizen science programme for malaria mosquito surveillance was implemented in five villages of the Ruhuha sector in Bugesera district, Rwanda. The aim of the research was to evaluate the value of citizen science in providing insight into potential malaria vector hotspots and other malaria related information, and to determine predictors of malaria vector abundance in a region where routine mosquito monitoring has not been established. In total, 112 volunteer citizens reported monthly data on mosquito nuisance and confirmed malaria cases. Using handmade carbon-dioxide baited traps they collected 3,793 female mosquitoes among which 10.8% were anophelines. For the entire period, 16% of the volunteers reported having at least one confirmed malaria case per month, but this varied by village and month. During the study year 66% of the households reported at least one malaria case. From a sector perspective, a higher mosquito and malaria vector abundance was observed in the two villages in the south of the study area. The findings revealed significant positive correlations among nuisance reported and confirmed malaria cases, as well as between total number of Culicidae and confirmed malaria cases, but not between the number of the malaria vector *An. gambiae* and malaria cases. At the sector level, of thirteen geographical risk factors considered for inclusion in a multiple regression, distance to the river network and elevation played a significant role in explaining mosquito (adjusted $R^2 = 57\%$) and malaria mosquito abundance (adjusted $R^2 = 28\%$). The study demonstrates that a citizen science approach can contribute to mosquito monitoring, and can identify areas that, in view of limited resources for control, are at higher risk of malaria.

Introduction

Malaria is a major public health concern in Rwanda, and a leading cause of morbidity and mortality (MOH 2019). Despite the progress made in reducing the malaria burden over the last decades (Karema et al. 2012), the country experienced an upsurge of malaria since 2012, putting the entire population, including an estimated 443,000 pregnant women per year and 1.8 million children less than five years old, at risk of malaria (PMI 2019). This increase of malaria cases was especially observed in the East and the South provinces of the country. According to the Health Management Information System of the Malaria and Other Parasitic Disease Division (MOPDD - HMIS), these two provinces accounted for 79% of the disease burden (PMI 2019).

Regardless of the malaria resurgence, Rwanda has made progress in vector monitoring by establishing 12 entomological sentinel sites that are involved in the surveillance of malaria vectors across the country (MOH 2013). This programme has given insight in the mosquito diversity, malaria vector and non-vector distribution and insecticide resistance status, as well as in entomological inoculation rates as a measure of transmission intensity (Hakizimana et al. 2018). Vector surveillance implies continuous monitoring of malaria mosquitoes (WHO 2017a). This involves long-term sustained funding and trained entomologists, and also the physical infrastructure to accomplish such activities (Fouet & Kamdem 2018; WHO 2017a, 2019). These activities receive external funding (70% from the Global Fund, President's Malaria Initiative, and End Malaria Fund). Funds required to extend vector surveillance to regions other than the 12 sentinel sites are not available (MOH 2013, 2019). Importantly, effective surveillance requires locality-specific information on the diversity, and the spatial and temporal distribution of malaria vectors. This information helps to inform decision-making for a response before outbreaks occur (Hay et al. 2010; Zahar 1984). Mostly, malaria prevention and control exist of early diagnosis and treatment and vector control by the use of insecticide-treated bed nets (ITNs) and indoor residual spraying (IRS) (WHO 2019). The effectiveness of vector control is increased by accurate identification of the malaria vector population at the local level. Not identifying malaria vector hotspots can cause malaria prevention and control to fail (Carter et al. 2000; Sinka et al. 2012).

Several citizen science initiatives have demonstrated that citizens or community members can contribute to the monitoring of disease-carrying mosquitoes (Bartumeus et al. 2019, 2018; Eritja et al. 2019; Jordan et al. 2017; Kampen et al. 2015; Oltra et al. 2016; Palmer et al. 2017; Vogels et al. 2015). Innovation in malaria mosquito surveillance is focusing on how local communities and stakeholders, especially in a rural African context, can participate in citizen science with the aim of ensuring implementation and sustainability. Citizen science programmes represent a unique opportunity to develop a new way to involve the public in the design, implementation, and evaluation of such vector surveillance programmes (Bartumeus et al. 2019).

The main goal of the current study was to evaluate the value of a citizen science programme in providing insight into potential malaria vector hotspots and other malaria related information, and to determine predictors of malaria vector abundance in a region where routine mosquito monitoring has not been established. For this purpose, the following two research questions were formulated: 1) what are the spatial and temporal dynamics in and correlations among (malaria) mosquito abundance, perceived mosquito nuisance and proportion of households reporting confirmed malaria cases in the study area and 2) what are the environmental drivers explaining the spatial and temporal distribution of the malaria vector *Anopheles gambiae* s.l. and other mosquitoes? The outcomes from this study will help to better understand malaria transmission dynamics in the study area based on citizen science data.

Materials and methods

Study area

The study was conducted in five selected villages of the Ruhuha sector (Figure 1) in the Bugesera district, of the Eastern province of Rwanda. The Ruhuha sector is composed of 35 villages grouped into five cells. The area covers 54 km^2 and is located 42 kilometers south from the capital Kigali (Kateera et al. 2015). The elevation varies from 1,300 m to 1,573 m above sea level. It is surrounded by lowland marshes and water streams draining into the Akagera river system, and is separated from Burundi by Lake Cyohoha in the south (Kateera et al. 2015). The sector has an estimated population of 24,000 people, living in approximately 5,000 households (Ingabire et al. 2017). Ruhuha has a predominantly rural agricultural setting and is known as a historical malaria endemic area (Kateera et al. 2015). Ruhuha experiences two malaria transmission peaks associated with the rainy seasons observed generally from October to November and March to May (Kateera 2016).

Study design

Recruitment of the participants and distribution of materials and tools

Volunteer participants were recruited through workshops conducted three months prior to the implementation of the citizen science programme for malaria mosquito surveillance (Asingizwe et al. 2019, Chapter 4). The list of volunteers consisted of the names of the participants and their contact details. Volunteers could indicate in which research activities they wanted to participate. This included filling forms with malaria related information and/or collecting mosquitoes. The citizen science programme kicked off with a launch event on 22 November 2018 in Ruhuha sector with participants from five selected villages of Ruhuha: Busasamana, Kagasera, Kibaza, Kiyovu, and Mubano (Figure 1). Based on their preference, volunteers were requested to report on a monthly basis the experienced mosquito nuisance and the number of malaria cases in their household, and to collect mosquitoes in their environment (Figure 2).

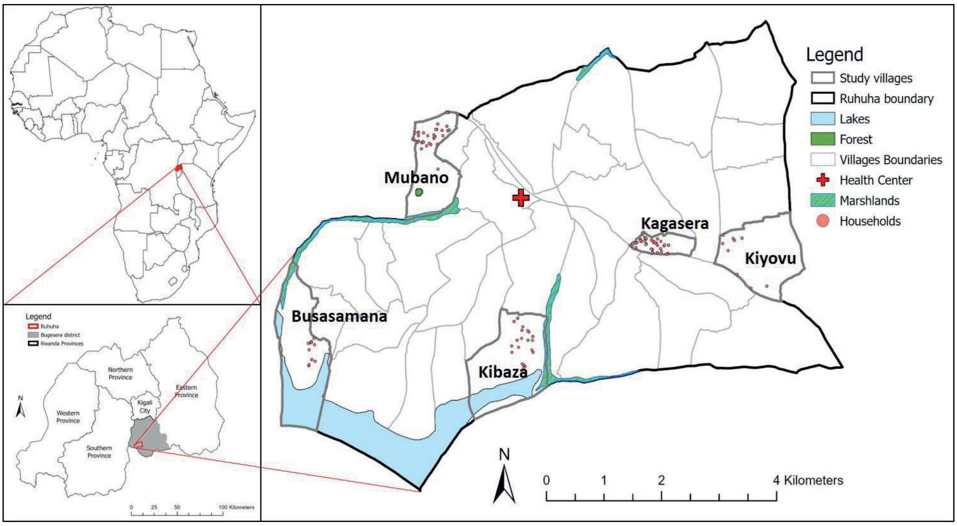


Figure 1. Map showing Ruhuha sector with the five selected villages where the citizen science programme was implemented (Busasamana, Kagasera, Kibaza, Kiyovu and Mubano). The pink dots represent the locations of the households (volunteers) from where observations were reported.

To facilitate data collection, volunteers were grouped into groups of households (called *isibo*) close to each other. Each *isibo* consisted of a few households (3 to 10). A phone call was made by the researcher to the *isibo* leaders one or two days prior to data collection to remind them of their tasks and to collect the data properly. After four consecutive rounds of data collection, a dissemination workshop was organized to share the results from the data collected previously by the volunteers, and to motivate them to continue their active participation. Additionally, researchers could also check whether the correct procedures were followed in terms of filling the requested information on the paper forms or when and how to capture and conserve mosquitoes.

During the launch workshop, the data collection and reporting schemes of the observations were determined by the researchers in consultation with the volunteers. Paper forms, handmade traps, ingredients for production of carbon dioxide (sugar and yeast) for baiting the traps, batteries and torches were distributed during the launch workshop. These materials were used for the duration of the study except for some materials that needed to be replenished such as sugar, yeast, forms, and batteries. These were distributed monthly every last Friday by the *isibo* leader and distributed to the participants (volunteers) three days prior to the data collection during the monthly *isibo* meeting. Instructions on how to fill the forms and how to set up the traps as well as how to label the containers containing the mosquitoes and to store all the data collected, were given during the workshop. In addition, the *isibo* leaders, who represented the volunteers enrolled for the study in each selected village, were elected during the launch workshop as field data collectors. They were asked to assemble the data collected by the volunteers, and to submit these data to the researchers at Ruhuha health center every last Friday of the month. *Isibo* leaders submitted the observations at the health center three weeks prior to the next date of data collection. They also submitted a short report summarizing the data collected for the month and the challenges faced by the volunteers.

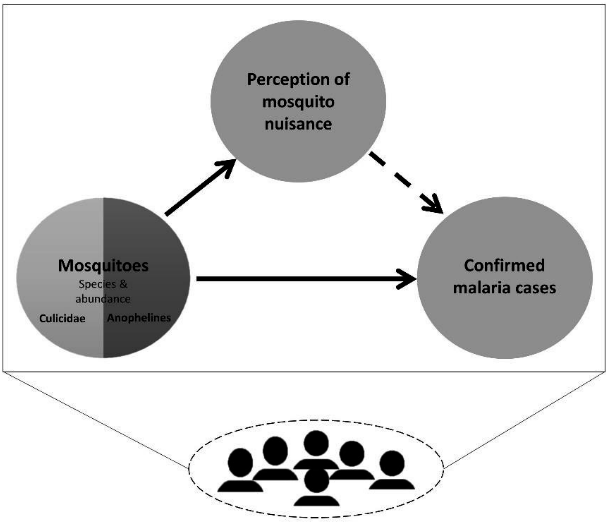


Figure 2. Schematic diagram showing the relations among nuisance, mosquito species and abundance and malaria cases, and the role of citizens in reporting data on these variables.

Data collection

Mosquito nuisance and confirmed malaria cases

The paper forms included two questions. One addressed the level of mosquito nuisance the participants perceived in their environment (indoors only, outdoors only and overall). The second question addressed whether participants had a confirmed malaria case in their household (diagnosed at the health center using a blood sample) within the two weeks prior to the date of data collection. Next to these two questions, participants included the date, and personal information on the forms.

Mosquito collection and laboratory processing

In each household, mosquitoes were collected by placing two handmade traps that were baited with CO_2 and a torch. The torch was hung approximately at 5 centimeters above the opening of the trap (see Chapter 5). One trap was placed indoors in the bedroom on the floor next to a human sleeping under a bed net, at the foot end of the bed. Another trap was placed outside of the house of the volunteers, preferably near the main entrance of the house and positioned on the ground against the wall. Carbon dioxide was provided by mixing 25 g brown sugar, two grams of yeast and 250 mL of water at 9:00 am. In each trap, a gauze net was inserted to prevent mosquitoes from drowning in the sugar-yeast solution. The bottle was wrapped with a black scotch tape (see also Chapter 5).

The next day, mosquitoes were transferred to a Petri dish, labelled with the collection date and time, and name of the volunteer. The observations were submitted to the *isibo* leader who gathered all the forms and mosquitoes and who submitted them later to the researcher at the Ruhuha health centre.

All mosquitoes were transported to the Mareba health center and identified to species level using standard taxonomic keys (Gillies & Coetzee 1987; Service 1993). Mosquitoes were also scored as fed or unfed, then pooled per study site and stored in Eppendorf tubes with silica gel for transportation to the national laboratory in Kigali for further molecular analysis. The head and thorax of each individual female *An. gambiae* s.l. was used to determine the presence of circumsporozoite protein (CSP) of *Plasmodium falciparum* using Enzyme-Linked Immunosorbent Assay (ELISA) techniques (Wirtz et al. 1987). The ELISA results were read visually (Beier et al. 1988). Additionally, 10% of the total *An. gambiae* s.l. collected were used for sibling species identification by Polymerase Chain Reaction (PCR) using the head and thorax of *Anopheles gambiae* s.l. (Scott et al. 1993). After DNA extraction, one microliter of the DNA sample was used as the template for PCR amplification. Each amplified sample was run in a 2.5% agarose gel and visualized by a UV transilluminator.

Remotely sensed environmental data

To investigate whether publicly available data on geographic features within the sector could explain the observed patterns in mosquito abundance, we first selected variables based on a review of relevant literature (Table 1). Geographical data were obtained from the best available sources including the Shuttle Radar Topography Mission (SRTM) that presents a 30 meter Digital Elevation Model (DEM) and Sentinel 2 L2A available on Sentinel hub. In addition to the geographical (satellite derived) data, the demographic variable population density was obtained from a dataset available at a resolution of 3 arc (100 meters at the equator) from WorldPop (2020).

Data analysis

All collected citizen science data were aggregated in Microsoft Excel, and included a unique code for each of the volunteers, as well as their location (latitude and longitude of the house of the volunteer), the collection date, the mosquito species (indoors and outdoors) and feeding status, the presence/absence of confirmed malaria cases in the two weeks prior to data collection, and the perceived mosquito nuisance expressed on a five-point Likert scale (from ‘no nuisance’ (0) to ‘very much nuisance’ (5)).

ArcGIS pro 2.4 (ESRI, Redlands, CA) was used to compile geographic data and create maps of the study area. Locations of the selected households were used to make an interpolation of mosquito abundance using the Inverse Distance Weighting (IDW) method (Garnero & Godone 2013).

Means, and proportions of confirmed malaria cases or perceived mosquito nuisance reported as well as mosquito species compositions were calculated. Spearman correlation tests were calculated using SPSS (Version 25.0, IBM Corporation, New York, USA) to determine the relationships among mosquito nuisance experienced, confirmed malaria cases reported and the number of mosquitoes (Culicidae) or number of *An. gambiae* s.l. collected (Figure 2). For all analysis, the results from the indoor and outdoor traps were summed per household. Calculations of these correlations were made at different levels of aggregation: with raw data for individual households, resulting in approximately 1,344 data points (i.e. 112 households x 12 months), or with village averages, resulting in 60 data points (i.e. 5 villages x 12 months). In addition, to investigate the variability/consistency of correlations among villages, we calculated correlations separately for each village, using the raw data at household level.

Environmental factors (Table 1) were selected to evaluate their impact on the abundance of mosquitoes (Culicidae) and *An. gambiae* s.l. as collected via our citizen science approach in the study area. To do so, Pearson correlations and multiple linear regressions were conducted to explore the relationship between mosquito abundance and selected environmental variables. The results were analysed at the sector level (all sampling points in the Ruhuha area). For the multiple regression analyses, we made use of the *leaps* package in R3.5, which uses an iterative process for finding one or more 'best subsets' of the explanatory variables (Sestelo et al. 2016). To visually inspect cross-correlations among the thirteen variables, we used principal component analysis (PCA).

Ethics statement

Informed consent was obtained from adult participants (volunteers) before the study was carried out. All information was processed anonymously. The Rwanda Institutional Review Board of the College of Medicine and Health Sciences (408/CMHS IRB/2016) and Rwandan Biomedical Center granted ethical clearance for the study. Furthermore, permission was obtained from CARITAS and Bugesera District office prior to conducting the research.

Table 1. Overview of the thirteen environmental variables selected for this study.

Variable	Source	Spatial resolution	Temporal resolution	Authors and year of publication
Elevation	Shuttle Radar Topography Mission (SRTM)	30 meters	-	(Zhou et al. 2007)
Slope	SRTM	30 meters	-	(Moss et al. 2011)
Aspect - sines	SRTM	30 meters	-	(Moss et al. 2011)
Aspect - cosines	SRTM	30 meters	-	(Moss et al. 2011)
Flow accumulation	SRTM	30 meters	-	(Cleckner et al. 2011)
Distance to marshlands	World Agroforestry Centre (ICRAF)	Shapefile	-	(Kursah 2017)
Distance to river networks	SRTM	30 meters	-	(Barták 2010)
Distance to open water bodies	Landviewer, Sentinel 2 L2A	10 meters	-	(Kursah 2017)
Topographic Position Index (TPI)	SRTM	30 meters	-	(Cleckner et al. 2011)
Topographic Wetness Index (TWI)	SRTM	30 meters	-	(Cleckner et al. 2011)
Normalised Difference Water Index (NDWI)	Landviewer, Sentinel 2 L2A	10 meters	-	(Kursah 2017)
Normalised Difference Vegetation Index (NDVI)	Landviewer, Sentinel 2 L2A	10 meters	27th of February 2019	(McFeeters 2013)
		10 meters	15th of September 2019	
Population density	Worldpop	100 meters	-	(Kursah 2017)

Results

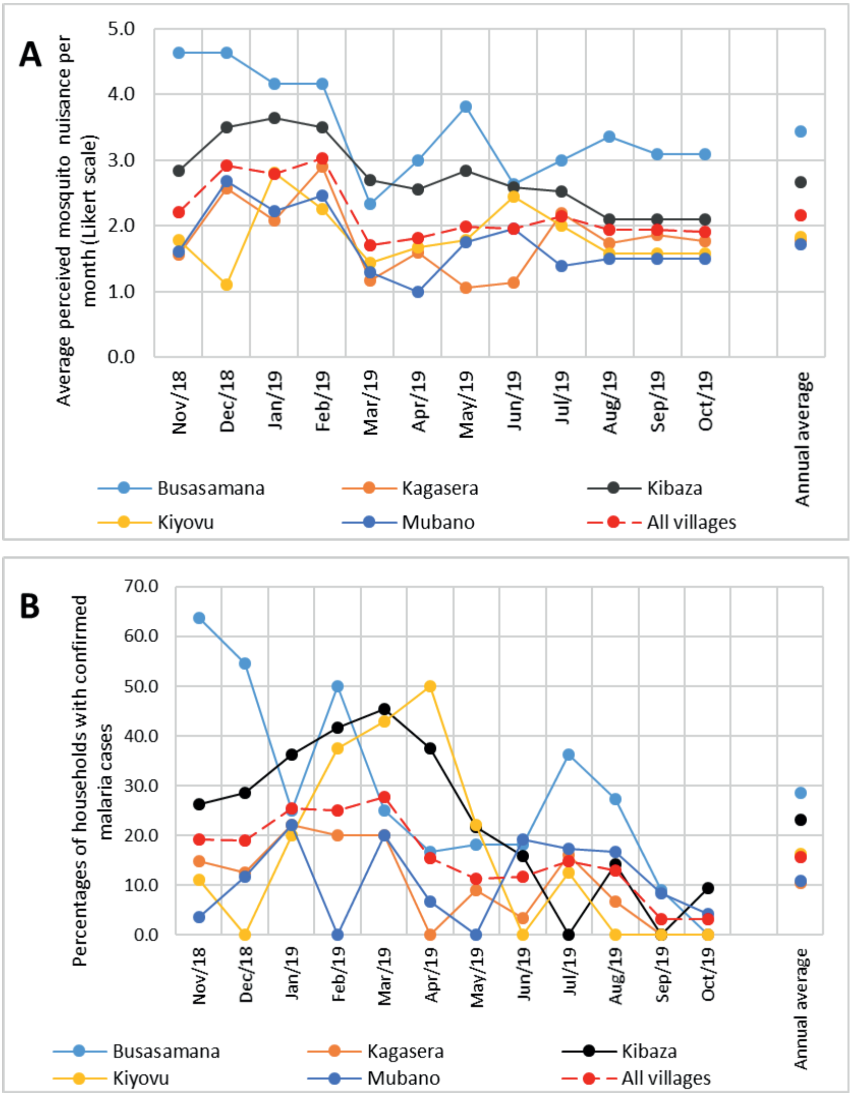
Overall, 112 volunteers participated actively in the current study by reporting mosquito nuisance perceived and confirmed malaria cases, and by submitting mosquitoes for a period of 12 months. Participation varied from village to village, and included 12 volunteers from Busasamana, 35 from Kagasera, 24 from Kibaza, 10 from Kiyovu, and 35 from Mubano (Figure 1). Data collected by two volunteers who moved away from the selected villages during the study were excluded in the analyses.

Perceived mosquito nuisance

At village level, there was a clear spatial and temporal variation in the perception of mosquito nuisance. The highest average (± standard deviation) perceived mosquito nuisance for the whole period was reported from Busasamana (3.4 ± 0.5), followed by Kibaza (2.7 ± 0.1), both in the south of the Ruhuha sector. Volunteers from Kagasera and Kiyovu in the western part of the sector experienced little nuisance (1.8 ± 0.4) followed by volunteers from Mubano in the north (1.8 ± 0.2). At a temporal scale, the highest average mosquito nuisance scores were reported in December (2.9 ± 1.3), January (3.0 ± 0.9), and February (3.1 ± 0.8) 2019 (Figure 3-A).

Confirmed malaria cases

Over the entire one-year study period, every month on average 16% of the volunteers reported having at least one confirmed malaria case in the two weeks prior to sampling. However, 66% of the households reported at least one confirmed malaria case in their household throughout the study period. The highest average monthly percentage of households over the entire year having a confirmed malaria case was reported in Busasamana (28.7%), followed by Kibaza (23.1%), Kiyovu (16.3%), Mubano (14%), and Kagasera (10%). Over the year of the study, the month with the highest percentage of households reporting having at least one confirmed malaria case was March (27.7%), followed by January (25.5%) and February (25%). The months with the lowest percentage of households having a confirmed malaria case were September and October 2019 with 3.2% each (Figure 3-B).


A
Average perceived mosquito nuisance per month (Likert scale)
5.0
4.0
3.0
2.0
1.0
0.0
Nov/18
Dec/18
Jan/19
Feb/19
Mar/19
Apr/19
May/19
Jun/19
Jul/19
Aug/19
Sep/19
Oct/19
Annual average
Busasamana
Kagasera
Kibaza
Kiyovu
Mubano
All villages
B
Percentages of households with confirmed malaria cases
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Nov/18
Dec/18
Jan/19
Feb/19
Mar/19
Apr/19
May/19
Jun/19
Jul/19
Aug/19
Sep/19
Oct/19
Annual average
Busasamana
Kagasera
Kibaza
Kiyovu
Mubano
All villages

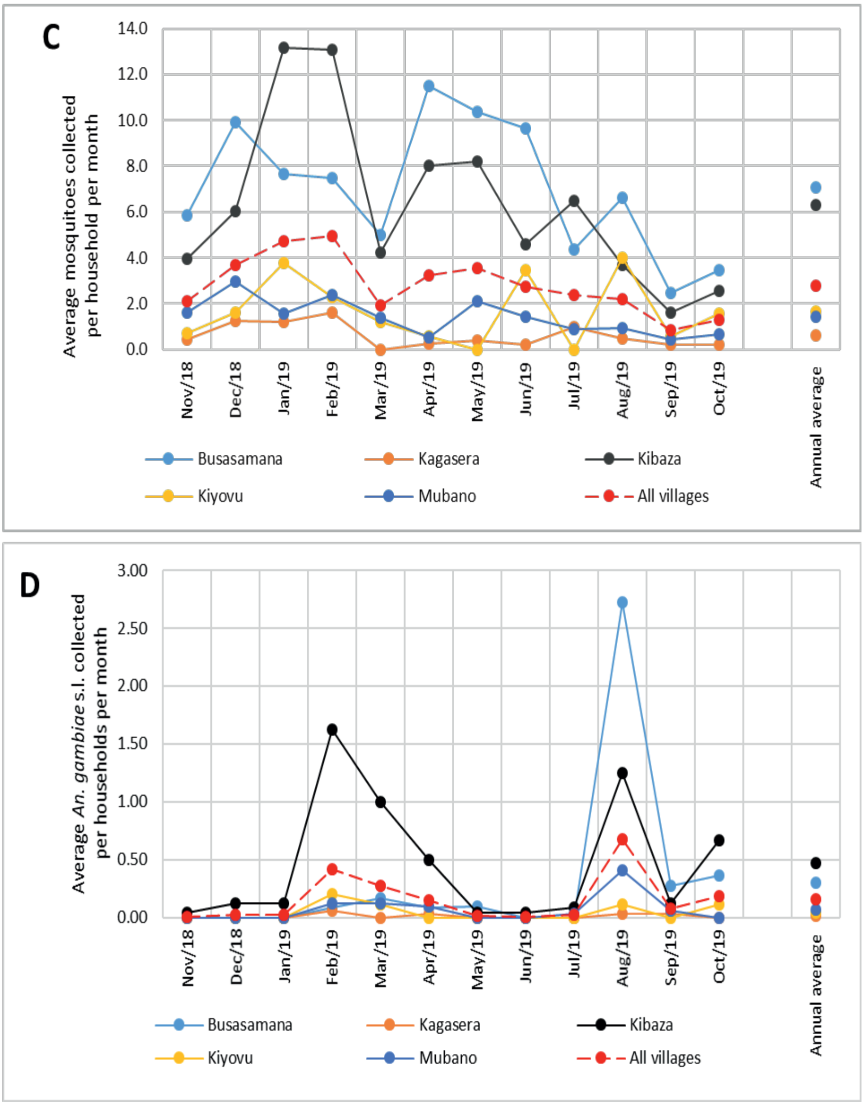


Figure 3. Spatial and temporal distribution of (A) average perceived mosquito nuisance, (B) proportion of households reporting at least one confirmed malaria case, (C) average number of mosquitoes (all species) per month, and (D) average number of *An. gambiae* s.l. per month reported in the five selected villages of Ruhuha sector, Rwanda.

Mosquito species composition and molecular identification of members of the An. gambiae complex

A total of 3,793 female mosquitoes were collected in the five selected villages using a handmade carbon dioxide baited trap over a period of one year. Of these, 51.7% (n = 1,964) were collected indoors and 48.2% (n = 1,829) were collected outdoors. These mosquitoes belonged to four genera and 10 species were identified. Of all mosquitoes, 89.4% (n = 3,390) was morphologically identified as culicine and 10.6% (n = 403) as anopheline (Table 2). Among female anopheline mosquitoes collected, 90.8% (n = 366) were unfed and 9.2% (n = 37) were fed. Of the *Anopheles* species, 49.6% (n = 200) were collected indoors and 50.4% (n = 203) were collected outdoors. Of the total culicines, 76.6% (n = 2,905) were *Culex* species, with *Culex quinquefasciatus* (74%) as the most abundant *Culex* species, followed by *Mansonia* (11.4%) and *Coquillettidia* species (1.3%).

Table 2. Species composition of mosquitoes collected during the citizen science programme in five villages in Ruhuha sector, Rwanda, November 2018 – October 2019

Village name	**Busasamana**	**Kagasera**	**Kibaza**	**Kiyovu**	**Mubano**	**Total**	**% Species composition**
An. gambiae s.l.	42	5	135	5	28	215	5.7
An. maculipalpis	0	0	5	0	3	8	0.2
An. pharoensis	4	0	12	0	1	17	0.4
An. squamosus	0	0	0	0	1	1	0.0
An. ziemanni	26	1	131	2	2	162	4.3
Total *Anopheles* spp	**72**	**6**	**283**	**7**	**35**	**403**	**10.6**
Coquillettidia spp	15	16	15	2	3	51	1.3
Culex spp	732	213	1,329	155	476	2,905	76.6
Mansonia spp	156	14	183	22	59	434	11.4
Total Culicinae	**903**	**243**	**1,527**	**179**	**538**	**3,390**	**89.4**
Total Culicidae	**975**	**249**	**1,810**	**186**	**573**	**3,793**	**100**
% *Anopheles* spp	17.9	1.5	70.2	1.7	8.7		
% Culicidae	25.7	6.6	47.7	4.9	15.1		

Of the *Anopheles* species, 53.3% were *An. gambiae* s.l. (n = 215), 40.2% were *An. ziemanni* Grunberg, and the other species were *An. pharoensis* Theobald (4.2%, n = 17), *An.*

maculipalpis Giles (2%; n = 8) and *An. squamosus* Theobald (0.2%; n = 1) (Table 2). Volunteers in Kibaza collected the highest proportion of *Anopheles* species (70.2%), followed by Busasamana (17.9%), Mubano (8.7%), Kiyovu (1.7%) and Kagasera (1.5%) (Table 2). Kibaza had the highest proportion of *An. gambiae s.l.* (33.5%), followed by Busasamana (10.4%), Mubano (6.9%), Kiyovu (1.2%) and Kagasera (1.2%).

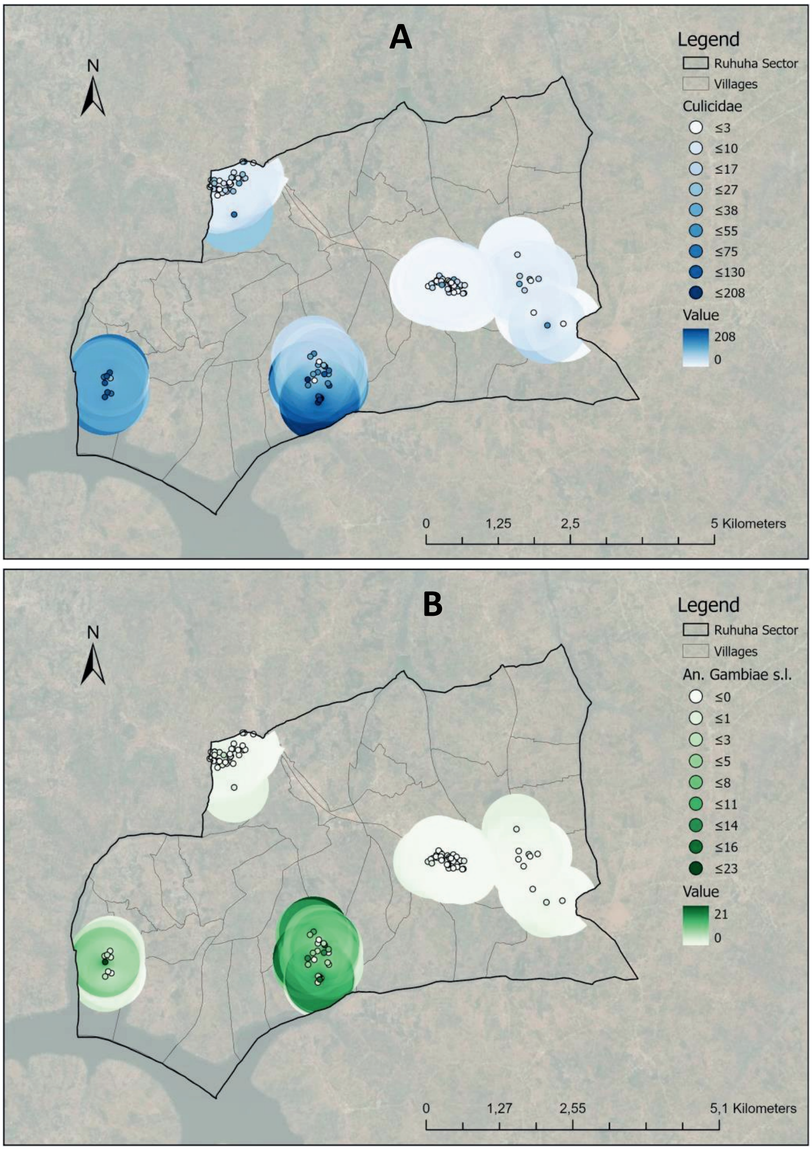


Figure 4. Map of total Culicidae collected (A) and total *An. gambiae* s.l. collected (B) between November 2018 and October 2019 in the Ruhuha sector, Rwanda. Intensity of colouring represents estimated abundance based on inverse distance weighting as interpolation method.

At sector level, relatively more mosquitoes were collected in the south of Ruhuha than in the north. Especially in Busasamana and Kibaza, the villages that also reported the highest nuisance levels, more Culicidae and *An. gambiae* s.l. were collected in comparison with the three other villages (Figure 4).

At sector level, the average (± standard deviation) number of mosquitoes (Culicidae) collected per household per month was 2.8 (± 1.2). Busasamana had the highest average number of mosquitoes (7.1 ± 6.3) per household per month, followed by Kibaza (6.3 ± 9.3). The lowest catch of mosquitoes was recorded in Kagasera (0.6 ± 1.6) followed by Kiyovu with (1.6 ± 3.0), mosquitoes and Mubano (1.4 ± 2.7) (Figure 3-C). For *An. gambiae* s.l., volunteers from Kibaza had the highest average of *An. gambiae* s.l. (0.47 ± 1.5) per household per month followed by Busasamana (0.30 ± 1.6), Mubano (0.07 ± 0.4), Kiyovu (0.04 ± 0.2) and Kagasera (0.01 ± 0.1) (Figure 3-D). At a temporal scale, most mosquitoes (Culicidae) were caught in January and February 2019 while the lowest numbers were caught in September and October 2019 (Figure 3-C). Both the months February and August 2019 had a peak in the number of *An. gambiae* s.l. in comparison with other months (Figure 3-D), and the number of *An. gambiae* s.l. dropped from March to May.

Sporozoite rates and molecular identification of members of the **An. gambiae** *complex*

Of 403 female *An. gambiae* tested, none tested positive for *P. falciparum* sporozoites. Of the 10% (41 out of 403) of *An. gambiae* s.l. tested, 63% (26/41) were *An. arabiensis*, 32% (13/41) were *An. gambiae* s.s. and 7% (3/41) did not yield a PCR product.

Correlation between number of mosquitoes collected and mosquito nuisance reported

Based on all data from the entire sampling period, there was a moderate, positive correlation between perceived mosquito nuisance reported per household per month and the number of mosquitoes (Culicidae) per household per month ($r_s = 0.459$; $P < 0.0001$; Figure 5-A) and a weak, positive correlation between nuisance and number of *An. gambiae* s.l. per household per month ($r_s = 0.121$; $P < 0.0001$; Figure 5-B). It should be noted that, in case of *An. gambiae* s.l. collections, only 7.2% of the collections contained one or more individuals in the trap (Figure 5-B), whereas this proportion was 42.5% in case of total Culicidae.

Interestingly, when the same data were aggregated and averaged by village, there was a strong correlation between the average nuisance level and average number of mosquitoes per month per village ($r_s = 0.798$; $P < 0.0001$; Figure 6-A). In other words, the average perceived mosquito nuisance level could be explained by the average number of mosquitoes collected. However, there was no significant correlation between the average nuisance level and the number of *An. gambiae* s.l. ($r_s = 0.225$; P = 0.084; Figure 6-B).

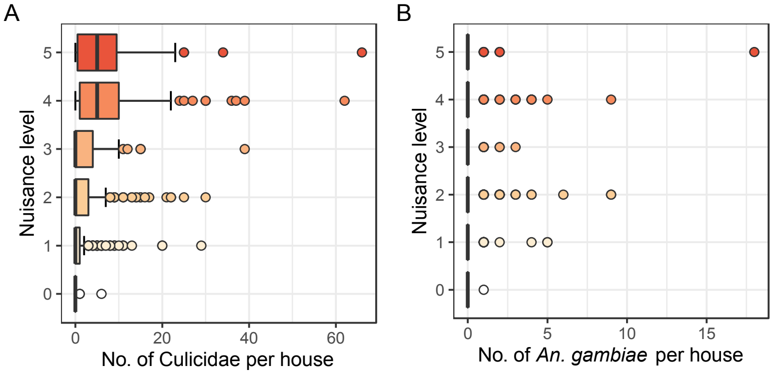


Figure 5. Boxplots showing the correlation between perceived mosquito nuisance experienced per household per month with the total number of mosquitoes (Culicidae) (A) or *An. gambiae* s.l. (B) collected per household per month in five selected villages in Ruhuha sector, Rwanda.

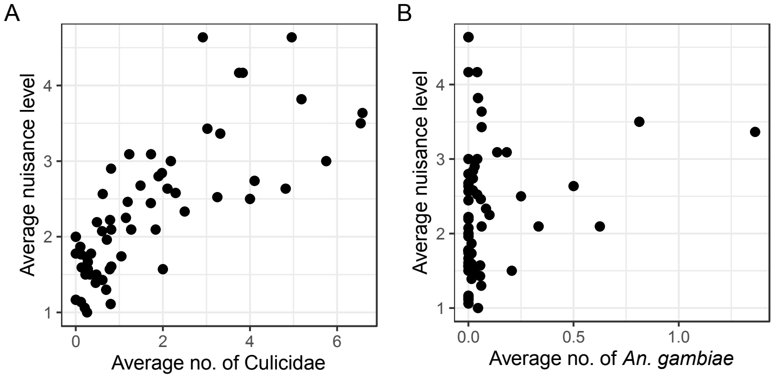


Figure 6. Scatter plots showing the correlation between average perceived mosquito nuisance per village per month and average number of mosquitoes (Culicidae, panel A) and *An. gambiae* s.l. (panel B) per village per month by the volunteers in five selected villages in Ruhuha sector, Rwanda.

When correlations were investigated for each village separately, there were significant correlations between the level of perceived mosquito nuisance and the total number of mosquitoes (Culicidae) collected per household per month, except for Busasamana where no significant correlation was found. Similar to the analyses for the entire sector, no significant correlations were observed between mosquito nuisance and the number of *An. gambiae* s.l. collected (Table 3).

Table 3. Spearman correlation coefficients for the relationship between nuisance level and the total number of Culicidae/*An. gambiae* s.l. separately for each village. In bold the significant correlations are highlighted.

	Mosquito group	**r_s**	***P***
Busasamana	Culicidae	0.117	0.175
	An. gambiae s.l.	0.044	0.613
Kagasera	Culicidae	0.266	**<0.001**
	An. gambiae s.l.	0.046	0.385
Kibaza	Culicidae	0.371	**<0.001**
	An. gambiae s.l.	0.056	0.374
Kiyovu	Culicidae	0.392	**<0.001**
	An. gambiae s.l.	0.057	0.583
Mubano	Culicidae	0.413	**<0.001**
	An. gambiae s.l.	0.037	0.507

Correlation between perception of mosquito nuisance and confirmed malaria cases

When data from households were aggregated and averaged for each village, a moderate, positive correlation between perceived mosquito nuisance and proportion confirmed malaria cases per households per month (r_s = 0.473, P <0.001) was found (Figure 7). When this correlation was investigated for each village separately, there was a significant, strong correlation for Kibaza (r_s = 0.643, *P* = 0.023), while for Busasamana (r_s = 0.567, *P* = 0.054), Kagasera (r_s = 0.261, *P* = 0.413), Kiyovu (r_s = 0.223, *P* = 0.486), and Mubano (r_s = -0.138, *P* = 0.670), correlations were not significant.

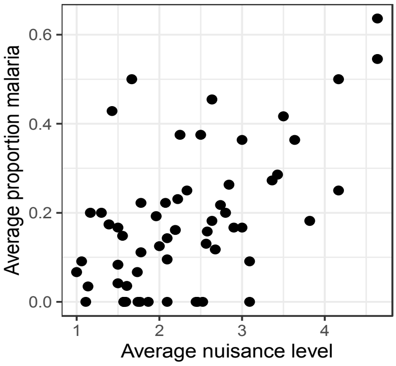


Figure 7. Scatterplot showing the correlation between average mosquito nuisance level and average proportion of confirmed malaria cases reported per village per month by the volunteers in five selected villages in Ruhuha sector, Rwanda.

Correlation between mosquitoes collected and confirmed malaria cases reported

At village level, a moderate, significant correlation (r = 0.468, *P* <0.0001) was found between the average number of mosquitoes and the proportion of confirmed malaria cases reported per village per month (Figure 8-A). No correlation was found between the average number of *An. gambiae* s.l. collected and the proportion of confirmed malaria cases reported per village per month (r = 0.204, *P* = 0.124; Figure 8-B). When data were analysed separately by village, no significant correlations were found between the number of mosquitoes or *An. gambiae* s.l. and the presence of confirmed malaria cases reported per village per month, except for Kibaza where a correlation (r = 0.581, *P* = 0.047) was found between number of Culicidae and confirmed malaria cases (Table 4).

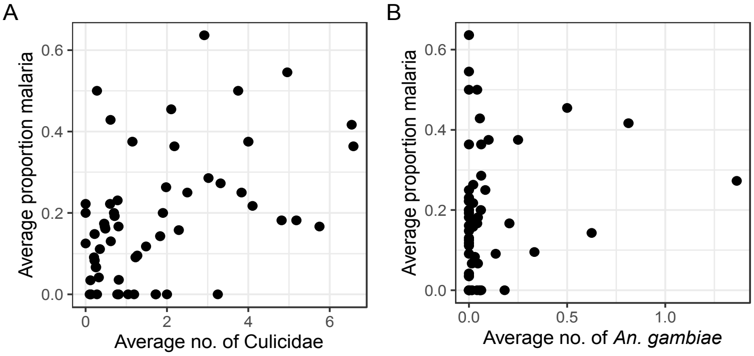


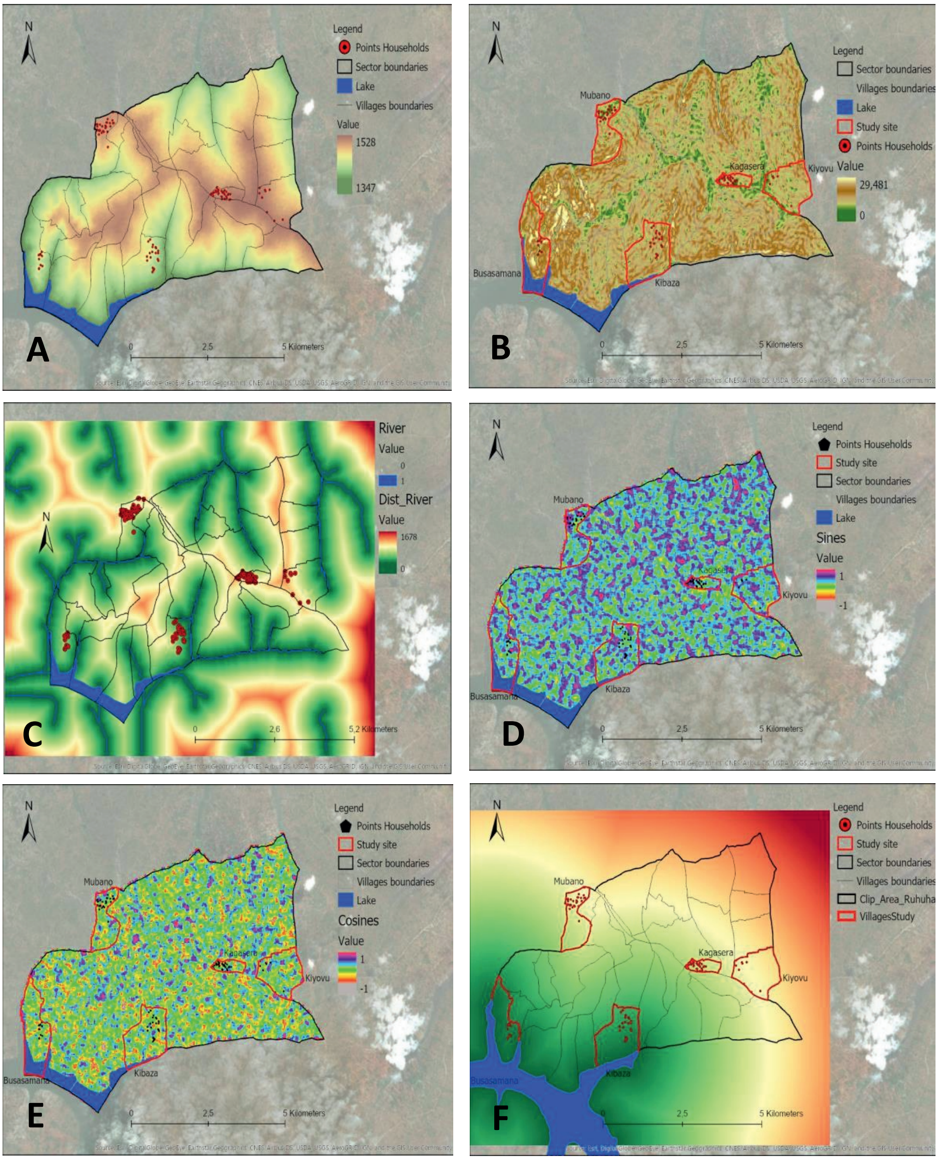
Figure 8. Scatter plots showing the correlation between average number of mosquitoes (Culicidae) (panel A) and *An. gambiae* s.l. (panel B) per village per month and average proportion of malaria in households per village per month as reported by the volunteers in five selected villages in Ruhuha sector, Rwanda.

Table 4. Pearson correlation coefficients between number of Culicidae/*An. gambiae* s.l. and presence of confirmed malaria cases in five villages of Ruhuha sector, Rwanda. In bold the significant correlations are highlighted.

Village name	**Mosquito group**	**r**	***P***
Busasamana	Culicidae	0.170	0.597
	An. gambiae s.l.	-0.126	0.696
Kagasera	Culicidae	0.560	0.058
	An. gambiae s.l.	-0.183	0.569
Kibaza	Culicidae	0.581	**0.047**
	An. gambiae s.l.	0.394	0.205
Kiyovu	Culicidae	-0.241	0.450
	An. gambiae s.l.	0.252	0.430
Mubano	Culicidae	-0.154	0.632
	An. gambiae s.l.	0.174	0.590

Environmental risk factors explaining the spatial and temporal distribution of mosquitoes and malaria vectors

Thirteen variables identified from the literature were selected (Table 1). These included 12 environmental variables: elevation, slope, distance to marshlands, distance to open water, distance to the river network, flow accumulation, cosines of the aspect, sines of the aspect, Normalized Difference Vegetation Index (NDVI), Normalized Difference Water Index (NDWI), Topographic Wetness Index (TWI), Topographic Position Index (TPI), as well as one demographic variable, population density. Data for these variables were extracted from different data sources (Table 1). Values for the different variables were derived and calculated from the extracted data specific to the area under study (Ruhuha sector; Figure 9) and linked to the locations of and data from the households under study.


A
Legend
Points Households
Sector boundaries
Lake
Villages boundaries
Value
1528
1347
0
2,5
5 Kilometers
B
Legend
Sector boundaries
Villages boundaries
Lake
Study site
Points Households
Value
29,481
0
Mubano
Kagasera
Kiyovu
Busasamana
Kibaza
C
River
Value
0
1
Dist_River
Value
1678
0
2,6
5,2 Kilometers
D
Legend
Points Households
Study site
Sector boundaries
Villages boundaries
Lake
Sines
Value
1
-1
E
Cosines
Value
1
-1
F
Legend
Points Households
Study site
Sector boundaries
Villages boundaries
Clip_Area_Ruhuha
VillagesStudy
N

]

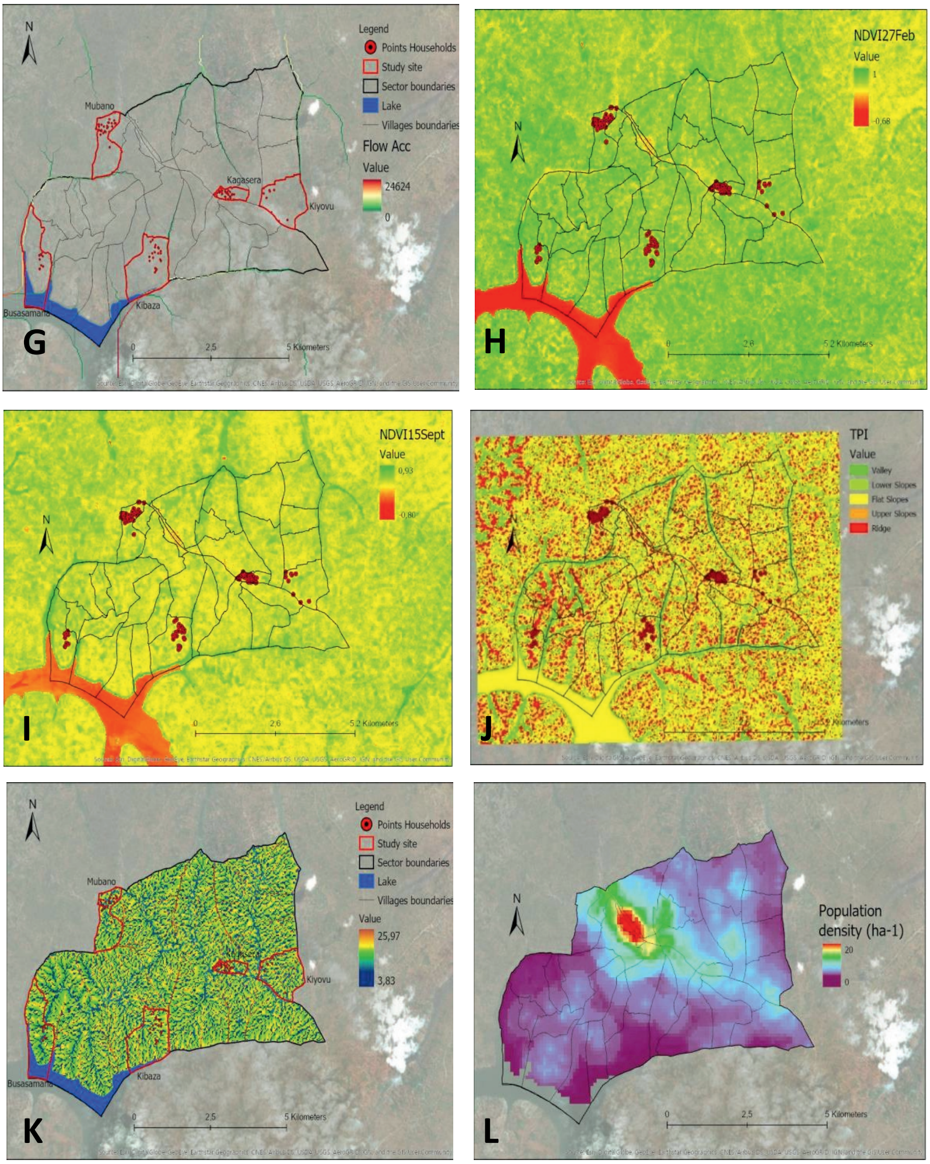


Figure 9. Maps showing different environmental factors: Elevation (A), slope (B), Distance to River(C), Sines Aspect (D), Cosines Aspect (E), Distance to lakes (F), Flow Accumulation (G), NDVI of the study area on the 27th of February 2019(H), NDVI of the study area on the 15th September 2019 (I), TPI (J), TWI(K), population density (L).

Investigation of the bivariate correlations between the selected environmental variables and the abundance of mosquitoes (Culicidae and *An. gambiae* s.l.) revealed that there were significant, correlations between distance to marshland, distance to open water, distance to rivers, NDVI and population density for both mosquito groups (Table 5). For Culicidae, also the sines, slope and TPI showed a significant correlation.

Table 5. Pearson correlation coefficient between mosquito group and environmental factors (sector scale); *: $P<0.05$. **: $P<0.01$.

Variables	**Culicidae**			***An. gambiae* s.l.**		
	r	R^2	P	r	R^2	P
Distance to marsh	- 0.51**	0.25	<0.01	- 0.40**	0.16	<0.01
Distance to water	-0.67**	0.48	<0.01	-0.45**	0.2	<0.01
Distance to river	-0.56**	0.30	<0.01	-0.42**	0.17	<0.01
Elevation	-0.69**	0.47	<0.01	-0.49**	0.24	<0.01
Flow Accumulation	-0.10	0.01	0.31	-0.02	0.00	0.81
Cosines of aspect	-0.15	0.02	0.12	-0.13	0.02	0.19
Sines of aspect	-0.21*	0.05	0.03	-0.15	0.02	0.12
NDVI	0.29**	0.09	<0.01	0.20*	0.04	0.04
NDWI	-0.11	0.01	0.26	-0.03	0.00	0.73
Slope	0.26**	0.07	0.01	0.18	0.03	0.06
TPI	0.20*	0.04	0.03	0.10	0.01	0.28
TWI	-0.14	0.02	0.15	-0.09	0.01	0.38
Population density	-0.66**	0.44	<0.01	-0.48*	0.23	<0.01

At sector level, there was a relationship between elevation and the total number of mosquitoes (Table 5) and in this case there was a clear distinction between the south and the north of the area. In Figure 10, the right oval encompasses data points from the villages in the north (Kagasera, Mubano and Kiyovu), whereas the left oval includes data points from the two villages in the south (Kibaza and Busasamana).

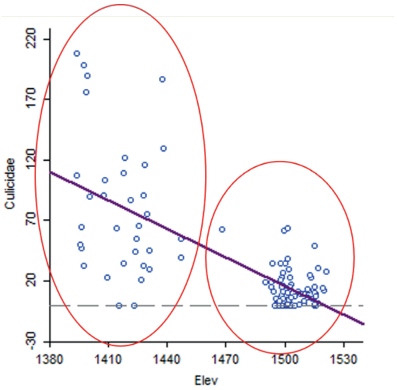


Figure 10. Scatterplot showing correlation between total Culicidae mosquitoes and elevation (in meters).

Prior to performing the multiple regression analysis, we visually inspected correlations among the thirteen selected variables by means of principal component analysis. This showed that NDWI and the sines of the aspect are highly correlated, as their direction and length of their vectors are similar. This also applies for correlations among the variables distance to river, distance to marshlands, elevation and population. On the other hand, the variables NDVI and NDWI showed a negative correlation as they diverge and form a large angle (Figure 10).

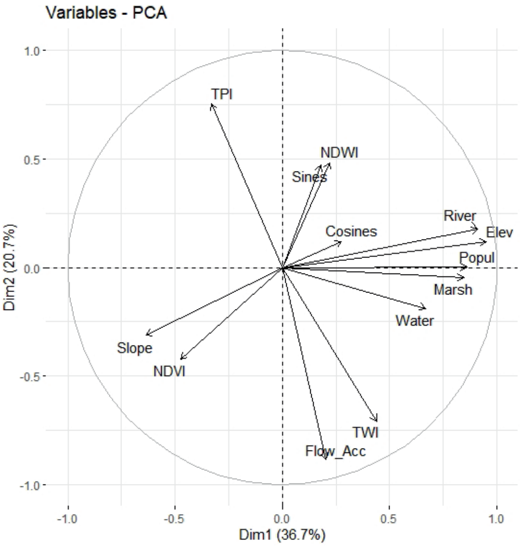


Figure 11. Biplot of the selected environmental variables: elevation (Elev), slope, distance to marshlands (Marsh), distance to open water (Water), distance to the river network (River), flow accumulation (Flow_Acc), cosines of the aspect (Cosines), sines of the aspect (Sines), Normalized Difference Vegetation Index (NDVI), Normalized Difference Water Index (NDWI), Topographic Wetness Index (TWI), Topographic Position Index (TPI), as well as one demographic variable, population density (Popul).

Multiple regression analyses, followed by 'best subset' model selection in the *leaps* package of R, revealed that distance to marshland, distance to the river network, elevation, population density, slope and TPI were all significant predictors in a final model for the total number of Culicidae based on the adjusted R^2 value (57%, Figure 12-A). However, when using the Bayesian Information Criterion (BIC), only distance to the river network and elevation remained in the final model (Figure 12-B). For the *An. gambiae* s.l. model, distance to the river network and elevation were also significant based on adjusted R^2 (28%; Figure 12-C). Using BIC, only elevation remained in the final model (Figure 12-D). Interestingly, both distance to river and elevation play an important role in explaining (malaria) mosquito abundance, despite the high correlation among the two variables (Figure 11). This could be interpreted as follows: the larger the distance is from the river network the lower the risk to encounter mosquitoes or collecting them, and the higher the elevation where the population in Ruhuha is concentrated, the lower is the risk to encounter mosquitoes including *An. gambiae* s.l..

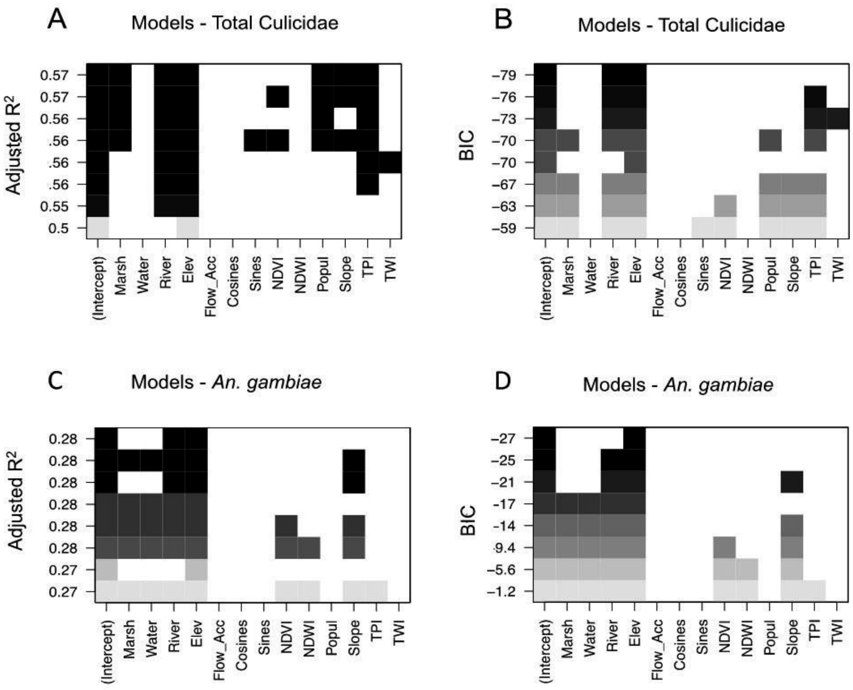


Figure 12. Multiple linear regression models showing selection of best subsets based on the *leaps* package in R. Filled squares indicate the inclusion of a variable in a model. Performance of a model increases from the bottom to the top of each panel based on adjusted R^2 value (panels A and C) or BIC (panels B and D).

Discussion

The findings from this study provide insights into the spatial and temporal dynamics of mosquitoes in five selected villages of Ruhuha based on citizen science data. This was further assessed for *An. gambiae* s.l. separately, as this is the most important malaria vector in the area (Nyirakanani et al. 2017). Furthermore, the study highlighted environmental risk factors that explain these spatial and temporal dynamics indicating areas with higher risk of malaria, especially in the south of the study area, although parasite infection rate was zero for the *An. gambiae* s.l. collected during the study. We show the potential of employing simple mosquito collection techniques with the aid of citizens. In combination with data on perception of mosquito nuisance denoted in a questionnaire, data from these traps can be used as a proxy for areas with higher risk of malaria.

All the anopheline species found in the current study were also reported from other parts of Rwanda (Hakizimana et al. 2018). *Anopheles gambiae* s.l. was the most abundant of all anopheline species and was recorded every month in all five villages. However, populations were not highly abundant throughout the season. This can be partially explained by the fact that the handmade trap has a lower trapping efficiency in comparison to other trapping technologies at household level (see Chapter 5). Most mosquitoes (Culicidae) were caught in the months of January and February 2019 during the short rainy season, but most malaria vector mosquitoes were collected in August 2019 (large dry season). Although larval habitats in this period have dried out, this is also the period that the second cycle of rice cultivation starts, leaving the irrigated fields with little vegetation, but with sufficient water for mosquito breeding, thereby increasing adult abundance. The villages of Busasamana and Kibaza (Figure 1), both located in the south or Ruhuha, are good examples in this regard, because the peak in *An. gambiae* s.l. was especially observed here. Busasamana has two irrigated fields, namely Nyaburiba, a rice field, and Nyagafunzo, used for irrigated cultivation of subsistence crops (Hakizimana 2019). Kibaza has also one irrigated rice field nearby (known under the same name, i.e. Kibaza). *Anopheles gambiae* s.l. is generally associated with irrigated rice, and irrigation elevates relative humidity that enhances survival of these vectors (Ijumba & Lindsay 2001). Although no *P. falciparum* infection was found in the *An. gambiae* s.l. collected in the studied area, our citizen science approach was able to identify areas with a relatively higher malaria vector abundance which are at higher risk for malaria as evidenced by the highest self-reported proportion of malaria cases in these two villages.

Similar to our results, a study from Ethiopia found that larval and adult abundance of the malaria vectors *An. arabiensis* and *An. pharoensis*, was higher in a village with nearby irrigation than in a village without nearby irrigation, as was malaria prevalence (Kibret et al. 2010). Another study conducted in Malawi showed that changes in the geography of breeding potential across irrigated spaces can have profound effects on the distribution of malaria risk for those living in close proximity to irrigated agricultural schemes (Frake et al. 2020; Mboera et al. 2010).

In the present study, Kagasera had the lowest number of mosquitoes including *An. gambiae* s.l.. The reason could be that the village is characterized by a higher quality of houses and that the village is located further away from the water network, which is not the case for Busasamana and Kibaza. A study conducted in rural Gambia demonstrated that incorporating a ceiling made from locally available materials significantly reduced house entry by *Anopheles gambiae* (Lindsay et al. 2003). Another study conducted in The Gambia demonstrated that there were lower vector survival rates and less malaria in villages with a higher proportion of metal roofs. The indoor climate of metal-roof houses, characterized by lower humidity and higher temperatures may reduce the survival of indoor-resting mosquitoes and may have even contributed to the observed reduction of malaria in parts of sub-Saharan Africa (Benelli & Beier 2017). Another reason could be that Kagasera has a higher elevation compared to Busasamana (see Figure 9-A) and this could also have had an impact on the presence of *An. gambiae* s.l.. A study conducted in Mambilla Plateau, Northeast Nigeria, demonstrated that indeed, altitude can influence mosquitoes and *Anopheles* species abundance (Garba et al. 2020).

Findings on the role of environmental factors showed that in particular elevation and distance to the river network contributed to the spatial distribution in numbers of mosquitoes and *An. gambiae* s.l.. A study conducted in Mara River basin located in the southwestern part of Kenya and the north-eastern side of Tanzania demonstrated that distance to nearby human habitation was another important factor influencing mosquito larval abundance. Most of the breeding habitats were recorded within a distance of 70–450 m from the nearest human habitation (Dida et al. 2018). Additionally, in the same study, it was found that in the river habitats, more mosquitoes were found in slow flowing streams and riverbeds with little vegetation as compared to open water, an indication that aquatic vegetation plays an important role in harbouring malaria transmitting vectors (Dida et al. 2018).

We conclude that perceived mosquito nuisance can be used as an indicator for mosquito density. However, although significant correlations between mosquito nuisance and the number of mosquitoes and *An. gambiae* s.l. were found when data were aggregated for all 12 months of the study, the correlations with *An. gambiae* s.l. were absent when analysed separately for each village or when using village level averages. In other words, nuisance seemed to be strongly driven by the total numbers of mosquitoes, and not by the abundance of *An. gambiae* s.l.. Interestingly, in our cross-sectional study conducted in 2017 and 2018, a moderate and significant correlation with nuisance was found for both mosquitoes and *An. gambiae* s.l. (Chapter 3). One of the reasons could be that the total number of mosquitoes for both years was almost three times higher (9,965) and almost two times higher for *An. gambiae* s.l. (974) than in the present study. Possibly, to detect correlations between mosquito numbers and nuisance a minimum number of mosquitoes needs to be collected.

Interestingly, our findings showed significant correlations of similar strength between mosquito nuisance and proportion of malaria cases, and between number of mosquitoes collected and malaria cases (both r = 0.47) (Figure 13). At the start, the study aimed to investigate whether nuisance level can be used as an indicator for malaria risk. Results suggest that, in our study, nuisance is an equally strong indicator for malaria risk as the number of mosquitoes collected. Although we expected to find a correlation between numbers of *An. gambiae* s.l. and malaria cases, the number of *Anopheles* individuals was low compared to, for example, what was collected during the baseline survey (Chapter 3), and therefore correlations were probably absent when using the citizen science data for this one species separately.

For the calculation of these correlations, it would have been preferred to have all 112 volunteers more homogeneously distributed over the sector, but for logistical reasons, this was not feasible, and hence we worked with citizens in five village clusters (Chapter 4). In the current study, the average number of *An. gambiae* collected per house was too low to expect a statistical result (there were many catch nights with zero mosquitoes). It is interesting that the proportion of confirmed malaria cases reported per village per month was correlated (r = 0.468, *P* <0.0001) (Figure 8-A)) with the average number of Culicidae collected, even though 89% of these mosquitoes were non-malaria vectors.

Busasamana and Kibaza both located in the south of the studied area, had the highest number of *An. gambiae* s.l., mosquito nuisance and the highest percentage of households having a confirmed malaria case indicating that the highest intensity of malaria transmission in the studied area strongly relates to distance to the river network and altitude (Figure 9-A and 9-C). A same proportion of host-seeking *An. gambiae* s.l. was collected both indoors and outdoors suggesting that transmission can take place both inside and outside.

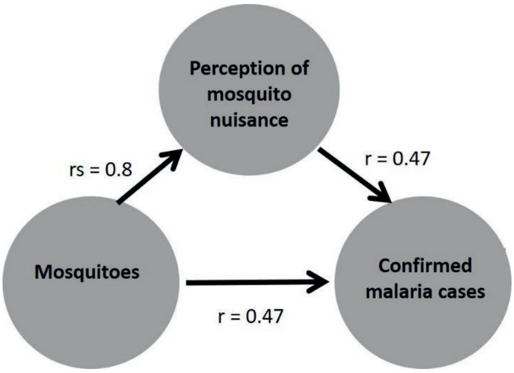


Figure 13. Strength of correlations between of mosquito nuisance (Culicidae) and proportion of malaria cases, and between number of mosquitoes (Culicidae) collected and malaria cases.

Conclusions

The results demonstrate that a well-established citizen science network provides valuable information on the bionomics of (malaria) mosquito species. In combination with reports on perceived mosquito nuisance, the citizen science network can give indications on the spatial and temporal variation in the risk of malaria. The study shows that especially elevation and distance to the river network explained the spatial and temporal variation of (malaria) mosquitoes at the sector level. The data collected through our citizen science programme can be valuable for the Malaria and Other Parasitic Diseases Division for the planning of control strategies for malaria vectors. They could consider to expand the current surveillance network of 12 sentinel sites with a citizen science network to areas where no monitoring is established. Such a citizen science network could contribute to more effective spending of limited resources for vector control.

Acknowledgements

The authors express sincerely thank the Mayor of Bugesera district, Ruhuha Health Centre, Ruhuha administrative sector. We also thank the laboratory of Entomology from Malaria and Other Parasitic Diseases Division and Wageningen University and Research for the technical support. Special thanks to Jeanine Loonen for technical assistance. We express our gratitude to the community members for their collaboration and involvement in this project. Wageningen University, The Netherlands through its Interdisciplinary Research and Education Fund (INREF) generously supported this study conducted in the framework of the "Environmental Virtual Observatories for Connective Action" (EVOCA) project.

Chapter 7

General discussion

Introduction

With the resurgence of malaria in Rwanda, there is an urgent need to improve vector surveillance for better implementation of malaria control efforts across the country (MOH 2020). The cornerstone of vector surveillance and control in Rwanda are the country-wide mosquito monitoring programmes established in 12 sentinel sites (Hakizimana et al. 2018). At these sites, mosquito population density and parasite infection rate of malaria vectors are monitored. These observations help to identify how malaria vectors spread the infection to humans, and help to determine the best intervention strategies to reduce the risk of infection (MOH 2019). Twelve sentinel sites are, however, not sufficient to identify all areas at risk of malaria. Consequently, deploying cost-effective vector control interventions are challenging to organize. Expanding the current monitoring programme to other regions of the country requires sustained domestic and international funding, which is currently inadequate to enable full programme implementation (MOH 2020). A possible cost-effective option to expand the monitoring programme could be the involvement of citizens to collect entomological data such as mosquito specimen, mosquito nuisance and biting intensity as a proxy for malaria transmission risk.

The main aim of this thesis was to investigate the opportunities for citizen science in malaria vector surveillance in low resource settings in Rwanda, especially in areas where mosquito monitoring is not established. Below, the main findings of the different chapters are discussed. Also, the implications of the findings for scaling up the malaria monitoring programme in Rwanda by using a citizen science approach are discussed.

Determinants of malaria in Rwanda and the role of citizen science to complement vector surveillance

The first objective of this thesis was to identify factors that contributed to the upsurge of malaria in Rwanda, and to provide a scientific basis to ensure the applicability of citizen science as a contribution to the current vector surveillance and control programme (Chapter 2). The findings in Chapter 2 demonstrated that the observed malaria resurgence of recent years has been attributed to various interrelated factors, including environmental, biological, socio-economic and institutional factors. Chapter 2 concluded that the upsurge of malaria in Rwanda is partially caused by the weakening of malaria control programmes. This, is mainly caused by resource constraints. Another contributing factor to the upsurge is a change in the intrinsic potential for malaria transmission, as well as a change in resistance of the mosquito vectors towards insecticides. The intrinsic potential for malaria transmission can evolve as a function of socio-economic development or environmental change. In Rwanda, agriculture is key in ensuring food security and economic growth. However, the introduction of intensive irrigation of rice during the past few years, especially in the south-eastern and western part of the country, has led to an important transformation of the ecological landscape, creating suitable habitats for malaria vectors, and thereby increasing opportunities for malaria transmission. This has jeopardized many efforts in vector prevention and control in place (MOH 2017b). This is supported by other studies which demonstrated that irrigation activities can contribute to the proliferation of suitable mosquito breeding habitats and hence increase the risk of malaria transmission (Hawaria et al. 2020; Ijumba & Lindsay 2001). Another factor that contributed to the upsurge is the resistance to insecticidal pyrethroids (lambda-cyhalothrin, deltamethrin, permethrin) and organochlorines (DDT) due to their extensive use for malaria vector control, which has also led to changes in feeding and biting behaviour of malaria vectors (Hakizimana et al. 2016). This finding is supported by many studies conducted elsewhere (Carrasco et al. 2019; Delenasaw & Kweka 2016; Ferreira et al. 2017; Killeen & Chitnis 2014; Killeen et al. 2016; Ranson & Lissenden 2016). Other factors that can explain the upsurge in malaria are socio-economic factors. A recent study conducted in Rwanda revealed that increased malaria prevalence was associated with lower income, non-compliance with bed-net usage and living below 1,700 m altitude, whereas season and type of residence were not associated with increased malaria incidence (Rudasingwa & Cho, 2020). Another study conducted in Rwanda found that people living in houses with poor roof and wall materials or with no bed nets are more likely to develop malaria (Bizimana et al. 2015).

Chapter 2 demonstrated that although malaria vector control is in place in Rwanda, current surveillance is costly, and there is limited capacity to scale up malaria vector surveillance. To lower the number of malaria cases, there is a need for more information on where malaria vector hotspots are and where action is most urgently needed. In this way, limited resources can be deployed more effectively. To identify the spatial and temporal variation in malaria risk, a citizen science approach may contribute to mosquito surveillance. Chapter 2 concluded that a citizen science programme implemented within the Rwandan context can potentially support malaria vector surveillance activities by providing data on malaria vector distribution and on other malaria related aspects, and thus aid in malaria risk assessment. In addition, enrolling citizens in this programme could contribute to changing behaviour towards the proper use of vector control measures (Asingizwe et al. 2018). We concluded that the integration of social and natural science disciplines in the design and coordination would be essential for successful implementation of the programme.

Mosquito nuisance as indicator for vector hotspots

The second objective of this thesis was to define the link between the perception of mosquito nuisance and actual vector abundance as an indicator of malaria vector hotspots (Chapter 3). CDC light traps were used in the houses of participants to determine the mosquito abundance and species composition. At the same time, household members were interviewed about their perceived level of mosquito nuisance and malaria risk factors. *An. gambiae* s.l. was the dominant *Anopheles* species with *An. gambiae* s.s. Giles as the main sibling species followed by *An. arabiensis*. *An. gambiae* s.s. is mainly endophilic (feeding indoor) and endophagic (resting indoor), whereas *An. arabiensis* displays higher diversity in these behaviours

(Coetzee et al. 2013). The number of *Anopheles gambiae* s.l. and the number of Culicidae collected indoors both correlated significantly with the perception of mosquito nuisance. However, this correlation varied across years and sectors. At the village level, nuisance was significantly correlated with *An. gambiae* s.l. and total mosquito density, but only in 2018 and not in 2017. The idea of recording mosquito nuisance (biting) via a questionnaire to determine the locations of and identify the responsible mosquito species has also been used in a several other studies (e.g. Heym et al. 2017; Kampen et al. 2015; Medlock et al. 2012; Samuelsen 2004). Medlock et al. (2012) also concluded that nuisance was attributed to non-invasive species (*Culiseta annulata*, *Ochlerotatus detritus*, *Culex pipiens* s.l., *Ochlerotatus cantans* and *Anopheles maculipennis* s.l.). We concluded that mosquito nuisance as reported by citizens could potentially be an indicator of malaria vector hotspots, but with only three weeks of mosquito monitoring in each of the two years of study, it was not possible to capture the temporal variation over the course of a year. In addition, the CDC light traps used for catching mosquitoes are expensive (Bijllaardt et al. 2009) and cannot be easily applied in a large scale set-up of a citizen science monitoring programme. An alternative trapping approach was thus desired. The results of Chapter 3 provided a solid basis for further exploring the potential of involving citizens in malaria mosquito surveillance and control.

Monitoring socio-economic factors that determine malaria risk

The questionnaires addressed for Chapter 3 not only provided an indication of the spatial and temporal variation in the population size of malaria vectors but also provided insight into which housing conditions impact mosquito abundance (Chapter 3). Poor structural housing, such as houses having tiled roofs or open eaves, and walls made of mud and wood, were contributors to mosquito and malaria vector densities indoors. With this type of houses, mosquitoes follow human odour plumes until they reach an external wall, then navigate upwards and enter the house through the open eaves (Njie et al. 2009; Spitzen & Takken 2018). Houses with walls of wood and mud may attract more mosquitoes, because they are more likely to contain holes, via which attractive human body odours emanate to the outside. Therefore, these findings suggest that improving the design and structure or building materials of the houses, such as using a metal roof, closing the eaves and tightly fitting doors and windows as well as adopting cemented wall, can reduce house entry by malaria vectors and reduce the burden of malaria as demonstrated by other studies (e.g. Bottomley et al. 2017; Lindsay et al. 2003; Lwetoijera et al. 2013; Mburu et al. 2018; Njie et al. 2009; Ondiba et al. 2018; Wanzirah et al. 2015). The answers regarding housing conditions provided by the participants in the study show the potential of citizen science networks to obtain a diversity of socio-economic and environmental data that could explain the variation in mosquito numbers and malaria incidence.

Co-designing the citizen science programme for malaria mosquito surveillance

Prior to the implementation of a citizen science programme for malaria mosquito surveillance in Rwanda, it was important to ensure its sustainability as a strategy that would be complementary to the existing mosquito monitoring programme. The third objective of this thesis was therefore to evaluate a co-design process for the sustained implementation of a citizen science programme for malaria mosquito surveillance. Applying a co-design approach in the development of the initiative is useful, because it maximizes the outputs of data collected by citizens (Liu et al. 2017). The results from Chapter 4 demonstrated that a citizen science programme in a rural context is feasible if citizens that have context-specific knowledge, experiences and skills, participate in the decision-making and co-design process. This is supported by a study by Shirk et al. (2012) who demonstrated that the design and implementation of every project requires decisions to be made about whose interests can and should be addressed, and how the end goals, or desired outcomes, are defined. The findings from Chapter 4 also demonstrated that participants joined the citizen science programme because they wanted to contribute to scientific knowledge, they wanted to learn, and they wanted to take part in malaria prevention and control. Participants felt empowered as they were given the opportunity to raise their voice. Their experiences were considered during the design process and this probably raised their motivation to participate in the citizen science programme. This finding agrees with West & Pateman (2016) who stated that, when recruiting participants, it is crucial to understand what motivates them to participate. If citizens feel that their needs are respected, they will continue to remain involved in the project (West & Pateman 2016). In Chapter 4, the findings demonstrated that for a sustained citizen science programme, local communities are a key actor and crucial resource in malaria mosquito surveillance. This finding agrees with other studies (Bartumeus et al. 2019; Jordan et al. 2017; Palmer et al. 2017). Volunteers took part in the different steps, including the development of the research questions, the technological design, the data interpretation process together with scientists, and the identification of individual and/or collective actions applicable to their context (e.g. increase of use of ITNs, collective management of mosquito breeding sites during community work etc.). This is also supported by other studies conducted in other domains, such as forest landscape management (Hovis et al. 2020; Shirk et al. 2009).

Retention mechanism for a sustained citizen science programme

In addition to the steps described in Chapter 4, providing feedback through the sharing of results also plays a crucial role in motivating participants. In Chapter 4, feedback of the results was provided through SMS, as well as during village meetings and dissemination workshops. Providing feedback to the participants throughout the project revealed new opportunities to improve the programme and its data quality and increased the educational potential of it. These findings are supported by other studies that demonstrated that a mechanism to sustain the programme is required (Dickinson & Paskewitz 2012; Gharesifard et al. 2017). In our case, two dissemination workshops were conducted after every 4 months to share the results and to adjust and sensitize citizens on the quality of the data collected. Immediate and continuous feedback of results in a visually attractive and easy to understand manner using PowerPoint presentations helped to keep citizens engaged in participating in the data collection activities. Strategies for collaborations and partnerships with the citizens are necessary to gather the resources and participants required to sustain a citizen science programme in the long run (thesis Asingizwe 2020).

Technical needs for citizen science

Different means of recruiting citizens and establishing networks of citizens for mosquito monitoring include articles contributed to magazines, flyers, newspaper interviews, personal meetings, published press releases, radio and TV (Kampen et al. 2015; Oltra et al. 2016; Walther & Kampen 2017). In Chapter 4, recruitment through personal contact during participatory workshops was used to stimulate public attention on malaria resurgence and to recruit citizens to become involved in citizen science. This approach was similar to strategies used in other studies for which participants were also recruited through workshops (Jordan et al. 2017; Sorensen et al. 2019). Many citizen science programmes use the internet, mobile phones and other technologies such as websites and social media to create and maintain a network and to collect observations (Franzoni & Sauermann 2014). Some of these have facilitated the assessment of disease risk over vast spatial and temporal scales, thereby advancing research to mitigate vector–borne disease risk (Hamer et al. 2018; Jordan et al. 2017). At the start of the research, one of our objectives was to use digital technology tools, such as smart phones, to report the observations, and to use a website to visualize the observations and to allow access to open data. However, very low or no internet coverage, no ownership of smartphones or mobile phones, and other constraints such as airtime and electricity shortage restricted the use of mobile phones in the studied areas (Chapter 3). Other methods, such as reporting via paper forms, were more preferred. Using the appropriate tools for monitoring indoor and outdoor mosquito populations is important for understanding the dynamics of malaria transmission, and thus needs to be tailored to the local context. Another aspect to emphasize regarding our context is that participants required tools to collect mosquitoes. Considering the local context where the study took place, a handmade trap was proposed as collection method. Being exposed to previous research on malaria by colleagues that used different mosquito collection tools in the study area (e.g. CDC light traps as used in Chapter 3) possibly restricted the innovative capability of participants in creating or designing mosquito collection tools.

The technological dimension is one of the pillars that has ensured success of many citizen science initiatives in developed countries (Braz et al. 2020; Fouet & Kamdem 2018; Kampen et al. 2015). The results presented in Chapter 4 are supported by other studies that demonstrated that emerging technologies in developing countries offer a unique opportunity for data collection within citizen science initiatives (Kampen et al. 2015). For example, studies conducted in Germany and The Netherlands demonstrated that interactive forms or questionnaires were completed with a special code that linked the mosquito samples to locations, and thus allowed to track the collection sites on an interactive map on the home page of the project (www.muggenradar.nl; www.mueckenatlas.de) (Vogels et al. 2015; Walther& Kampen 2017). The choice of these components depends on the aim of the citizen science project and the context in which the project has been implemented.

Efficiency of a handmade mosquito trap for the citizen science programme

Participants in the first participatory workshop requested a tool to collect mosquitoes. We opted to evaluate a handmade plastic bottle trap with sugar fermenting yeast, because carbon dioxide produced from sugar and yeast was proven to attract *Anopheles* mosquito species in the laboratory as well as in the field (Smallegange et al. 2010). Therefore, the fourth objective of the thesis was to assess the efficiency of a handmade, carbon-dioxide baited trap for collecting adult malaria vectors for use in a citizen science programme for malaria mosquito surveillance. Results in Chapter 5 demonstrated that a handmade plastic bottle trap baited with CO_2 and light was able to capture malaria and non-malaria mosquitoes. This finding is supported by other studies that demonstrated that CO_2 used as bait attracts mosquitoes including malaria vectors (Abdon-liwanag & Tansengco 2015; Laguna-Aguilar et al. 2012; Rosanti et al. 2017; Smallegange et al. 2010). The number of *An. gambiae* s.l. in the handmade trap baited with CO_2 and light was about 8-10% of the number caught with a CDC light trap. This suggests that about 10 volunteers with a handmade trap could capture a similar-sized sample of *An. gambiae* as one CDC light trap would collect. In other words, if sufficient volunteers can be recruited in a citizen science project, this could be an alternative option to use. Hence, the trap was selected as a tool for mosquito surveillance activities in the citizen science programme that was initiated in the final stage of the project (Chapter 6).

The presence of mosquitoes captured using the CO_2 trap with light enhanced the motivation of citizens to continue their participation. However, the limited capability of the CO_2 trap to catch a large number of mosquitoes also caused citizens to come up with their own way, tool or idea for collecting mosquitoes, such as using light to hypnotize the mosquito while feeding on the volunteer, or to actively collect it using the net that was part of the CO_2 trap. This needs further exploration in case of future expansion of the citizen science programme. For example, to increase the effectiveness of capturing, a small fan can be incorporated in the trap which operates on a miniature solar panel. In this way challenges for changing the batteries for the torch during the night will be overcome and the number of mosquitoes possibly may increase.

Citizen science and remote sensing to evaluate the spatial and temporal distribution of mosquitoes and malaria vectors

After all preparations were completed (Chapters 2 to 5, Figure 1) we started our citizen science programme on malaria in November 2018. In total, 112 households participated in the monthly reporting of (i) mosquitoes collected with the hand-made CO_2 trap, (ii) mosquito nuisance levels and (iii) the number of confirmed malaria cases in their household in the two weeks prior to submission of data. The 12 months of observations and their analyses provided the basis for addressing the fifth objective of the thesis which was to evaluate the value of a citizen science programme in providing insight into potential malaria vector hotspots and other malaria related information, and to determine predictors of malaria vector distribution.

Chapter 5 already showed the potential of hand-made traps in catching mosquitoes by volunteers. In the twelve monitoring rounds, our 112 citizen scientists caught 3,793 female mosquitoes. This large number of mosquitoes provided by the citizen science programme provided sufficient basis for determining the spatial and temporal distribution of malaria vectors, and pinpoints to areas that, in view of limited resources for control, may be at higher risk of malaria. This finding is supported by a study conducted in West Baltimore (USA) that demonstrated that citizen scientists, recruited through neighbourhood workshops and festivals, provided equally reliable data on adult mosquito distribution as data collected by experts (Jordan et al. 2017). In our study, relatively more mosquitoes were collected in the south of Ruhuha than in the north, especially in Busasamana and Kibaza. These villages also reported the highest nuisance levels. At a temporal scale, findings from our citizen science programme showed that most mosquitoes (Culicidae) were caught in January and February 2019, while the lowest numbers were caught in September and October 2019. Both the months February and August 2019 had a peak in the number of *An. gambiae* s.l. in comparison with other months, and the number of *An. gambiae* s.l. dropped from March to May. These findings demonstrated that data collected through citizen science can reveal spatial and temporal variability in mosquito dynamics at a different scale than the 12 sentinel sites for the whole of Rwanda (Hakizimana et al. 2018).

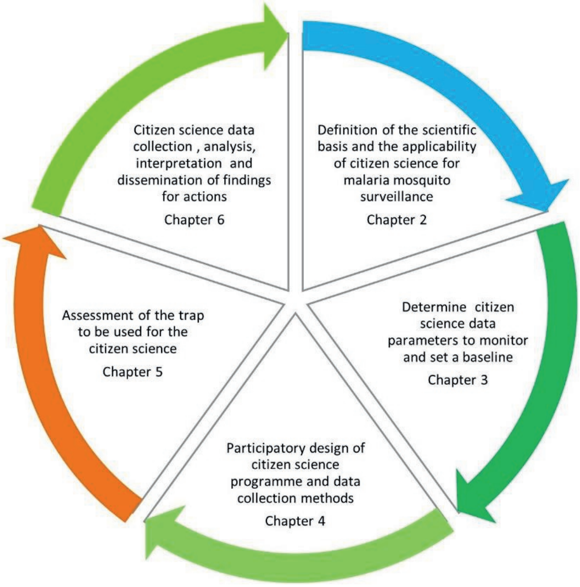


Figure 1. Process for implementing the citizen science programme.

The conclusion of Chapter 3 was that the level of mosquito nuisance partly correlated with the number of mosquitoes collected using CDC light traps. During the one-year citizen science programme, nuisance levels were also correlated with mosquito numbers caught by the citizens. The results show a clear spatial and temporal variation in the perception of mosquito nuisance with the highest average mosquito nuisance reported in the south of the study area from Busasamana and Kibaza, while in the western part of the sector little nuisance was experienced. The highest average mosquito nuisance scores were reported in December, January, and February. Based on all data collected for one year, there was a moderate, positive correlation between perceived mosquito nuisance per household per month and the number of mosquitoes (Culicidae) per household per month and a weak, positive correlation between nuisance and the number of *An. gambiae* s.l. per household per month. These findings imply that monitoring the perception of mosquito nuisance by citizens over a longer time period would allow to highlight areas with mosquito hotspots and hence malaria vectors. On its turn, this can identify areas with heightened malaria risk. Longer term citizen science studies (i.e. longer than one year) may help to study the impact of changes in weather and climate on mosquito populations and to study the impact of malaria preventive measures on mosquito numbers in and outside houses.

In terms of confirmed malaria cases, the findings demonstrate that the southern part of the studied area scored the highest average monthly percentage of households having a confirmed malaria case. Over one year, every month on average 16% of the volunteers reported having at least one confirmed malaria case in the two weeks prior to sampling.

However, 66% of the households reported at least one confirmed malaria case in their household throughout the entire study period. March, January and February had the highest percentage of households having at least one confirmed malaria case. Busasamana and Kibaza had the highest percentage of households having a confirmed malaria case over the entire year followed by Kiyovu, Mubano and Kagasera. Findings demonstrated significant positive correlations between nuisance and confirmed malaria cases, as well as between total number of Culicidae and confirmed malaria cases, but not between number of *An. gambiae* and malaria cases. According to citizens in our programme and according to the national malaria control programme, an indoor spraying campaign was carried out in March 2019 in most of the selected houses of volunteers during the period of the study, and this could explain the low number of *An. gambiae* s.l. collected. Although the number of malaria vectors was quite low, the percentage of households having a confirmed malaria case remained large, leading to the conclusion that with a low number of malaria vectors as a results of insecticide application, malaria transmission can still be maintained. An important aspect regarding these self-reported confirmed malaria cases, is to perform a cross-check at the health center to confirm the positivity of the malaria cases. In the present study, such validation was technically not feasible. For example, reports of malaria cases in our study were done at the household level, whereas the confirmed malaria cases at household level could be from other (visiting) members of the household, or not necessarily belonging to the volunteer's family. Also, the confirmed cases reported by the volunteer could be tested at Ruhuha health center, or by the community health worker at the community level or at the health sentinel post. Reports from our study could thus not be matched with cases recorded elsewhere.

Spatial and temporal distribution of malaria vectors and its association with environmental risk factors

Citizen science data in Chapter 6 were coupled with environmental factors from other sources (remotely sensed data). The findings in Chapter 6 revealed that factors such as distance to the river network and elevation played a significant role in explaining mosquito and malaria vector abundance, whereas several other parameters (e.g. greenness of the area and the wetness index) did not. Citizens from villages in the north of the study area and far away from lake Cyohoha and the irrigated fields, experienced little nuisance and had a lower risk to encounter mosquitoes. Similar results have been found in studies elsewhere that demonstrated that people living closer to wetlands are at higher risk of contracting malaria than people living further away (Amaechi et al. 2018; Hawaria et al. 2020; G. Zhou et al 2007b).

Recommendations

Improving housing quality and stimulating ITN use can reduce malaria risk. However, these measures are likely to be insufficient to address residual malaria transmission. Sherrard-Smith et al. (2019) found that about 5% to 40% of residual malaria transmission in Africa occurs when people are awake and less protected by bet nets. Another study in Malawi demonstrated that a considerable proportion of biting by malaria vectors occurred at times in the evening when many people are likely still active and not protected by bed nets (Mburu et al. 2019). These above cited examples demonstrate that the application of other vector control measures targeting the interruption of residual malaria transmission such as larval control with *Bacillus thuringiens* var. *israelensis* (Bti) in regions predominated by irrigated fields is essential to complement bed net usage/coverage as well as the earlier mentioned house improvement.

Citizen science as a complementary strategy to ongoing malaria control initiatives presents an opportunity for malaria mosquito surveillance in Rwanda. Prior to the collection of the data by the citizens, involvement of different stakeholders (decision-makers, scientists and citizens who constitute the core of the programme) is required for it success. In the development of our citizen science programme, close collaboration of natural and social scientists was key for understanding the social dynamics of the programme (PhD thesis Asingizwe, 2020). Close collaboration between citizens and the researchers helped to improve the quality of the data and learning process. Seeing the value that the citizen science data present, a continuation and further expansion of the citizen science program would be recommended. This would require a central coordinating office connected to the current vector surveillance currently in place for malaria prevention and control. This ensures visibility of the outcomes, integration of monitoring and analysis of the citizen science programme, as well as implementation of malaria control actions. Incorporating digital technologies, such as a website that allows free and open access to the citizen science data, remain an advantage and an option to further explore. Beforehand, a step by step guideline on how to establish a citizen science programme is necessary to consider when implementing it.

To ensure a sustained citizen science programme and to achieve a better understanding of the factors that explain malaria incidence in Ruhuha and other sectors of Rwanda, further research is recommended on the following topics:

First, given the low catches of malaria vectors collected by the handmade carbon dioxide baited trap, improving the trap by incorporating a low-cost fan that operates on a miniature solar panel and applying a more attractive odour bait to increase the effectiveness to catch more mosquitoes would be helpful. Another option that can be explored is conducting a citizen science programme in areas where research on mosquito monitoring has not been conducted and by requesting volunteers to catch mosquitoes themselves. Such study will help to discover other avenues or approaches for collecting mosquitoes especially in rural settings.

Second, considering the added value of citizen science, expanding the programme by including other villages or by recruiting more participants in malaria endemic risk zones will help to improve the robustness of the citizen science programme, and will help to validate the predictors of the spatial and temporal distribution of malaria vectors. By enlarging the group and asking them to have a different monitoring moment in the month could also help to better capture the spatial and temporal distribution of malaria vectors. The 12 existing sentinel sites also monitor only once a month. A citizen science network with a sufficient number of participants would allow a weekly monitoring of mosquito populations. Adopting mechanisms of retention of citizens, such as feedback provision and/or rewards (e.g. Jordan et al. 2017; Yoshioka 2013) can ensure that multi-year patterns in (malaria) mosquito abundance are captured via citizen science.

Third, linking citizen science data to socio-economic factors, such as type of livestock kept indoors, will help to determine their role in mosquito abundance hence malaria risk. In our study, the impact of livestock on malaria vector distribution was not clear. Therefore, an evaluation of the impact of livestock kept indoor on mosquito density and occurrence in households with or without bed nets will help to understand more the risk of malaria indoor. Mayagaya et al. (2015) in their study in southern Tanzania demonstrated that the presence of livestock at the household level can significantly alter the local species composition, feeding and resting behaviour of malaria vectors.

Fourth, even citizen science programmes require funding (although less than conventional surveillance programmes), and further mechanisms ensuring a sustainable source of funds need to be investigated. Braz et al. (2020) stated that, based on a comparison of a citizen science programme with professional programmes, citizen science mosquito surveillance may be less expensive at similar geographical scales. Demonstrating the public health benefits of citizen science for mosquito surveillance will be central to ensure sustainable funding and maximum impact of citizen science (Braz et al. 2020). Financial resources are necessary in any citizen science programme in the long-term (Hovis et al. 2020), in particular for application development, monitoring equipment, and staff members (Bonney et al. 2014; Shirk et al. 2012). The primary challenges for most programmes include maintaining funding for the materials, cyberinfrastructure, databases, and project leadership (Dickinson & Paskewitz 2012). Gharesifard et al. (2017) defined several revenue streams that could sustain citizen science initiatives. Several of these could be explored in our setting. The first revenue stream is government sponsorship. In this stream, the government plans to use the data and thus allocates the resources (e.g., from research budget) to establish and maintain a citizen science programme. The second revenue stream is combining data contributed by citizens with other sources such as satellite data and sell these data products to individuals or organizations. The aim is to generate new information such as disease forecasts, warnings, maps etc., and make profit from selling such products. This category of revenue streams is referred to as data or information usage fee. The third revenue stream is a subscription fee which refers to membership fees that platform users may have to pay to gain continuous access to services provided by the platform. The fourth revenue stream is the asset sale and refers to selling physical products. The fifth revenue stream is in advertising: some networks might offer advertisement opportunities on their platform. A sixth revenue stream is licensing, which is generated as result of providing intellectual property rights to data sharers or the public. A seventh revenue stream is via donations and indicates the network's full or partial dependence on the contributions of others (donors or the public) to sustain the initiative (Gharesifard et al. 2017). Apart from government sponsorship, the six other revenue streams are most often applied in a bottom-up approach as the set-up targets more profit-driven organizations (Gharesifard et al. 2017). With a vision to improve the accessibility of real time (entomological) data collected via citizen science, the 'government sponsorship' and 'donation' revenue stream may be further explored to sustain a citizen science programme for malaria vector surveillance.

Conclusions

This thesis has shown that (i) a citizen science approach can complement malaria vector surveillance as it provided insight into the spatial and temporal distribution of malaria vectors in areas where routine mosquito monitoring programmes are not established, (ii) both *An. gambiae sensu stricto* and *Anopheles arabiensis* contribute to indoor and outdoor malaria transmission in areas dominated by irrigated fields, (iii) perception of mosquito nuisance can be used as an indicator for mosquito and malaria vector density, (iv) a handmade plastic bottle trap baited with sugar fermenting yeast and light represents an option for inclusion as mosquito collection trap for surveillance activities in a citizen science context in rural areas, (v) socio-economic factors such as house design and building materials explain mosquito and *An. gambiae* abundance, and (vi) environmental factors such as distance to the river network and elevation play an important role in explaining mosquito and malaria vector abundance.

In conclusion, my research demonstrated that citizen science can play an important role in malaria vector surveillance. Based on observations from citizens, it is possible to identify villages and periods of the year in which they are at higher risk of malaria. This knowledge can help government authorities to identify areas that are in most need of vector control operations.

Summary

Rwanda made tremendous achievements in reducing malaria morbidity and mortality through the scale up of vector control interventions including long lasting insecticide nets (LLINs) and indoor residual spraying (IRS) from 2005 up to 2011. However, malaria remains a major public health concern in the country. With the resurgence of the disease during the last few years, improving vector control is becoming urgent. Inadequate financial resources and ecological changes which increase the malaria transmission potential of mosquitoes are among the factors that have caused this upsurge. This has hindered the sustainability of the progress made in malaria reduction and control.

Vector control is one of the most important strategies for malaria prevention and control being undertaken in Rwanda. Currently, the national vector surveillance programme involves 12 sentinel sites. There is a need for more widespread mosquito surveillance to produce maps of malaria risk for the whole country. In this way available resources for malaria control can be deployed in an efficient way. However, there are significant costs associated with the expansion of the surveillance programme. The implementation of a citizen science programme for (malaria) mosquito surveillance has the potential to overcome these limitations. The main objective of this thesis was to investigate the opportunities for using citizen science in malaria vector surveillance in low resource settings in Rwanda, especially in areas where mosquito monitoring is not established.

Chapter 2 describes factors that have contributed to the malaria resurgence with a focus on Rwanda. These factors are categorized into biological, environmental and institutional factors. In addition, the chapter examines the current challenges for the vector control surveillance system in Rwanda, and explores the opportunity and feasibility of a citizen science approach as a new and alternative strategy to contribute to the improvement of malaria mosquito surveillance in low resource settings. Results in Chapter 2 showed that key information on the spatio-temporal distribution of mosquito nuisance and malaria vectors collected through citizen science can provide insight into the ecology of malaria vectors, and can thereby help to better understand malaria transmission patterns in Rwanda. The implementation of a sustainable citizen science programme for malaria vector surveillance requires the participation of citizens.

In Chapter 3, the mosquito abundance and species composition of malaria vectors is assessed using Centers for Disease Control and Prevention (CDC) light traps inside houses and by conducting entomological and household surveys in 2017 and 2018. In addition, factors that can explain malaria vector abundance collected indoors are determined. Socio-economic factors such as design and the building materials of the houses (for example tiled roofs, walls made of mud and wood), as well as the number of occupants in the house, predict the number of mosquitoes (Culicidae) in the houses, while the presence of eaves plus walls made of mud and wood predict malaria vector abundance. Secondly, household members were interviewed about malaria risk factors and their perceived level of mosquito nuisance. Perception of mosquito nuisance reported indoors tended to be significantly correlated with the number of *Anopheles gambiae* s.l. and Culicidae collected indoors, but this varied across years and sectors. At village level, nuisance also significantly correlated with *An. gambiae* s.l. and total mosquito density, but only in 2018 and not in 2017. I conclude that the perception of mosquito nuisance denoted in a questionnaire could be used as a global indicator of malaria vector hotspots. Hence, involving citizens in reporting mosquito nuisance can complement malaria mosquito surveillance and control.

To develop and ultimately implement a citizen science programme for sustained malaria mosquito surveillance, in Chapter 4 the co-design process initiated in collaboration with communities in Ruhuha, Rwanda, through participatory design workshops is evaluated. Both the technical and social components are relevant for a sustained citizen science programme. This should entail the inclusion of participants from different backgrounds, and community members should participate in the decision-making on the technical tools for collecting and reporting mosquito species, mosquito nuisance, and confirmed malaria cases. In the development of our citizen science programme, community members set up a social structure to gather the data by nominating representatives to collect the reports and send them to the researchers. These results demonstrate that co-designing a citizen science programme (CSP) with citizens allows for decision on the content, the tool, and the frequency in reporting the observations. The decisions that the citizens took, demonstrate that they have valuable context-specific knowledge and skills, and hence demonstrate that implementing a citizen science programme in a rural area is feasible.

In Chapter 5 the results of the effectiveness of a simple handmade trap with different chemical and physical attractants for capturing malaria vectors in the laboratory and in the field are reported. The trap baited with CO_2 (produced by yeast fermenting sugar) and light collected higher numbers of mosquitoes compared to traps baited with light alone or CO_2 alone. The number of *An. gambiae* s.l. collected in the handmade trap with light and CO_2 was about 8-10% of the number caught with a CDC light trap. So, about 10 volunteers with a handmade trap capture a similar-sized sample of *An. gambiae* as one CDC light trap collects. The results suggest that the handmade plastic bottle trap baited with CO_2 and light represents an option for inclusion in mosquito surveillance activities in a citizen science context in rural areas.

Chapter 6 presents the results of a one-year citizen science programme aimed at determining the spatial and temporal variation of malaria vectors and non-malaria vector mosquitoes, mosquito nuisance and percentages of households having malaria cases in five selected villages of Ruhuha. The results on the applicability of using remote sensing and citizen science data in determining the environmental predictors of malaria vector distribution in the studied areas are also presented. It is shown that through the citizen science approach, the spatial and temporal distribution of malaria vectors were elucidated. Malaria hotspots were found more in the southern part of Ruhuha, especially in Busasamana and Kibaza, the villages that also reported the highest mosquito abundance and nuisance levels.

Chapter 7 underscores the societal and scientific relevance of the thesis and places the research in a wider perspective. I show that a citizen science approach can contribute to mosquito monitoring, and can identify areas that, in view of limited resources for control, are at higher risk of malaria. The overall conclusion of this thesis is that a citizen science programme for malaria vector surveillance has potential for extending the Rwandese malaria vector surveillance into areas where mosquito monitoring is not established. It allows for a more detailed insight in the spatial and temporal variation of malaria risks, and therefore helps to better deploy the limited resources available for malaria control.

References

Abdon-liwanag, B., & Tansengco, M. L. (2015). Feasibility of Brown Sugar and Yeast Solution as a Potential Organic Mosquito Trap (OMT). *Biological and Chemical Research*, *2015*, 357–361.

Abong'O, B., Yu, X., Donnelly, M. J., Geier, M., Gibson, G., Gimnig, J., Ter Kuile, F., Lobo, N. F., Ochomo, E., Munga, S., Ombok, M., Samuels, A., Torr, S. J., Hawkes, F. M. (2018). Host Decoy Trap (HDT) with cattle odour is highly effective for collection of exophagic malaria vectors. *Parasites and Vectors*, *11*(1), 1–11.

Akogbéto, M. C., Dagnon, F., Aïkpon, R., Ossé, R., Salako, A. S., Ahogni, I., Akinro, B., Sominahouin, A., Sidick, A., Tokponnon, F., Padonou, G. G. (2020). Lessons learned, challenges and outlooks for decision-making after a decade of experience monitoring the impact of indoor residual spraying in Benin, West Africa. *Malaria Journal*, *19*(1), 1–16.

Alaii, J. A., Hawley, W. A., Kolczak, M. S., Ter Kuile, F. O., Gimnig, J. E., Vulule, J. M., Odhacha, A., Oloo, A. J., Nahlen, B. L., Phillips-Howard, P. A. (2003). Factors affecting use of permethrin-treated bed nets during a randomized controlled trial in western Kenya. *The American Society of Tropical Medicine and Hygiene*, *68*(Suppl 4), 137–141.

Alipour, H., Ladonni, H., Abai, M. (2005). The Significance of Excito-Repellency Phenomenon in Chemical Control of Malaria Vectors. *Fifth International Conference on Urban Pests*, 495–496.

Alonso, P. L., Brown, G., Arevalo-Herrera, M., Binka, F., Chitnis, C., Collins, F., Doumbo, O. K., Greenwood, B., Hall, B. F., Levine, M. M., Mendis, K., Newman, R. D., Plowe, C. V., Rodriguez, M. H., Sinden, R., Slutsker, L., Tanner, M. (2011). A research Agenda to underpin Malaria Eradication. *PLoS Medicine*, *8*(1), 8.

Amaechi, E. C., Ukpai, O. M., Ohaeri, C. C., Ejike, U. B., Irole-eze, O. P., Egwu, O., Nwadike, C. C. (2018). Distribution and seasonal abundance of Anopheline mosquitoes and their association with rainfall around irrigation and non-irrigation areas in Nigeria. *Cuademos de Investigación UNED Research*, *10*(2), 267–272.

Anderson, E., Ottavianelli, G., Girard, T., Pinnock, S. (2009). Integrated space technology monitoring of mosquitoes and malaria epidemics. *IEEE Potentials*, *28*(5), 18–23.

Asale, A., Kussa, D., Girma, M., Mbogo, C., Mutero, C. M. (2019). Community based integrated vector management for malaria control: Lessons from three years' experience (2016-2018) in Botor-Tolay district, southwestern Ethiopia. *BMC Public Health*, *19*(1), 1–14.

Asidi, A. N., N' Guessan, R., Koffi, A. A., Curtis, C. F., Hougard, J.-M., Chandre, F., Corbel, V., Darriet, F., Zaim, M., Rowland, M. W. (2005). Experimental hut evaluation of bednets treated with an organophosphate. *Malaria Journal*, *4*(1), 1-25.

Asiimwe, C., Gelvin, D., Lee, E., Amor, Y. Ben, Quinto, E., Katureebe, C., Sundaram, L., Bell, D., Berg, M. (2011). Use of an innovative , affordable , and open-source short message service – based tool to monitor malaria in remote areas of Uganda. *American Journal of Tropical Medicine and Hygiene*, *85*(1), 26–33.

Asingizwe, D. (2020). *Citizen science for malaria control in Rwanda: Engagment, motivation, and behaviour change*. PhD thesis, Wageningen University.

Asingizwe, D., Murindahabi, M. M., Koenraadt, C. J. M., Poortvliet, P. M., Vliet, A. J. H. Van, Ingabire, C. M., Hakizimana, E., Mutesa, L., Takken, W., Leeuwis, C. (2019). Co-designing a citizen science program for malaria control in Rwanda. *Sustainability*, *11*(24), 1–17.

Asingizwe, D., Poortvliet, P. M., Koenraadt, C. J. M., Vliet, A. J. H. Van, Ingabire, C. M., Mutesa, L., Leeuwis, C. (2019). Role of individual perceptions in the consistent use of malaria preventive measures : mixed methods evidence from rural Rwanda. *Malaria Journal*, 1–19.

Asingizwe, D., Poortvliet, P. M., Koenraadt, C. J. M., Vliet, A. J. H. Van, Murindahabi, M. M., Ingabire, C., Mutesa, L., Feindt, P. H. (2018). Applying citizen science for malaria prevention in Rwanda : An integrated conceptual framework. *NJAS - Wageningen Journal of Life Sciences*, *86*(87), 111–122.

Barrington, J., Wereko-brobby, O., Ward, P., Mwafongo, W., Kungulwe, S. (2010). SMS for Life : a pilot project to improve anti-malarial drug supply management in rural Tanzania using standard technology. *Malaria Journal*, *9*(1), 298.

Barron, P., Pillay, Y., Fernandes, A., Sebidi, J. (2016). The MomConnect mHealth initiative in South Africa: Early impact on the supply side of MCH services. *Journal of Public Health Policy*, *37*(2), 201–212.

Barták, V. (2010). New computer program for digital terrain analysis. *GIS Ostrava*, *24*, 20.

Bartumeus, F., Costa, G. B., Eritja, R., Id, A. H. K., Finda, M., Lezaun, J., Okumu, F., Quinlan, M. M., Thizy, C. D., Paré Toé, L., Vaughan, M. (2019). Sustainable innovation in vector control requires strong partnerships with communities. *PLOS Negleted Tropical Diseases*, *13*(4), 1–5.

Bartumeus, F., Oltra, A., Palmer, J. R. B. (2018). Citizen Science: A gateway for innovation in disease-carrying mosquito management? *Trends in Parasitology*, 1–3.

Bates, I., Fenton, C., Gruber, J., Lalloo, D., Lara, A. M., Squire, S. B., Theobald, S., Thomson, R., Tolhurst, R. (2004). Vulnerability to malaria , tuberculosis , and HIV / AIDS infection and disease . Part 1 : household level. *Lancet Infectious Disease*, *4*(5), 267–277.

Bazin, M., & Williams, C. R. (2018). Mosquito traps for urban surveillance : collection efficacy and potential for use by citizen scientists. *Vector Ecology*, *43*(1), 98–103.

Beier, J. C., & Koros, J. K. (1991). Visual assessment of sporozoite and bloodmeal ELISA samples in malaria field studies. *Journal of Medical Entomology*, *28*(6), 805–808.

Beier, J. C., Perkins, P. V., Wirtz, R. A., Koros, J., Diggs, D., Gargan, T. P., Koech, D. K. (1988). Bloodmeal identification by direct enzyme-linked immunosorbent assay (ELISA), tested on *Anopheles* (Diptera: Culicidae) in Kenya. *Journal of Medical Entomology*, *25*(1), 9–16.

Benallal, K. E., Garni, R., Bouiba, L., Harrat, Z. (2019). Case report first detection of *Aedes* (*Stegomyia*) *albopictus* (Diptera: Culicidae) in Algiers, the capital city of Algeria. *J Arthropod-Borne Dis*, *13*(4), 420–425.

Benelli, G., & Beier, J. C. (2017). Current vector control challenges in the fight against malaria. *Acta Tropica*, *174*, 91–96.

Beza, E., Reidsma, P., Poortvliet, P. M., Misker, M., Sjors, B. (2018). Exploring farmers ' intentions to adopt mobile Short Message Service (SMS) for citizen science in agriculture. *Computers and Electronics in Agriculture*, *151*, 295–310.

Bhatt, S., Weiss, D. J., Cameron, E., Bisanzio, D., Mappin, B., Dalrymple, U., Battle, K. E., Moyes, C. L., Henry, A., Eckhoff, P. A., Wenger, E. A., Briët, O., Penny, M. A., Smith, T. A., Bennett, A., Yukich, J., Eisele, T. P., Griffin, J. T., Fergus, C. A., Lynch, M., Lindgren, F., Cohen10, J. M., Murray, C. L. J., Smith, D. L., Hay, S.I., Cibulskis, R. E., Gething, P. W. (2015). The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015. *Nature*, *526*(7572), 207–211.

Bhunu, C. P., Masocha, M., Mashonjowa, E., Ngarakana-Gwasira, E. T. (2016). Assessing the Role of Climate Change in Malaria Transmission in Africa. *Malaria Research and Treatment*, *2016*, 7.

Bijllaardt, W. Van Den, Shekalaghe, S., Otieno, S., Mahande, A., Sauerwein, R., Takken, W., Bousema, T. (2009). The suitability of clay pots for indoor sampling of mosquitoes in an arid area in northern Tanzania. *Acta Tropica*, *111*(2), 197–199.

Binagwaho, A., Farmer, P. E., Nsanzimana, S., Karema, C., Gasana, M., Ngirabega, J. de D., Ngabo, F., Wagner, C. M., Nutt, C. T., Nyatanyi, T., Gatera, M., Kayiteshonga, Y., Mugeni, C., Mugwaneza, P., Shema, J., Uwaliraye, P., Gaju, E., Muhimpundu, M. A., Dushime, T., Senyana, F., Muzayire Gaju, C., Rusanganwa, V., Umutoni, A., Kankindi, I., Ntizimira, C., Ruton, H., Mugume, N., Nkunda, D., Ndenga, E., Mubiligi, J. M ., Mukherjee, J. S., Bukhman, G., van der Hoof Holstein, C., Bonds, M. H., Wilkes, M. S., Lu, C., Smith-Fawzi, M. C., Mazarati, J. B., Mutabazi, V., Nyemazi, J. P., Kyamanywa, P., Rusingiza, E., Kakoma, J. B., Karita, E., Sekabaraga, C., Rich, M. L., Bertrand-Farmer, D., Habinshuti, A., Dahl, O., Drobac, P. C., Rhatigan, J. Cancedda, C., Stulac, S. N., Tapela, N. M., Swain, J. D., Shulman, L. N., Murphy, M. P., Ricks, A., Kerry, V. B., Bush, B. P., Siegler, R. W., Stern, C. S., Sliney, A., Nuthulaganti, T., Karangwa, I., Pegurri, E., Tuyisenge, M. (2014). Rwanda 20 years on: investing in life. *Lancet*, *384*(9940), 371–375.

Binagwaho, A., & Karema, C. (2015). A call for international accountability-preserving hope amid false protection. *The Lancet Global Health*, *3*(4), 188–189.

Bizimana, J.-P., Twarabamenye, E., Kienberger, S. (2015). Assessing the social vulnerability to malaria in Rwanda. *Malaria Journal*, *14*(1), 2.

Bonney, R., Cooper, C. B., Dickinson, J., Kelling, S., Phillips, T., Rosenberg, K. V., Shirk, J. (2009). Citizen Science: A Developing Tool for Expanding Science Knowledge and Scientific Literacy. *BioScience*, *59*(11), 977–984.

Bonney, Rick, Phillips, T. B., Ballard, H. L., Enck, J. W. (2016). Can citizen science enhance public understanding of science? *Public Understanding of Science*, *25*(1), 2–16.

Bonney, Rick, Shirk, J. L., Phillips, T. B., Wiggins, A., Ballard, H. L., Miller-rushing, A. J., Parrish, J. K. (2014). Next steps for citizen science. *Science*, *343*(6178), 1436–1437.

Bottomley, C., Tusting, L. S., Bottomley, C., Gibson, H., Kleinschmidt, I., Tatem, J., Lindsay, S. W., Gething, P. W. (2017). Housing improvements and malaria risk in Sub-Saharan Africa: A multi-housing improvements and malaria Risk in Sub-Saharan Africa: A multi-country analysis of survey data. *PLoS Medicine*, *14*(2). 1-15.

Bradley, J., Lines, J., Fuseini, G., Schwabe, C., Monti, F., Slotman, M., Vargas, D., Garcia, G., Hergott, D., Kleinschmidt, I. (2015). Outdoor biting by *Anopheles* mosquitoes on Bioko Island does not currently impact on malaria control. *Malaria Journal*, *14(1)*, 1-8.

Braz, L. S., Fricker, S. R., Doherty, S. S., Webb, C. E., Baldock, K. L., Williams, C. R. (2020). Citizen science and smartphone e-entomology enables low-cost upscaling of mosquito surveillance. *Science of the Total Environment*, *704*, 1-10.

Bucagu, M. (2016). Improving maternal health in Rwanda: The role of community-based interventions: a systematic review (2005-2015). *Journal of Community Medicine & Health Education*, *6*(3), 1–6.

Busula, A. O., Takken, W., Loy, D. E., Hahn, B. H., Mukabana, W. R., Verhulst, N. O. (2015). Mosquito host preferences affect their response to synthetic and natural odour blends. *Malaria Journal*, *14*(133), 1-9.

Caley, P., Welvaert, M., Barry, S. C. (2020). Crowd surveillance: estimating citizen science reporting probabilities for insects of biosecurity concern: Implications for plant biosecurity surveillance. *Journal of Pest Science*, *93*(1), 543–550.

Campos, A. S. De, Hartley, S., Koning, C. De, Lezaun, J. (2017). Responsible Innovation and political accountability : genetically modified mosquitoes in Brazil. *Journal of Responsible Innovation*, *4(1)*, 5-25.

Carrasco, D., Lefèvre, T., Moiroux, N., Pennetier, C., Chandre, F., Cohuet, A. (2019). Behavioural adaptations of mosquito vectors to insecticide control. *Current Opinion in Insect Science*, *34*, 48–54.

Carter, R., Mendis, K. N., Roberts, D. (2000). Spatial targeting of interventions against malaria. *Bulletin of the World Health Organization*, *78*(12), 1401–1411.

Castro, M. C., Kanamori, S., Kannady, K., Mkude, S., Killeen, G. F., Fillinger, U. (2010). The importance of drains for the larval development of lymphatic filariasis and malaria vectors in dares salaam, United Republic of Tanzania. *PLoS Neglected Tropical Diseases*, *4*(5).

Ceccato, P., Connor, S. J., Jeanne, I., Thomson, M. C. (2005). Application of Geographical Information Systems and Remote Sensing technologies for assessing and monitoring malaria risk. *Parassitologia*, *47*, 81–96.

Chaki, P. P., Dongus, S., Fillinger, U., Kelly, A., Killeen, G. F. (2011). Community-owned resource persons for malaria vector control: enabling factors and challenges in an operational programme in Dar es Salaam, United Republic of Tanzania. *Human Resources for Health*, *9*(1),1-21.

Chaki, P. P., Govella, N. J., Shoo, B., Hemed, A., Tanner, M., Fillinger, U., Killeen, G. F. (2009). Achieving high coverage of larval-stage mosquito surveillance: challenges for a community-based mosquito control programme in urban Dar es Salaam, Tanzania. *Malaria Journal*, *8*(1), 311.

Chandre, F., Dabire, R. K., Hougard, J.-M., Djogbenou, L. S., Irish, S. R., Rowland, M., N'Guessan, R. (2010). Field efficacy of pyrethroid treated plastic sheeting (durable lining) in combination with long lasting insecticidal nets against malaria vectors. *Parasites &*

Vectors, *3*(1), 65.

Charlwood, J. D., Pinto, J., Ferrara, P. R., Sousa, C. A., Ferreira, C., Gil, V., Rosário, V. E. (2003). Raised houses reduce mosquito bites. *Malaria Journal*, *2*(45), 1–6.

Chaves, L. F., Satake, A., Hashizume, M., Minakawa, N. (2012). Indian ocean dipole and rainfall drive a moran effect in east Africa malaria transmission. *Journal of Infectious Diseases*, *205*(12), 1885–1891.

Chizema-Kawesha, E., Miller, J. M., Steketee, R. W., Mukonka, V. M., Mukuka, C., Mohamed, A. D., Miti, S. K., Campbell, C. C. (2010). Scaling up malaria control in Zambia: Progress and impact 2005-2008. *American Journal of Tropical Medicine and Hygiene*, *83*(3), 480–488.

Cieslik, K. J., Leeuwis, C., Dewulf, A. R. P. J., Lie, R., Werners, S. E., Wessel, M. Van, Feindt, P., Struik, P. C. (2018). Addressing socio-ecological development challenges in the digital age: Exploring the potential of Environmental Virtual Observatories for Connective Action (EVOCA). *NJAS - Wageningen Journal of Life Sciences*, 86–87, 2–11.

Cleckner, H. L., Allen, T. R., Scott Bellows, A. (2011). Remote sensing and modeling of mosquito abundance and habitats in Coastal Virginia, USA. *Remote Sensing*, *3*(12), 2663–2681.

Coetzee, M., Hunt, R. H., Wilkerson, R., Torre, A. Della, Coulibaly, M. B., Besansky, N. J. (2013). *Anopheles coluzzii* and *Anopheles amharicus*, new members of the *Anopheles gambiae* complex. *Zootaxa*, *3619*(3), 246–274.

Coleman, M., & Hemingway, J. (2008). The implications of entomological monitoring and evaluation for arthropod vector-borne disease control programs. In *Vector-Borne Diseases: Understanding the Environmental, Human Health, and Ecological Connections, Workshop Summary* (p. 350). Institute of Medicine.

Condo, J., Mugeni, C., Naughton, B., Hall, K., Tuazon, M. A., Omwega, A., Nwaigwe, F., Drobac, P., Hyder, Z., Ngabo, F., Binagwaho, A. (2014). Rwanda's evolving community health worker system: a qualitative assessment of client and provider perspectives. *Human Resources for Health*, *12*(71), 71.

Crain, R., Cooper, C., Dickinson, J. L. (2014). Citizen Science: a tool for integrating studies of human and natural systems. *Annual Review of Environment and Resources*, *39*(1), 641–665.

Davis, R. G., Kamanga, A., Castillo-Salgado, C., Chime, N., Mharakurwa, S., Shiff, C. (2011). Early detection of malaria foci for targeted interventions in endemic southern Zambia. *Malaria Journal,* 10(1), 2-10.

De Silva, P. M., & Marshall, J. M. (2012). Factors contributing to urban malaria transmission in sub-saharan Africa: A systematic review. *Journal of Tropical Medicine*, *2012*, 1-10.

Delenasaw, Y., & J. Kweka, E. (2016). Insecticide resistance in East Africa — History , distribution and drawbacks on malaria vectors and disease control. *Insecticide Resistance*. *InTech*, p29.

Dhiman, S. (2019). Are malaria elimination efforts on right track ? An analysis of gains achieved and challenges ahead. *Infectious Diseases of Poverty*, *8*(14), 1–19.

Diabaté, A., Bilgo, E., Dabiré, R. K., Tripet, F. (2013). Environmentally friendly tool to control mosquito populations without risk of insecticide resistance: the Lehmann's funnel entry trap. *Malaria Journal*, *12*(196), 8.

Diagne, N., Fontenille, D., Konate, L., Faye, O., Lamizana, M. T., Legros, F., Molez, J. F., Trape, J. F. (1994). Les *Anopheles* du Senegal. *Bull. Soc. Path. Ex.*, *87*, 267–277.

Dickinson, J. L., Shirk, J., Bonter, D., Bonney, R., Crain, R. L., Martin, J., Phillips, T., Purcell, K. (2012). The current state of citizen science as a tool for ecological research and public engagement. *Citizen Science*, *10*(6), 291–297.

Dickinson, K., & Paskewitz, S. (2012). Willingness to pay for mosquito control: how Important is West Nile Virus risk compared to the nuisance of mosquitoes? *Vector-Borne and Zoonotic Diseases*, *12*(10), 886–892.

Dida, G. O., Anyona, D. N., Abuom, P. O., Akoko, D., Adoka, S. O., Matano, A., Owuor, P. O., Ouma, C. (2018). Spatial distribution and habitat characterization of mosquito species during the dry season along the Mara River and its tributaries, in Kenya and Tanzania. *Infection, Genetics and Evolution*, *7*(2), 1–16.

Dinku, T., Cousin, R., Corral, J., Ceccato, P., Thomson, M. C., Faniriantsoa, R., Khomyakov, I., Vadillo, A. (2016). The ENACTS Approach: Transforming climate services in Africa one country at a time. In *World Policy Papers,* 1-24*.*

Doolan, D. L., Dobaño, C., Baird, J. K. (2009). Acquired immunity to Malaria. *Clinical Microbiology Reviews*, *22*(1), 13–36.

Dotson, E. M., Linser, P., Koekemoer, L., Benedict, M. Q. (2011). *Methods in Anopheles* (2nd Edition).

Durnez, L., & Coosemans, M. (2013). Residual transmission of malaria: an old issue for new approaches. *InTech*, 1-35.

Dykstra, L. (2008). Guidance for surveillance, prevention, and control of mosquito-borne disease. 2008 Edition*, Washington State Department of Health, 1-137.*

ECDC. (2012). Guidelines for the surveillance of invasive mosquito species in Europe. Stockholm, Sweden. p100

Eisele, T. P., Thwing, J., Keating, J. (2011). Claims about the misuse of insecticide-treated mosquito nets: Are these evidence-based? *PLoS Medicine*, *8*(4), 2.

ENACTS. (2016). *Meteo Rwanda Map Room*. http://maproom.meteorwanda.gov.rw/maproom/

Entsieh, A. A., Emmelin, M., Pettersson, K. O. (2015). Learning the ABCs of pregnancy and newborn care through mobile technology. *Global Health Action*, *8*(29340), 1–10.

Eritja, R., Ruiz-arrondo, I., Delacour-estrella, S., Schaffner, F., Álvarez-chachero, J., Bengoa, M., Puig, M.- Ángeles, Melero-Alcíbar, R., Oltra, A., Bartumeus, F. (2019). First detection of *Aedes japonicus* in Spain : an unexpected finding triggered by citizen science. *Parasites & Vectors*, 1–9.

Eskenazi, B., Quirós-Alcalá, L., Lipsitt, J. M., Wua, L. D., Kruger, P., Ntimbane, T., Nawn, J. B., Bornman, M. S. R., Seto, E. (2014). mSpray: a mobile phone technology to improve malaria control efforts and monitor human exposure to malaria control pesticides in Limpopo, South Africa. *Environment International*, *68*(510), 219–226.

Feachem, R. G., Phillips, A. A., Targett, G. A., Snow, R. W. (2010). Call to action: Priorities for malaria elimination. *The Lancet*, *376*(9752), 1517–1521.

Fenny, A. P., Yates, R., Thompson, R. (2018). Social health insurance schemes in Africa leave out the poor. *International Health*, *10*(1), 1–3.

Ferguson, H. M., Dornhaus, A., Beeche, A., Borgemeister, C., Gottlieb, M., Mulla, M. S., Gimnig, J. E., Fish, D., Killeen, G. F. (2010). Ecology: A prerequisite for malaria elimination and eradication. *PLoS Medicine*, *7*(8), 1–8.

Ferreira, C. P., Lyra, S. P., Azevedo, F., Greenhalgh, D., Massad, E. (2017). Modelling the impact of the long-term use of insecticide-treated bed nets on *Anopheles* mosquito biting time. *Malaria Journal*, *16*(1), 1–11.

Fisher, M. (1985). Malaria at high altitudes in Africa. *British Medical Journal*, *291*(6487), 56.

Fletcher, M., Teklehaimanot, A., Yemane, G. (1992). Control of mosquito larvae in the port city of Assab by an indigenous larvivorous fish, *Aphanius dispar*. *Acta Tropica*, *52*(2–3), 155–166.

Fouet, C., & Kamdem, C. (2018). Integrated mosquito management : Is precision control a luxury or necessity ? *Trends in Parasitology*, *35(1)*, 85–95.

Frake, A. N., Namaona, W., Walker, E. D., Messina, J. P. (2020). Estimating spatio-temporal distributions of mosquito breeding pools in irrigated agricultural schemes: A case study at the Bwanje Valley Irrigation Scheme. *Malaria Journal*, *19*(1), 1–21.

Franzoni, C., & Sauermann, H. (2014). Crowd science: The organization of scientific research in open collaborative projects. *Research Policy*, *43*(1), 1–20.

Frederick, J., Saint Jean, Y., Lemoine, J. F., Dotson, E. M., Mace, K. E., Chang, M., Slutsker, L., Le Menach, A., Beier, J. C., Eisele, T. P., Okech, B. A., Beau de Rochars, V. M., Carter, K. H., Keating, J., Impoinvil, D. E. (2016). Malaria vector research and control in Haiti: a systematic review. *Malaria Journal*, *15*(1), 17.

Gahutu, J.-B., Steininger, C., Shyirambere, C., Zeile, I., Cwinya-Ay, N., Danquah, I., Larsen, C. H., Eggelte, T. A., Uwimana, A., Karema, C., Musemakweri, A., Harms, G., Mockenhaupt, F. P. (2011). Prevalence and risk factors of malaria among children in southern highland Rwanda. *Malaria Journal*, *10*(1), 1–12.

Garba, L. C., Oyieke, F. A., Owino, E. A., Mwansat, G. S., D.G.W, W. C. (2020). Molecular characterization and species composition of anopheline vectors of malaria along an altitudinal gradient on the highlands of Mambilla plateau Northeast, Nigeria. *African Journal of Biological Sciences*, *02*(01), 18.

Garnero, G., & Godone, D. (2013). Comparisons between different interpolation techniques. *International Archives of the Photogrammetry, Remote Sensing and Spatial Information*

Sciences - ISPRS Archives, *40*(5W3), 139–144.

Gharesifard, M., Wehn, U., van der Zaag, P. (2017). Towards benchmarking citizen observatories: Features and functioning of online amateur weather networks. *Journal of Environmental Management*, *193*, 381–393.

Gibson, D. G., Ochieng, B., Kagucia, E. W., Were, J., Hayford, K., Moulton, L. H., Levine, O. S., Odhiambo, F., Brien, K. L. O., Feikin, D. R., Bill, F., Foundation, M. G. (2017). Mobile phone-delivered reminders and incentives to improve childhood immunisation coverage and timeliness in Kenya (M-SIMU): a cluster randomised controlled trial. *The Lancet Global Health*, *5*(4), e428–e438.

Gillies, M. T. (1980). The role of carbon dioxide in host-finding by mosquitoes. *Bulletin of Entomological Research*, *70*(1940), 525–532.

Gillies, M. T., & Coetzee, M. (1987). A Supplement to the Anophelinae of Africa South of the Sahara. *Publications of the South African Institute for Medical Research*, *55*(55), 63.

Gimnig, J. E., Walker, E. D., Otieno, P., Kosgei, J., Olang, G., Ombok, M., Williamson, J., Marwanga, D., Abong'o, D., Desai, M., Kariuki, S., Hamel, M. J., Lobo, N. F., Vulule, J., Bayoh, M. N. (2013). Incidence of malaria among mosquito collectors conducting human landing catches in western kenya. *American Journal of Tropical Medicine and Hygiene*, *88*(2), 301–308.

Githeko, A. K., Lindsay, S. W., Confalonieri, U. E., Patz, J. A., Lindsay, S. W., Confalonieri, U. E., Patz, J. A., (2000). Climate change and vector-borne diseases: A regional analysis. In *Bulletin of the World Health Organization* 78*(9*), 1136–1147.

Githinji, S., Kigen, S., Memusi, D., Nyandigisi, A., Wamari, A., Muturi, A., Jagoe, G., Ziegler, R., Snow, R. W., Zurovac, D. (2014). Using mobile phone text messaging for malaria surveillance in rural Kenya. *Malaria Journal*, *13*, 107.

Govella, Okumu, F. O., Killeen, G. F. (2010). Short report: Insecticide-treated nets can reduce malaria transmission by mosquitoes which feed outdoors. *American Journal of Tropical Medicine and Hygiene*, *82*(3), 415–419.

Guilbaud, C. S. E., & Guilbaud, T. G. D. P. V. (2017). Mosquito Mapper : a phone application to map urban mosquitoes. *Scientific Phone Apps and Mobile Devices*, *3*(6), 6.

Haddow, A. J., & Ssenkubuge, Y. (1973). The mosquitoes of Bwamba County, Uganda. IX. Further studies on the biting behaviour of an outdoor population of the *Anopheles gambiae* Giles complex. *Bulletin of Entomological Research*, *62*(3), 407–414.

Hakizimana, E. (2019). Integrated vector management (IVM) as a tool for community empowerment towards malaria elimination in Rwanda. PhD thesis, Wageningen University.

Hakizimana, E., Cyubahiro, B., Rukundo, A., Kabayiza, A., Mutabazi, A., Beach, R., Patel, R., Tongren, J. E., Karema, C. (2014). Monitoring long-lasting insecticidal net (LLIN) durability to validate net serviceable life assumptions, in Rwanda. *Malaria Journal*, *13*(1), 1–8.

Hakizimana, E., Karema, C., Munyakanage, D., Githure, J., Mazarati, J. B., Tongren, J. E., Takken, W., Binagwaho, A., Koenraadt, C. J. M. (2018). Spatio-temporal distribution of mosquitoes and risk of malaria infection in Rwanda. *Acta Tropica*, *182*(February), 149–157.

Hakizimana, E., Karema, C., Munyakanage, D., Iranzi, G., Githure, J., Tongren, J. E., Takken, W., Binagwaho, A., Koenraadt, C. J. M. (2016). Susceptibility of *Anopheles gambiae* to insecticides used for malaria vector control in Rwanda. *Malaria Journal*, *15*(1), 582.

Hamainza, B., Killeen, G. F., Kamuliwo, M., Bennett, A., Yukich, J. O. (2014). Comparison of a mobile phone-based malaria reporting system with source participant register data for capturing spatial and temporal trends in epidemiological indicators of malaria transmission collected by community health workers in rural Zambia. *Malaria Journal*, *13*, 489.

Hamer, S. A., Curtis-Robles, R., Hamer, G. L. (2018). Contributions of citizen scientists to arthropod vector data in the age of digital epidemiology. *Science Direct*, *28*, 98–104.

Hawaria, D., Demissew, A., Kibret, S., Lee, M., Yewhalaw, D. (2020). Effects of environmental modification on the diversity and positivity of anopheline mosquito aquatic habitats at Arjo-Dedessa irrigation development site , Southwest Ethiopia. *Infectious Diseases of Poverty*, *9*(9), 1–11.

Hawkes, F. M., Dabiré, R. K., Sawadogo, S. P., Torr, S. J., Gibson, G. (2017). Exploiting *Anopheles* responses to thermal, odour and visual stimuli to improve surveillance and control of malaria. *Scientific Reports*, *7*(1), 17283.

Hay, S. I., Cox, J. C., Rogers, D. J., Randolph, S. E., Stern, D. I., Shanks, G. D., Myers, M. F., Snow, R. W. (2002). Climate change and the resurgence of malaria in the East African highlands. *Nature*, *415*(6874), 905–909.

Hay, S. I., Sinka, M. E., Okara, R. M., Kabaria, C. W., Mbithi, P. M., Carolynn, C., Benz, D., Gething, P. W., Howes, R. E., Patil, A. P., Temperley, W. H., Bangs, M. J., Chareonviriyaphap, T., Elyazar, I. R. F., Harbach, R. E., Manguin, S., Mbogo, C. M., Rubio-palis, Y., Godfray, H. C. J. (2010). Developing global maps of the dominant *Anopheles* vectors of human Malaria. *PLoS Med*, *7*(2), 1–6.

Head, M. G., Goss, S., Gelister, Y., Alegana, V., Brown, R. J., Clarke, S. C., Fitchett, J. R. A., Atun, R., Scott, J. A. G., Newell, M. L., Padmadas, S. S., Tatem, A. J. (2017). Global funding trends for malaria research in sub-Saharan Africa: a systematic analysis. *The Lancet Global Health*, *5*(8), e772–e781.

Hecker, S., Bonney, R., Haklay, M., Hölker, F., Hofer, H., Goebel, C., Gold, M., Makuch, Z., Ponti, M., Richter, A., Robinson, L., Iglesias, J. R., Owen, R., Peltola, T., Sforzi, A., Shirk, J., Vogel, J., Vohland, K., Witt, T., Bonn, A. (2018). Innovation in citizen science – perspectives on science-policy advances. *Citizen Science: Theory and Practice*, *3*(1), 1–14.

Heym, E. C., Schröder, J., Kampen, H., Walther, D. (2017). The Nuisance Mosquito *Anopheles plumbeus* (Stephens, 1828) in Germany—A Questionnaire survey may help support surveillance and control. *Frontiers in Public Health*, *5*(October), 1–8.

Himeidan, Y. E., & Kweka, E. J. (2012). Malaria in East African highlands during the past 30 years: impact of environmental changes. *Frontiers in Physiology*, (August), 1–11.

Hinzoumbé, C. K., Péka, M., Antonio-, C., Donan-gouni, I., Awono-ambene, P., Samè-, A., Simard, F. (2009). Malaria vectors and transmission dynamics in Goulmoun, a rural city in south-western Chad. *BMC Infectious Diseases*, *11*(9:71), 1–11.

Hiscox, A., Otieno, B., Kibet, A., Mweresa, C. K., Omusula, P., Geier, M., Rose, A., Mukabana, W. R., Takken, W. (2014). Development and optimization of the suna trap as a tool for mosquito monitoring and control. *Malaria Journal*, *13*(1), 257.

Hoel, D. F., Dunford, J. C., Kline, D. L., Irish, S. R., Weber, M., Richardson, A. G., Doud, C. W., Wirtz, R. A., Richardson, A. G., Doud, C. W., Wirtz, R. A. (2015). A Comparison of carbon dioxide sources for mosquito capture in Centers for Disease Control and Prevention Light Traps on the Florida Gulf Coast. *Journal of the American Mosquito Control Association*, *31*(3), 248–257.

Hoel, D. F., Marika, J. A., Dunford, J. C., Irish, S. R., Geier, M., Obermayr, U., Wirtz, R. A. (2014). Optimizing collection of *Anopheles gambiae* s.s. (Diptera: Culicidae) in biogents sentinel Traps. *Journal of Medical Entomology*, *51*(6), 1268–1275.

Homan, T. (2015). Impact of odour-baited mosquito traps for malaria control: design and evaluation of a trial using solar-poweed mosquito trapping systems in Western kenya. PhD thesis, WageningenUniversity. *2015* (Vol. 1).

Honjo, K., Chaves, L. F., Satake, A., Kaneko, A., Minakawa, N. (2013). When they don't bite, we smell money: understanding malaria bednet misuse. *Parasitology*, *140*(5), 580–586.

Hovis, M., Cubbage, F., Rashash, D. (2020). Designing a Citizen Science Project for Forest Landscapes : A Case from Hofmann forest in Eastern North Carolina. *Scientific Research Publishing*, *10*(2), 187–203.

Ijumba, J. N., & Lindsay, S. W. (2001). Impact of irrigation on malaria in Africa: paddies paradox. *Medical and Veterinary Entomology*, *15*(1), 1–11.

Ingabire, C. M., Alaii, J., Hakizimana, E., Kateera, F., Muhimuzi, D., Nieuwold, I., Bezooijen, K., Rulisa, S., Kaligirwa, N., Muvunyi, C., Koenraadt, C. J. M., Mutesa, L., Van Vugt, M., Van Den Borne, B. (2014). Community mobilization for malaria elimination: application of an open space methodology in Ruhuha sector, Rwanda. *Malaria Journal*, *13*(1), 167.

Ingabire, C. M., Hakizimana, E., Kateera, F., Rulisa, A., Van Den Borne, B., Nieuwold, I., Muvunyi, C., Koenraadt, C. J. M., Van Vugt, M., Mutesa, L., Alaii, J. (2016). Using an intervention mapping approach for planning, implementing and assessing a community-led project towards malaria elimination in the Eastern Province of Rwanda. *Malaria Journal*, *15*(1), 594.

Ingabire, C. M., Hakizimana, E., Rulisa, A., Kateera, F., Van Den Borne, B., Muvunyi, C. M., Mutesa, L., Van Vugt, M., Koenraadt, C. J. M., Takken, W., Alaii, J. (2017). Community-based biological control of malaria mosquitoes using *Bacillus thuringiensis* var. *israelensis* (*Bti*) in Rwanda: Community awareness, acceptance and participation. *Malaria Journal*, *16*(1), 1–13.

Ingabire, C. M., Kateera, F., Hakizimana, E., Rulisa, A., Muvunyi, C., Mens, P., Koenraadt, C. J. M., Mutesa, L., Van Vugt, M., Van Den Borne, B., Alaii, J. (2016). Determinants of prompt and adequate care among presumed malaria cases in a community in eastern Rwanda: a cross sectional study. *Malaria Journal*, *15*(1), 227.

Ingabire, C. M., Rulisa, A., Van Kempen, L., Muvunyi, C., Koenraadt, C. J. M., Van Vugt, M., Mutesa, L., Van Den Borne, B., Alaii, J. (2015). Factors impeding the acceptability and use of malaria preventive measures: implications for malaria elimination in eastern Rwanda. *Malaria Journal*, *14*(1), 1–11.

Jaenisch, T., Sullivan, D. J., Dutta, A., Deb, S., Ramsan, M., Othman, M. K., Gaczkowski, R., Tielsch, J., Sazawal, S. (2010). Malaria incidence and prevalence on Pemba island before the onset of the successful control intervention on the Zanzibar archipelago. *Malaria Journal*, *9*(1), 32.

Jager, C. de, Kruger, T., Tosh, C. (2019). Research, Innovation and Education Towards Malaria Elimination: Improving Quality of Life in Africa. In Irma Eloff Editor (Ed.), *Handbook of quality of life in African Societies* (p. 455). Springer.

Jatta, E., Jawara, M., Bradley, J., Jeffries, D., Kandeh, B., Knudsen, J. B., Wilson, A. L., Pinder, M., Alessandro, U. D. (2015). Articles How house design affects malaria mosquito density , temperature , and relative humidity : an experimental study in rural Gambia. *The Lancet Planetary Health*, *2*(11), e498–e508.

Johnson, B. A., Mader, A. D., Dasgupta, R., Kumar, P. (2020). Citizen science and invasive alien species: An analysis of citizen science initiatives using information and communications technology (ICT) to collect invasive alien species observations. *Global Ecology and Conservation*, *21*.

Johnson, B. J., Brosch, D., Christiansen, A., Wells, E., Wells, M., Bhandoola, A. F., Milne, A., Garrison, S., Fonseca, D. M. (2018). Neighbors help neighbors control urban mosquitoes. *Scientific Reports*, *8*(1), 1–6.

Jordan, R. C., Gray, S., Sorensen, A., Newman, G., Mellor, D., Newman, G., Hmelo-silver, C., Ladeau, S., Biehler, D., Crall, A. (2016). Studying citizen science through adaptive management and learning feedbacks as mechanisms for improving conservation. *Conservation Biology*, *30*(3), 487–495.

Jordan, R. C., Sorensen, A. E., Ladeau, S. (2017). Citizen Science as a tool for mosquito control. *Journal of the American Mosquito Control Association*, *33*(3), 241–245.

Kabanywanyi, A. M., Mulure, N., Migoha, C., Malila, A., Lengeler, C., Schlienger, R., Genton, B. (2010). Experience of safety monitoring in the context of a prospective observational study of artemether-lumefantrine in rural Tanzania: lessons learned for pharmacovigilance reporting. *Malaria Journal*, *9*, 205.

Kaindoa, E. W., Finda, M., Kiplagat, J., Mkandawile, G., Nyoni, A., Coetzee, M., Okumu, F. O. (2018). Housing gaps , mosquitoes and public viewpoints : a mixed methods assessment of relationships between house characteristics, malaria vector biting risk and community perspectives in rural Tanzania. *Malaria Journal*, 17(1), 1–16.

Kamanga, A., Moono, P., Stresman, G., Mharakurwa, S., Shiff, C. (2010). Rural health centres, communities and malaria case detection in Zambia using mobile telephones: a means to detect potential reservoirs of infection in unstable transmission conditions. *Malaria*

Journal, *9*, 96.

Kamau, L., Mulaya, N., Vulule, J. M. (2006). Evaluation of Potential Role of *Anopheles ziemanni* in malaria transmission in Western Kenya. *Journal of Medical Entomology*, *43*(4), 774–776.

Kampen, H., Medlock, J. M., Vaux, A. G., Koenraadt, C. J., van Vliet, A. J., Bartumeus, F., Oltra, A., Sousa, C. A., Chouin, S., Werner, D. (2015). Approaches to passive mosquito surveillance in the EU. *Parasites & Vectors*, *8*(1), 1–13.

Kantele, A., & Jokiranta, T. S. (2011). Review of cases with the emerging fifth human malaria parasite, *Plasmodium knowlesi*. *Clinical Infectious Diseases*, *52*(11), 1356–1362.

Karema, C., Aregawi, M. W., Rukundo, A., Kabayiza, A., Mulindahabi, M., Fall, I. S., Gausi, K., Williams, R. O., Lynch, M., Cibulskis, R., Fidele, N., Nyemazi, J.-P., Ngamije, D., Umulisa, I., Newman, R., Binagwaho, A. (2012). Trends in malaria cases, hospital admissions and deaths following scale-up of anti-malarial interventions, 2000–2010, Rwanda. *Malaria Journal*, *11*(1), 1–13.

Karina, L., Qualls, W., Brown, S., Beier, J. (2015). Expanding integrated vector management to promote healthy environments. *Trends in Parasitology*, *30*(8), 394–400.

Kateera, F. . (2016). Determinants of malaria control in a rural community in Eastern Rwanda. PhD thesis, Universiteit van Amsterdam.

Kateera, F., Ingabire, C. M., Hakizimana, E., Kalinda, P., Mens, P. F., Grobusch, M. P., Mutesa, L., Vugt, M. (2015). Malaria, anaemia and under-nutrition: three frequently co-existing conditions among preschool children in rural Rwanda. *Malaria Journal*, *14*(1), 1–11.

Kateera, F., Ingabire, C. M., Hakizimana, E., Rulisa, A., Karinda, P., Grobusch, M. P., Mutesa, L., van Vugt, M., Mens, P. F. (2015). Long-lasting insecticidal net source, ownership and use in the context of universal coverage: a household survey in eastern Rwanda. *Malaria Journal*, *14*(1), 390.

Kateera, F., Mens, P. F., Hakizimana, E., Ingabire, C. M., Muragijemariya, L., Karinda, P., Grobusch, M. P., Mutesa, L., van Vugt, M. (2015). Malaria parasite carriage and risk determinants in a rural population: a malariometric survey in Rwanda. *Malaria Journal*, *14*(1), 16.

Keiser, J., De Castro, M. C., Maltese, M. F. (2005). Effect of irrigation and large dams on the burden of malaria on a global and regional scale. *The American Journal of Tropical Medicine and Hygiene*, *72*(4), 392–406.

Kelly-Hope, L. A., Hemingway, J., McKenzie, F. E. (2009). Environmental factors associated with the malaria vectors *Anopheles gambiae* and *Anopheles funestus* in Kenya. *Malaria Journal*, *8*(1), 268.

Kibret, S., Alemu, Y., Boelee, E., Tekie, H., Alemu, D., Petros, B. (2010). The impact of a small-scale irrigation scheme on malaria transmission in Ziway area , Central Ethiopia. *Tropical Medicine and International Health*, *15*(1), 41–50.

Kibret, S., Lautze, J., Boelee, E., Mccartney, M. (2012). How does an Ethiopian dam increase malaria ? Entomological determinants around the Koka reservoir. *Tropical Medicine and*

International Health, 17(1), 1320–1328.

Kienberger, S., & Hagenlocher, M. (2014). Spatial-explicit Modeling of Social Vulnerability to malaria in East Africa. *International Journal of Health Geographics*, *13*(1), 29.

Kilama, M., Smith, D. L., Hutchinson, R., Kigozi, R., Yeka, A., Lavoy, G., Kamya, M. R., Staedke, S. G., Donnelly, M. J., Drakeley, C., Greenhouse, B., Dorsey, G., Lindsay, S. W. (2014). Estimating the annual entomological inoculation rate for *Plasmodium falciparum* transmitted by *Anopheles gambiae* s.l. using three sampling methods in three sites in Uganda. *Malaria Journal*, *13*(1), 111.

Killeen, G. F. (2014). Characterizing, controlling and eliminating residual malaria transmission. *Malaria Journal*, *13*(1), 330.

Killeen, G. F., & Chitnis, N. (2014). Potential causes and consequences of behavioural resilience and resistance in malaria vector populations: A mathematical modelling analysis. *Malaria Journal*, *13*(1), 1–16.

Killeen, G. F., Govella, N. J., Lwetoijera, D. W., Okumu, F. O. (2016). Most outdoor malaria transmission by behaviourally-resistant *Anopheles arabiensis* is mediated by mosquitoes that have previously been inside houses. *Malaria Journal*, *15*(1), 1–10.

Killeen, G. F., Kihonda, J., Lyimo, E., Oketch, F. R., Kotas, M. E., Mathenge, E., Schellenberg, J. A., Lengeler, C., Smith, T. A., Drakeley, C. J. (2004). Quantifying behavioural interactions between humans and mosquitoes : Evaluating the protective efficacy of insecticidal nets against malaria transmission in rural Tanzania. *BMC Infectious Diseases*, *10*, 1–10.

Kiware, S. S., Russell, T. L., Mtema, Z. J., Malishee, A. D., Chaki, P., Lwetoijera, D., Chanda, J., Chinula, D., Majambere, S., Gimnig, J. E., Smith, T. A., Killeen, G. F. (2016). A generic schema and data collection forms applicable to diverse entomological studies of mosquitoes. *Source Code for Biology and Medicine*, *11*, 4.

Kleinschmidt, I., Bagayoko, M., Clarke, G. P., Craig, M., le Sueur, D. (2000). A spatial statistical approach to malaria mapping. *International Journal of Epidemiology*, *29*(2), 355–361.

Kolff, C. A., Scott, V. P., Stockwell, M. S., Kolff, C. A., Scott, V. P., Stockwell, M. S. (2018). The use of technology to promote vaccination : A social ecological model based framework. *Human Vaccines & Immunotherapeutics ISSN:*, *14*(7), 1636–1646.

Kuhn, K., Campbell-Lendrum, D., Haines, A., Cox, J. (2004). Using Climate to Predict Infectious Disease Outbreaks : A Review. In *Communicable Diseases Surveillance and Response Protection of the Human Environment Roll Back Malaria*. Geneva, Switzerland. 55p

Kullenberg, C., & Kasperowski, D. (2016). What is citizen science? - A scientometric meta-analysis. *PLoS ONE*, *11*(1), 1–16.

Kursah, M. B. (2017). GIS and correlation analysis of geo-environmental variables influencing malaria prevalence in the Saboba district of Northern Ghana. *Ghana Journal of Geography*, *9*(3), 112–131.

Kweka, E. J., Kamau, L., Munga, S., Lee, M.-C., Githeko, A. K., Yan, G. (2013). A first report of *Anopheles funestus* sibling species in western Kenya highlands. *Acta Tropica*, *128*(1), 158–161.

Laguna-Aguilar, M., Alvarado-Moreno, M. S., Sánchez-Rodríguez, O. S., Ramírez-Jiménez, R., Zárate-Nahón, E. A., Sánchez-Casas, R. M., Fernández-Salas, I., Rebollar-Téllez, E. A. (2012). Field evaluation of a novel trap baited with carbon dioxide produced by yeast for the collection of female *Aedes aegypti* mosquitoes in Mexico. *Southwestern Entomologist*, *37*(4), 495–504.

Lansana, K. (2008). Rwanda entomology monitoring: technical report presidential malaria initiative (PMI), Kigali-Rwanda.

Larman, C. (2004). Applying UML and patterns: An introduction to object-oriented analysis and design and the unified process (3rd Edition). In *8th Conference on the Advances of Computer Entertainment Technology*.

Leeuwis, C., Cieslik, K. J., Aarts, M. N. C., Dewulf, A. R. P. J., Ludwig, F., Werners, S. E., Struik, P. C. (2018). Reflections on the potential of virtual citizen science platforms to address collective action challenges : lessons and implications for future research. *NJAS - Wageningen Journal of Life Sciences*, 86-87, 146-157.

Lemire, G. F. (1995). Field evaluation of heat as an added attractant to traps baited with carbon dioxide and octenol for *Aedes Taeniorynchus*. *Joumal of the American Mosquito Control Association, 11*(4), 454–456.

Lima, J. B. P., Rosa-Freitas, M. G., Rodovalho, C. M., Santos, F., Lourenço-de-Oliveira, R. (2014). Is there an efficient trap or collection method for sampling *Anopheles darlingi* and other malaria vectors that can describe the essential parameters affecting transmission dynamics as effectively as human landing catches? - A Review. *Memórias Do Instituto Oswaldo Cruz*, *109*(5), 685–705.

Lindsay, S., Kirby, M., Baris, E., Bos, R. (2004). Environmental management for malaria control in the East Asia and pacific (EAP) region (Issue April). 1-66.

Lindsay, S. W., & Martens, W. J. M. (1998). Malaria in the African highlands: past, present and future. *Bulletin of the World Health Organization*, *76*(1), 33–45.

Lindsay, Steve W., Jawara, M., Paine, K., Pinder, M., Walraven, G. E. L., Emerson, P. M. (2003). Changes in house design reduce exposure to malaria mosquitoes. *Tropical Medicine and International Health*, *8*(6), 512–517.

Liu, H., Grossberndt, S., Kobernus, M. (2017). Citizen science and citizens 'observatories : trends , roles , challenges and development needs for science and environmental governance. In *Mapping and the Citizen Sensor* (pp. 351–376).

Loevinsohn, M. E. (1994). Climatic warming and increased malaria incidence in Rwanda. *Lancet*, *343*(8899), 714–718.

Lozano-Fuentes, S., Wedyan, F., Hernandez-Garcia, E., Sadhu, D., Ghosh, S., Bieman, J. M., Tep-Chel, D., García-Rejón, J. E., Eisen, L., Garcia-Rejon, J. E., Eisen, L. (2013). Cell phone-based system (Chaak) for surveillance of immatures of dengue virus mosquito vectors. *Journal of Medical Entomology*, *50*(4), 879–889.

Lwetoijera, D. W., Kiware, S. S., Mageni, Z. D., Dongus, S., Harris, C. (2013). A need for better housing to further reduce indoor malaria transmission in areas with high bed net coverage. *Parasites & Vectors*, *6*(1), 1.

Lynch, C. A., Bruce, J., Bhasin, A., Roper, C., Cox, J., Abeku, T. A. (2015). Association between recent internal travel and malaria in Ugandan highland and highland fringe areas. *Tropical Medicine and International Health*, *20*(6), 773–780.

Mahajan, S., Kumar, P., Antonino, J., Riccetti, A., Schaaf, K., Camprodon, G., Smári, V., Passani, A., Forino, G. (2019). A citizen science approach for enhancing public understanding of air pollution. *Sustainable Cities and Society*, *52*(August), 1-12.

Maharaj, R., Kissoon, S., Lakan, V., Kheswa, N. (2019). Rolling back malaria in Africa - Challenges and opportunities to winning the elimination battle. *South African Medical Journal*, *109*(11b), 53–56.

Maier, A. G., Matuschewski, K., Zhang, M., Rug, M. (2019). *Plasmodium falciparum*. *Trends in Parasitology*, *35*(6), 481–482.

Maliti, D. V, Govella, N. J., Killeen, G. F., Mirzai, N., Johnson, P. C. D., Kreppel, K., Ferguson, H. M. (2015). Development and evaluation of mosquito - electrocuting traps as alternatives to the human landing catch technique for sampling host - seeking malaria vectors. *Malaria Journal*, 1–15.

Mangam, K., Fiekowsky, E., Bagayoko, M., Norris, L., Belemvire, A., Longhany, R., Fornadel, C., George, K. (2016). Feasibility and effectiveness of mHealth for mobilizing households for indoor residual spraying to prevent malaria: a case study in Mali. *Global Health: Science and Practice*, *4*(2), 222–237.

Maria, E., Santos, M. De, Melo-santos, M. A. V. De, Maria, C., Oliveira, F. De, Correia, J. C., Maria, C., Albuquerque, R. De. (2012). Evaluation of a sticky trap (AedesTraP), made from disposable plastic bottles , as a monitoring tool for *Aedes aegypti* populations. *Parasites & Vectors*, *5*(195), 2–11.

Massey, N. C., Garrod, G., Wiebe, A., Henry, A. J., Huang, Z., Moyes, C. L., Sinka, M. E. (2016). A global bionomic database for the dominant vectors of human malaria. *Scientific Data*, *3*(160014), 1–13.

Matowo, N. S., Koekemoer, L. L., Moore, S. J., Mmbando, A. S., Mapua, S. A., Coetzee, M., Okumu, F. O. (2016). Combining synthetic human odours and low-cost electrocuting grids to attract and kill outdoor-biting mosquitoes: field and semi-field evaluation of an improved mosquito landing box. *PloS One*, *11*(1), 1-25.

Maxwell, C. A., Wakibara, J., Tho, S., Curtis, C. F. (1998). Malaria-infective biting at different hours of the night. *Medical and Veterinary Entomology*, *12*(3), 325–327.

Mboera, L.E.G., Knols, B. G. J., Takken, W., Torre, A. della. (1997). The response of A*nopheles gambiae* s.l. and *A. funestus* (Diptera: Culicidae) to tents baited with human odour or carbon dioxide in Tanzania . *Bulletin of Entomological Research*, *87*(2), 173–178.

Mboera, L E G, Kihonda, J., Braks, M. a H., Knols, B. G. J. (1998). Short report: influence of centers for disease control light trap position, relative to a human-baited bednet, on catches of *Anopheles gambiae* and *Culex quinqufasciatus* in Tanzania. *American Journal of Tropical Medicine and Hygiene*, *59*(4), 5.

Mboera, L. E. G., Mazigo, H. D., Rumisha, S. F., Kramer, R. A. (2013). Towards malaria elimination and its implication for vector control , disease management and livelihoods in Tanzania. *MalariaWorld Journal*, *4*(19), 18–20.

Mboera, L.. E. G., Senkoro, K. P., Mayala, B. K., Rumisha, S. F., Rwegoshora, R. T., Mlozi, M. R. S., Shayo, E. H. (2010). Spatio-temporal variation in malaria transmission intensity in five agro-ecosystems in Mvomero district , Tanzania. *Geospatial Health*, *4*(2), 167–178.

Mburu, M. M., Juurlink, M., Spitzen, J., Moraga, P., Hiscox, A., Mzilahowa, T., Takken, W., McCann, R. S. (2018). Impact of partially and fully closed eaves on house entry rates by mosquitoes. *Parasites & Vectors*, *11*(1), 383.

Mburu, M. M., Mzilahowa, T., Amoah, B., Chifundo, D., Phiri, K. S., Berg, H. Van Den, Takken, W., Mccann, R. S. (2019). Biting patterns of malaria vectors of the lower Shire valley , southern Malawi. *Acta Tropica*, *197*(2019), 1-9.

McFeeters, S. K. (2013). Using the normalized difference water index (ndwi) within a geographic information system to detect swimming pools for mosquito abatement: A practical approach. *Remote Sensing*, *5*(7), 3544–3561.

Medlock, J. M., Hansford, K. M., Anderson, M., Mayho, R., Snow, K. R. (2012). Mosquito nuisance and control in the UK – A questionnaire-based survey of local authorities. *European Mosquito Bulletin*, *30*(15–29), 29.

Menger, D. J., Omusula, P., Wouters, K., Oketch, C., Carreira, A. S., Durka, M., Derycke, J., Loy, D. E., Hahn, B. H., Mukabana, W. R., Mweresa, C. K., Loon, J. J. A. Van, Takken, W., Hiscox, A. (2016). Eave screening and push-pull tactics to reduce house entry by vectors of malaria. *American Journal of Tropical Medicine and Hygiene*, *94*(4), 868–878.

Minakawa, N., Dida, G. O., Sonye, G. O., Futami, K., Kaneko, S. (2008). Unforeseen misuses of bed nets in fishing villages along Lake Victoria. *Malaria Journal*, *7*(1), 165.

Minakawa, N., Mutero, C. M., Githure, J. I., Beier, J. C., Yan, G. (1999). Spatial distribution and habitat characterization of Anopheline mosquito larvae in Western Kenya. *American Journal of Tropical Medicine and Hygiene*, *61*(6), 1010–1016.

Mmbando, B. P., Vestergaard, L. S., Kitua, A. Y., Lemnge, M. M., Theander, T. G., Lusingu, J. P. A. (2010). A progressive declining in the burden of malaria in north-eastern Tanzania. *Malaria Journal*, *9*(1), 1–9.

Mnzava, A., Macdonald, M., Knox, T., Temu, E., Shiff, C. (2014). Malaria vector control at a crossroads: public health entomology and the drive to elimination. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *108*(9), 550–554.

MOH. (2013). National Strategic Plan for Integrated Vector Management (2013 - 2017). Kigali, Rwanda.

MOH. (2015). Rwanda Demographic and Health Survey 2014-15. Kigali, Rwanda.

MOH. (2016). Mid term review final report of Rwanda malaria strategic plan 2013-2018. Kigali, Rwanda.

MOH. (2017a). Malaria Indicator Survey (MIS) 2017. Kigali, Rwanda.

MOH. (2017b). Revised National Malaria Contingency Plan 2016-2020. Kigali, Rwanda.

MOH. (2017c). Rwanda Malaria Control Extended National Strategic Plan (Issue July 2013).

MOH. (2019). Rwanda Malaria and Neglected Tropical Diseases Annual Report 2018-2019.

MOH. (2020). Rwanda malaria strategic plan 2020-2024.

Moon, Y. M., Metoxen, A. J., Leming, M. T., Whaley, M. A., Tousa, J. E. O. (2015). Rhodopsin management during the light-dark cycle of *Anopheles gambiae* mosquitoes. *Journal of Insect Physiology*, *70*, 88–93.

Malaria and Other Parasitic diseases Division (MOPDD). (2013). Rwanda Malaria Cases Surveillance Guidelines. Kigali, Rwanda.

Moss, W. J., Hamapumbu, H., Kobayashi, T., Shields, T., Kamanga, A., Clennon, J., Mharakurwa, S., Thuma, P. E., Glass, G. (2011). Use of remote sensing to identify spatial risk factors for malaria in a region of declining transmission: A cross-sectional and longitudinal community survey. *Malaria Journal*, *10*, 1–10.

Mouchet, J. (1998). L'origine des épidémies de paludisme sur les Plateaux de Madagascar et les montagnes d'Afrique de L'est et du Sud. *Bulletin De La Societe De Pathologie Exotique*, *91*(1), 64–66.

Mukundarajan, H., Hol, F. J. H., Castillo, E. A., Newby, C., Prakash, M. (2017). Using mobile phones as acoustic sensors for high-throughput surveillance of mosquito ecology. *BioRxiv*, 1–38.

Muñoz, J. P., Boger, R., Dexter, S., Low, R. (2020). Mosquitoes and public health: improving data validation of citizen science contributions using computer vision. In *Delivering Superior Health and Wellness Management with IoT and Analytics. Healthcare Delivery in the Information Age* (p. 686).

Munyaneza, F., Hirschhorn, L. R., Amoroso, C. L., Nyirazinyoye, L., Birru, E., Mugunga, J. C., Murekatete, R. M., Ntaganira, J. (2014). Leveraging community health worker system to map a mountainous rural district in low resource setting: a low-cost approach to expand use of geographic information systems for public health. *International Journal of Health Geographics*, *13*, 49.

Munyantore, S. (1989). Historique de la lutte antipaludique au Rwanda. *Revue Medicale Rwandaise*, *21*(14–28).

Murindahabi, M. M., Asingizwe, D., Poortvliet, P. M., Vliet, A. J. H. Van, Hakizimana, E., Mutesa, L., Takken, W., Koenraadt, C. J. M. (2018). A citizen science approach for malaria mosquito surveillance and control in Rwanda. *NJAS - Wageningen Journal of Life Sciences*, *86–87*, 101–110.

Musabe, B. L. (2012). Crop Intensification Program as a tool of poverty reduction in Rwanda. University of Rwanda.

Mutabazi, A. (2010). Assessment of operational framework related to climate change in Rwanda (Issue March).

Mwangungulu, S. P., Sumaye, R. D., Limwagu, A. J., Siria, D. J., Kaindoa, E. W., Okumu, F. O. (2016). Crowdsourcing vector surveillance: using community knowledge and experiences to predict densities and distribution of outdoor-biting mosquitoes in rural Tanzania. *PloS One*, *11*(6), e0156388.

Mweresa, C. K., Omusula, P., Otieno, B., van Loon, J. J. A., Takken, W., Mukabana, W. R. (2014). Molasses as a source of carbon dioxide for attracting the malaria mosquitoes *Anopheles gambiae*, and *Anopheles funestus*. *Malaria Journal*, *13*(1), 160.

Mayagaya, V. S., Ntamatungiro, A. J., Moore, S. J., Wirtz, R. A., Dowell, F. E., Maia, M. F. (2015). Evaluating preservation methods for identifying *Anopheles gambiae* s.s. and *Anopheles arabiensis* complex mosquitoes species using near infra-red spectroscopy. *Parasites & Vectors*, *8*, 60.

Najera, J. a, Koumetsov, R., Delacollette, C. (1998). Malaria epidemics, detection and control, forecasting and prevention. In *Malaria epidemics detection and control forecasting and prevention* (Vol. 81).

Nchoutpouen, E., Talipouo, A., Djiappi-tchamen, B., Djamouko-, L., Kopya, E., Ngadjeu, C. S., Doumbe-belisse, P., Awono-ambene, P., Kekeunou, S., Wondji, C. S., Id, C. A. (2019). Culex species diversity , susceptibility to insecticides and role as potential vector of Lymphatic filariasis in the city of Yaounde Cameroon. *PLoS Negl Trop Dis*, *13*(4), 1–16.

Ngabo, F., Nguimfack, J., Nwaigwe, F., Mugeni, C., Muhoza, D., Wilson, D. R., Kalach, J., Gakuba, R., Karema, C., Binagwaho, A. (2012). designing and implementing an innovative sms-based alert system (RapidSMS-MCH) to monitor pregnancy and reduce maternal and child deaths in Rwanda. *The Pan African Medical Journal*, *13*, 31.

National Institute of Statistics Rwanda. (2017). The Fifth Integrated Household Living Conditions Survey EICV5.

Njie, M., Dilger, E., Lindsay, S. W., Kirby, M. J. (2009). Importance of Eaves to House Entry by Anopheline , But Not Culicine , Mosquitoes. *J. Med. Entomol*, *46*(3), 505–510.

Norris, D. E. (2004). Mosquito-borne Diseases as a Consequence of Land Use Change. *EcoHealth*, *1*(1), 19–24.

Nov, O., Arazy, O., Anderson, D. (2014). Scientists@Home: what drives the quantity and quality of online citizen science participation? *PLOS ONE*, *9*(4), e90375.

Nsanzimana, S., Ruton, H., Lowrance, D. W., Cishahayo, S., Nyemazi, J. P., Muhayimpundu, R., Karema, C., Raghunathan, P. L., Binagwaho, A., Riedel, D. J. (2012). Cell phone-based and internet-based monitoring and evaluation of the national antiretroviral treatment program during rapid scale-up in Rwanda: TRACnet, 2004-2010. *Journal of Acquired Immune Deficiency Syndromes*, *59*(2), 17–23.

Nyirakanani, C., Chibvongodze, R., Kariuki, L., Habtu, M., Masika, M., Mukoko, D., Njunwa, K. J. (2017). Characterization of malaria vectors in Huye District, Southern Rwanda. *Tanzania Journal of Health Research*, *19*(3), 1–10.

O'Meara, Mangeni, J. N., Steketee, R., Greenwood, B. (2010). Changes in the burden of malaria in sub-Saharan Africa. *The Lancet Infectious Diseases*, *10*(8), 545–555.

Ogoma, S. B., Lweitoijera, D. W., Ngonyani, H., Furer, B., Tanya, L., Mukabana, W. R., Killeen, G. F., Moore, S. J. (2010). Screening mosquito house entry points as a potential method for integrated control of endophagic filariasis , arbovirus and malaria vectors. *PLoS Neglected Tropical Diseases*, *4*(8), 1–8.

Oltra, A., Palmer, J. R. B., Bartumeus, F. (2016). *AtrapaelTigre.com*: enlisting citizen-scientists in the war on tiger mosquitoes. In *European Handbook of Crowdsourced Geographic Information*. London: Ubiquity Press, (pp. 295–308).

Omumbo, J., Lyon, B., Waweru, S. M., Connor, S. J., Thomson, M. C. (2011). Raised temperatures over the Kericho tea estates: revisiting the climate in the East African highlands malaria debate. *Malaria Journal*, *10*(1), 12.

Ondiba, I. M., Oyieke, F. A., Ong, G. O., Olumula, M. M., Nyamongo, I. K., Estambale, B. B. A. (2018). Malaria vector abundance is associated with house structures in Baringo County , Kenya. *PLoS ONE*, *13*(6), 1–12.

Onyango, S. A., Kitron, U., Mungai, P., Muchiri, E. M., Kokwaro, E., King, C. H., Mutuku, F. M. (2013). Monitoring malaria vector control interventions: effectiveness of five different adult mosquito sampling methods. *Journal of Medical Entomology*, *50*(5), 1140–1151.

Otten, M., Aregawi, M., Were, W., Karema, C., Medin, A., Bekele, W., Jima, D., Gausi, K., Komatsu, R., Korenromp, E., Low-Beer, D., Grabowsky, M. (2009). Initial evidence of reduction of malaria cases and deaths in Rwanda and Ethiopia due to rapid scale-up of malaria prevention and treatment. *Malaria Journal*, *8*(1), 1–8.

Palmer, J. R. B., Brocklehurst, M., Tyson, E., Bowser, A., Pauwels, E., Bartumeus, F., Editor, B., Haklay, M., Bowser, A., Makuch, Z., Vogel, J., Pauwels, E., Bartumeus, F. (2018). Global mosquito alert. In *Citizen Science,* UCL Press, (p. 8).

Palmer, J. R. B., Oltra, A., Collantes, F., Delgado, J. A., Lucientes, J., Delacour, S., Bengoa, M., Eritja, R., Bartumeus, F. (2017). Citizen science provides a reliable and scalable tool to track disease-carrying mosquitoes. *Nature Communications*, *8*(1), 1–12.

Parham, P. E., Waldock, J., Christophides, G. K., Hemming, D., Agusto, F., Evans, K. J., Fefferman, N., Gaff, H., Gumel, A., LaDeau, S., Lenhart, S., Mickens, R. E., Naumova, E. N., Ostfeld, R. S., Ready, P. D., Thomas, M. B., Velasco-Hernandez, J., Michael, E. (2015). Climate, environmental and socio-economic change: weighing up the balance in vector-borne disease transmission. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *370*(1665).

Patz, J. A., Graczyk, T. K., Geller, N., Vittor, A. Y. (2000). Effects of environmental change on emerging parasitic diseases. *Int J Parasitol*, *30*(12–13), 1395–1405.

PMI. (2013). President’s Malaria Initiative. Malaria Operational Plan: Rwanda FY 2013.

PMI. (2014). President’s Malaria Initiative Rwanda.. Malaria Operational Plan, Rwanda FY 2014.

PMI. (2015). President’s Malaria Initiative. Malaria Operational Plan: Rwanda FY 2015.

PMI. (2018). President’s Malaria Initiative. Malaria Operational Plan: Rwanda FY 2018.

PMI. (2017). President’s Malaria Initiative Rwanda. (2017). Rwanda - Malaria Operational Plan FY 2017.

PMI. (2019). *President’s Malaria Initiative. Malaria Operational Plan: Rwanda FY 2019*.

Protopopoff, N., Van Bortel, W., Speybroeck, N., Van Geertruyden, J.-P., Baza, D., D’Alessandro, U., Coosemans, M. (2009). Ranking malaria risk factors to guide malaria control efforts in African highlands. *PloS One*, *4*(11), e8022.

Rabinovich, R. N., Drakeley, C., Djimde, A. A., Hall, B. F., Hay, S. I., Hemingway, J., Kaslow, D. C., Noor, A., Okumu, F., Steketee, R., Tanner, M., Wells, T. N. C., Whittaker, M. A., Winzeler, E. A., Wirth, D. F., Whitfield, K., Alonso, P. L. (2017). malERA: An updated research agenda for malaria elimination and eradication. *PLoS Medicine*, *14*(11), 1–17.

Raja, A., Tridane, A., Gaffar, A., Lindquist, T., Pribadi, K. (2014). Android and ODK based data collection framework to aid in epidemiological analysis. *Online Journal of Public Health Informatics*, *5*(3), 228.

Raji, J.I. & DeGennaro, M. (2018). Genetic Analysis of Mosquito Detection of Humans Joshua. *Current Opinion in Insect Science*, *10*(1), 1–13.

Rambonnet, L., Vink, S. C., Land-zandstra, A. M., Bosker, T. (2019). Making citizen science count : Best practices and challenges of citizen science projects on plastics in aquatic environments. *Marine Pollution Bulletin*, *145*, 271–277.

Randrianasolo, L., Raoelina, Y., Ratsitorahina, M., Ravolomanana, L., Andriamandimby, S., Heraud, J., Rakotomanana, F., Ramanjato, R., Randrianarivo-solofoniaina, A. E., Richard, V. (2010). Sentinel surveillance system for early outbreak detection in Madagascar. *BMC Public Health*, *10*(31), 8.

Ranson, H., & Lissenden, N. (2016). Insecticide Resistance in African *Anopheles* Mosquitoes: A Worsening Situation that Needs Urgent Action to Maintain Malaria Control. *Trends in Parasitology*, *32*(3), 187–196.

Reddy, M. R., Overgaard, H. J., Abaga, S., Reddy, V. P., Caccone, A., Kiszewski, A. E., Slotman, M. A. (2011). Outdoor host seeking behaviour of *Anopheles gambiae* mosquitoes following initiation of malaria vector control on Bioko Island, Equatorial Guinea. *Malaria Journal*, *10(1)*, 184.

REMA. (2006). National Adaptation Programmes of Actionto Climate Change. In *Report* (Issue December 2006).

Rickard, D. G., Dudovitz, R. N., Wong, M. D., Jen, H. C., Osborn, D., Fernandez, H. E., Donkor, C. I. (2011). Closing the Gap Between Insecticide Treated Net Ownership and Use for the Prevention of Malaria. *Progress in Community Health Partnerships: Research, Education, and Action*, *5*(2), 123–131.

Rosanti, T. I., Mardihusodo, S. J., Artama, W. T. (2017). Effectiveness of environmentally friendly mosquito trap containing sugar yeast solution. *KEMAS*, *1*(1), 1–11.

Rotman, D., Hammock, J., Preece, J., Hansen, D., Boston, C., Bowser, A., Yurong, H. (2014). Motivations Affecting Initial and Long-Term Participation in Citizen Science Projects in Three Countries. *IConference 2014 Proceedings*, 110-124.

Rotman, D., Preece, J., Hammock, J., Procita, K., Hansen, D., Parr, C., Lewis, D., Jacobs, D. (2012). Dynamic Changes in Motivation in Collaborative Citizen-Science Projects. *ACM 2012 Conference on Computer Supported Cooperative Work-CSCW*, 217–226.

Rudasingwa, G., & Cho, S. Il. (2020). Determinants of the persistence of malaria in Rwanda.

Malaria Journal, *19*(1), 1–9.

Rulisa, S., Kateera, F., Bizimana, J. P., Agaba, S., Dukuzumuremyi, J., Baas, L. (2013). Malaria prevalence, spatial clustering and risk factors in a low endemic area of Eastern Rwanda: a cross sectional study. *PLoS One*, *8*.

Russell, T. L., Govella, N. J., Azizi, S., Drakeley, C. J., Kachur, S. P., Killeen, G. F. (2011). Increased proportions of outdoor feeding among residual malaria vector populations following increased use of insecticide-treated nets in rural Tanzania. *Malaria Journal*, *10*, 80.

Ruyange, M. M., Condo, J., Karema, C., Binagwaho, A., Rukundo, A., Muyirukazi, Y. (2016). Factors associated with the non-use of insecticide-treated nets in Rwandan children. *Malaria Journal*, *15*(355), 7.

Rwanyiziri, G., & Rugema, J. (2013). Climate Change Effects on Food Security in Rwanda: Case Study of Wetland Rice Production in Bugesera District. *Rwanda Journal*, *1*(1), 35–51.

Ryan, S. F., Adamson, N. L., Aktipis, A., Andersen, L. K., Austin, R., Barnes, L., Beasley, M. R., Bedell, K. D., Briggs, S., Chapman, B., Cooper, C. B., Corn, J. O., Creamer, N. G., Delborne, J. A., Domenico, Dunn, R. R., J. O., Landis, E. A., Madden, A. A., Mckenney, E. A., Nichols, L. M., Reading, B. J., Russell, S., Sengupta, N., Shapiro, L. R., Shell, L. K., Sheard, J. K., Shoemaker, D. D., Sorger, D. M., Starling, C., P., Sullivan, Thakur, S., Vatsavai, R. R., Weinstein, M., Winfrey, P., (2018). The role of citizen science in addressing grand challenges in food and agriculture research. *Proceedings Royal Society*, 285, 891.

Samuelsen, H. (2004). Prevention of mosquito nuisance among urban populations in Burkina Faso. *Social Science & Medicine*, *59*, 2361–2371.

Sanford, M. R., Cornel, A. J., Nieman, C. C., Dinis, J., Marsden, C. D., Weakley, A. M., Han, S., Rodrigues, A., Lanzaro, G. C., Lee, Y. (2014). *Plasmodium falciparum* infection rates for some *Anopheles* spp . from Guinea-Bissau , West Africa. *F1000Reseach*, 3 (243), 1–10.

Sanoff, H. (2007). Special issue on participatory design. *Design Studies*, *28*(3), 213–215.

Sattler, M. a, Mtasiwa, D., Kiama, M., Premji, Z., Tanner, M., Killeen, G. F., Lengeler, C. (2005). Habitat characterization and spatial distribution of *Anopheles sp*. mosquito larvae in Dar es Salaam (Tanzania) during an extended dry period. *Malaria Journal*, *4*, 4.

Scott, J. A., Brogdon, W. G., Collins, F. H. (1993). Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. *The American Society of Tropical Medicine and Hygiene*, *49*(4), 520–529.

Stockolm Environment Institute (SEI). (2009). *Economics of Climate Change in Rwanda*.

Service, M. W. (1993). *Mosquito Ecology: Field Sampling Methods.* (Elsevier).

Sestelo, M., Villanueva, N. M., Meira-Machado, L., Roca-Pardiñas, J. (2016). FWDselect: An R package for variable selection in regression models. The *R Journal*, *8*(1), 132–148.

Sherrard-Smith, E., Skarp, J. E., Beale, A. D., Fornadel, C., Norris, L. C., Moore, S. J., Mihreteab, S., Charlwood, J. D., Bhatt, S., Winskill, P., Griffin, J. T., Churcher, T. S. (2019). Mosquito feeding behavior and how it influences residual malaria transmission across Africa. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(30), 15086–15096.

Shirk, J., Ballard, H. L., Wilderman, C. C., Phillips, T., Wiggins, A., Jordan, R. (2012). Public participation in scientific research : a framework for deliberate design. *Ecology and Society*, *17*(2), 17.

Shirk, J., Ballard, H., Wiggins, A., Phillips, T., Jordan, R., Wilderman, C., Mccallie, E., Bonney, R. (2009). public participation in scientific research : converging on effective design strategies. Conference proceedings, *Informal Science*, 5, 1.

Sikaala, C. H., Chinula, D., Chanda, J., Hamainza, B., Mwenda, M., Mukali, I., Kamuliwo, M., Lobo, N. F., Seyoum, A., Killeen, G. F. (2014). A cost-effective, community-based, mosquito-trapping scheme that captures spatial and temporal heterogeneities of malaria transmission in rural Zambia. *Malaria Journal*, *13*(1), 225.

Silver, J. B. (2008). Mosquito ecology: field sampling methods. *Springer* (3rd ed.).

Sinka, M. E., Bangs, M. J., Manguin, S., Coetzee, M., Mbogo, C. M., Hemingway, J., Patil, A. P., Temperley, W. H., Gething, P. W., Kabaria, C. W., Okara, R. M., Boeckel, T. Van, Godfray, H. C. J., Harbach, R. E., Hay, S. I. (2010). The dominant *Anopheles* vectors of human malaria in Africa , Europe and the Middle East : occurrence data , distribution maps and bionomic précis. *Parasites & Vectors*, *3*(1), 117.

Sinka, M. E., Bangs, M. J., Manguin, S., Rubio-palis, Y., Chareonviriyaphap, T., Coetzee, M., Mbogo, C. M., Hemingway, J., Patil, A. P., Temperley, W. H., Gething, P. W., Kabaria, C. W., Burkot, T. R., Harbach, R. E., Hay, S. I. (2012). A global map of dominant malaria vectors. *Parasites & Vectors*, *5*(1), 69.

Smallegange, R., Schmied, W. H., Roey, K. J. van, Verhulst, N. O., Spitzen, J., Mukabana, W. R., Takken, W. (2010). Sugar-fermenting yeast as an organic source of carbon dioxide to attract the malaria mosquito *Anopheles gambiae*. *Bulletin of Entomological Research*, *70*(4), 525–532.

Snyman, K., Mwangwa, F., Bigira, V., Kapisi, J., Clark, T. D., Osterbauer, B., Greenhouse, B., Sturrock, H., Gosling, R., Liu, J., Dorsey, G. (2015). Poor housing construction associated with increased malaria incidence in a cohort of young ugandan children. *American Journal for Troical Medicine and Hygiene.*, *92*(6), 1207–1213.

Sorensen, A. E., Jordan, R. C., Ladeau, S. L., Biehler, D., Pitas, J., Leisnham, P. T. (2019). Reflecting on efforts to design an inclusive citizen science project in west baltimore. *Citizen science: Theory and Practice 4*(1), 1–12.

Spitzen, J., Smallegange, R. C., Takken, W. (2008). Effect of human odours and positioning of CO2 release point on trap catches of the malaria mosquito *Anopheles gambiae sensu stricto* in an olfactometer. *Physiological Entomology*, *33*(2), 116–122.

Spitzen, J., Spoor, C. W., Grieco, F., Braak, C., Beeuwkes, J., Van, S. P., Kranenbarg, S., Noldus, L. P. J. J., Leeuwen, J. L. Van, Takken, W. (2013). A 3D Analysis of Flight Behavior of *Anopheles gambiae sensu stricto* Malaria Mosquitoes in Response to Human Odor and Heat. *PLoS One*, *8*(5), 1–12.

Spitzen, J., & Takken, W. (2018). Keeping track of mosquitoes: A review of tools to track, record and analyse mosquito flight. *Parasites and Vectors*, *11*(1), 1–11.

Stern, D. I., Gething, P. W., Kabaria, C. W., Temperley, W. H., Noor, A. M., Okiro, E. A., Dennis Shanks, G., Snow, R. W., Hay, S. I. (2011). Temperature and malaria trends in highland East Africa. *PLoS ONE*, *6*(9), 4–12.

Sturrock, H. J. W., Roberts, K. W., Wegbreit, J., Ohrt, C., Gosling, R. D. (2015). Tackling imported malaria: An elimination endgame. *American Journal of Tropical Medicine and Hygiene*, *93*(1), 139–144.

Sukumaran, D., Ponmariappan, S., Sharma, A. K., Jha, H. K., Wasu, Y. H., Sharma, A. K. (2015). Application of biogenic carbon dioxide produced by yeast with different carbon sources for attraction of mosquitoes towards adult mosquito traps. *Parasitology Research*, *115*(4), 1453–1462.

Tabue, R. N., Nem, T., Atangana, J., Bigoga, J. D., Patchoke, S., Tchouine, F., Fodjo, B. Y., Leke, R. G. F., Fondjo, E. (2014). *Anopheles ziemanni* a locally important malaria vector in Ndop health district , north west region of Cameroon. *Parasites & Vectors*, *7*(262), 11.

Takken, W., & Knols, B. G. J. (1999). Odor-Mediated Behavior of Afrotropical Malaria Mosquitoes. *Annual Review of Entomology*, *44*(1), 131–157.

Takken, W., & Knols, B. G. J. (2010). *Olfaction in vector-host interactions: Ecology and control of vector-borne diseases* (Vol. 2).

Takken, W., & Koenraadt, C. J. M. (2013). Ecology of Parasite-Vector Interactions. In *Ecology and Control of Vector-Borne Diseases* (Vol. 3).

Tanner, M., & Savigny, D. de. (2008). Malaria eradication back on the table. *Bulletin of the World Health Organization*, *86*(2), 82.

Tarter, K. D., Levy, C. E., Yaglom, H. D., Adams, L. E., Plante, L., Casal, M. G., Gouge, D. H., Rathman, R., Stokka, D., Weiss, J. (2019). Uing Citizen science to enhance surveillance of *Aedes aegypti* in Arizona, 2015-17. *Journal of the American Mosquito Control Association*, *35*(1), 11–18.

Tusting, L. S., Bousema, T., Smith, D. L., Drakeley, C. (2014). Measuring changes in *Plasmodium falciparum* transmission. Precision, accuracy and costs of metrics. In *Advances in Parasitology* Vol. 84, pp. 151–208.

Tuyishimire, J. (2013). Spatial modelling of malaria risk factors in ruhuha. *Rwanda Journal Series - Life and Natural Sciences*, *1*(February), 17.

Tyson, E., Bowser, A., Palmer, J., Kapan, D., Bartumeus, F., Brocklehurst, M. (2018). Global mosquito alert : building citizen science capacity for surveillance and control of disease-vector mosquitoes. Workshop report, Vol. 2, p27.

USAID. (2012a). National Policy Guidelines for Integrated Vector Mangement—Rwanda - Ministry of Health.

USAID. (2012b). National Strategic Plan on Integrated Vector Republic of Rwanda Ministry of Health (Issue June).

Vogels, C. B. F., van de Peppel, L. J. J., van Vliet, A. J. H., Westenberg, M., Ibañez-Justicia, A., Stroo, A., Buijs, J. A., Visser, T. M., Koenraadt, C. J. M. (2015). Winter activity and aboveground hybridization between the two biotypes of the west nile virus vector *Culex pipiens*. *Vector-Borne and Zoonotic Diseases*, *15*(10), 619–626.

Wal, R. evan der, Sharma, N., Mellish, C., Robinson, A., Siddharthan, A. (2016). The role of automated feedback in training and. *Conservation Biology*, *30*(3), 550–561.

Walther, D., & Kampen, H. (2017). The Citizen Science Project “Mueckenatlas” Helps monitor the distribution and spread of invasive mosquito species in germany. *Journal of Medical Entomology*, *54*(6), 1790–1794.

Wandiga, S. O., Opondo, M., Olago, D., Githeko, A., Githui, F., Marshall, M., Downs, T., Opere, A., Oludhe, C., Ouma, G. O., Yanda, P. Z., Kangalawe, R., Kabumbuli, R., Kathuri, J., Apindi, E., Olaka, L., Ogallo, L., Mugambi, P., Sigalla, R., Nanyunja, R., Baguma, T., Achola, P. (2010). Vulnerability to epidemic malaria in the highlands of lake Victoria basin: The role of climate change/variability, hydrology and socio-economic factors. *Climatic Change*, *99*(3), 473–497.

Wanzirah, H., Tusting, L. S., Arinaitwe, E., Katureebe, A., Maxwell, K., Rek, J., Bottomley, C., Staedke, S. G., Kamya, M., Dorsey, G., Lindsay, S. W. (2015). Mind the gap : house structure and the risk of malaria in Uganda. *PLoS ONE*, *10*(1), 1–15.

Watentena, A., & Okoye, C. I. (2019). Species specificity of carbon dioxide , 1-octen-3-ol , l-lactic acid and 2-butanone as mosquito chemo- attractants in mosquito surveillance : A review. *International Journal of Mosquito Research*, *6(1)*(March), 138–142.

West, S., & Pateman, R. (2016). Recruiting and retaining participants in citizen science: what can be learned from the volunteering literature? *Citizen Science: Theory and Practice*, *1*(2), 15.

White, G. B. (1974). *Anopheles gambiae* cmplex and disease transmission in Africa. *Royal Society of Tropical Medicine and Hygiene*, *68*(4), 278–298.

White, J. N., Pukrittayakamee, S., Hien, T. T., Faiz, M. A., Mokuolu, O. A., Dondorp, A. M. (2014). Malaria. *The Lancet*, *383*(9918), 723-735

WHO. (2009). Recommended insecticides for indoor residual spraying against malaria vectors. October, 2009.

WHO. (2015). World malaria report 2015. Geneva: World Health Organization; 2015.

WHO. (2015). Recommended long-lasting insecticidal nets Product name Deltamethrin coated on polyester. (p. 1).

WHO. (2016a). Artemisinin and artemisinin-based combination therapy resistance (Issue October).

WHO. (2016b). World Malaria Report 2016. Geneva: World Health Organization; 2017.

WHO. (2017a). Global vector control response 2017–2030. Geneva: World Health Organization; 2017.

WHO. (2017b). World Malaria report 2017. Geneva: World Health Organization; 2017.

WHO. (2018). World malaria report 2018. Geneva: World Health Organization; 2018.

WHO. (2019). World Malaria Report. Geneva: World Health Organization; 2019.

Wijngaarden, J. D. H. Van, Scholten, G. R. M., Wijk, K. P. Van. (2012). Strategic analysis for health care organizations : the suitability of the SWOT-anaylsis. *International Journal of Health Planning and Management*, *27*(July 2015), 34–49.

Wirth, D. F., Winzeler, E. A., Hall, B. F. ("Lee"), Bopp, S. E., LaMonte, G., Rabinovich, N. R., Shaw, W. R. (2017). The malERA Refresh consultative panel on basic science and enabling technologies (2017) malera: an updated research agenda for basic science and enabling technologies in malaria elimination and eradication. *PLoS Medicine*, *14*(11), 1–29.

Wirtz, R. A., Zavala, F., Charoenvit, Y., Campbell, G. H., Burkot, T. R., Schneider, I., Esser, K. M., Beaudoin, R. L., Andre, R. G. (1987). Comparative testing of monoclonal antibodies against *Plasmodium falciparum* sporozoites for ELISA development. *Bulletin of the World Health Organization*, *65*(1), 39–45.

Wong, J., Bayoh, N., Olang, G., Killeen, G. F., Hamel, M. J., Vulule, J. M., Gimnig, J. E. (2013). Standardizing operational vector sampling techniques for measuring malaria transmission intensity: Evaluation of six mosquito collection methods in western Kenya. *Malaria Journal*, *12*(1), 1–11.

Worthington, J. P., Silvertown, J., Cook, L., Cameron, R., Dodd, M., Greenwood, R. M., Mcconway, K., Skelton, P. (2012). Evolution megaLab : a case study in citizen science methods. *Method in Ecology and Evolution*, *3*, 303–309.

Wu, T.-P., Tian, J.-H., Xue, R.-D., Fang, Y.-L., Zheng, A.-H. (2016). Mosquito (Diptera: Culicidae) habitat surveillance by android mobile devices in guangzhou, China. *Insects*, *7*(4), 79.

Yewhalaw, D., Legesse, W., Van Bortel, W., Gebre-Selassie, S., Kloos, H., Duchateau, L., Speybroeck, N. (2009). Malaria and water resource development: the case of Gilgel-Gibe hydroelectric dam in Ethiopia. *Malaria Journal*, *8*, 21.

Yoshioka, K. (2013). Impact of a community-based bug-hunting campaign on Chagas disease control: A case study in the department of Jalapa, Guatemala. *Memorias Do Instituto Oswaldo Cruz*, *108*(2), 205–211.

Yujia He. (2017). Global Mosquito Alert: UN Backed Citizen Science Platform to Fight Mosquito-Borne Diseases | Wilson Center. https://www.wilsoncenter.org/blog-post/global-mosquito-alert-un-backed-citizen-science-platform-to-fight-mosquito-borne-0

Yukich, J. O., Butts, J., Miles, M., Berhane, Y., Nahusenay, H., Malone, J. L., Dissanayake, G., Reithinger, R., Keating, J. (2014). A description of malaria sentinel surveillance : a case study in Oromia regional State , Ethiopia. *Malaria Journal*, *13*(88), 1–13.

Zahar, A. R. (1984). Vector bionomics in the epidemiology and control of malaria. WHO.

Zhou, G., Munga, S., Minakawa, N., Githeko, A. K., Yan, G. (2007a). Spatial Relationship between Adult Malaria Vector Abundance and Environmental Factors in Western Kenya Highlands. *American Journal for Tropical Medecine and Hygiene*, *77*(1), 29–35.

Zhou, G., Munga, S., Minakawa, N., Githeko, A. K., Yan, G. (2007b). Spatial Relationship between Adult Malaria Vector Abundance and Environmental Factors in Western Kenya Highlands Spatial Relationship between Adult Malaria Vector Abundance and Environmental Factors in Western Kenya Highlands. *Am. J. Trop. Med. Hyg.*, *77*(1), 29–35.

Zhou, Y. H., Zhang, Z. W., Fu, Y. F., Zhang, G. C., Yuan, S. (2018). Carbon dioxide, odorants, heat and visible cues affect wild mosquito landing in open spaces. *Frontiers in Behavioral Neuroscience*, *12*(May), 1–8.

Zurovac, D., Talisuna, A. O., Snow, R. W. (2012). Mobile phone text messaging: Tool for malaria control in Africa. *PLoS Medicine*, *9*(2), 1–6.

Zwiebel, L. J., & Takken, W. (2004). Olfactory regulation of mosquito-host interactions. *Insect Biochemistry and Molecular Biology*, *34*(7), 645–652.

ART	Anti-retroviral treatment
ECDC	European Centre for Disease Prevention and Control
IVM	Integrated Vector Management
MOH	Rwandan Ministry of Health
MOPDD	Malaria and Other Parasitic Diseases Division
NMCP	National Malaria Control Programme
PAR	Participatory Action Research
PMI	President’s Malaria Initiative
REMA	Rwanda Environmental Management Authority
RDT	Rapid Diagnostic Test

Acknowledgements

As my PhD journey has come to an end, I had a great opportunity to be surrounded by amazing people who provided me extensive personal and professional guidance and taught me a lot about scientific research and life in general. I would like to convey my heartfelt gratefulness and sincere appreciation to all the people around me who contributed and made my PhD journey, though challenging, possible and enjoyable.

My deepest gratitude goes to my promotor Prof. Willem Takken for the continuous support, patience, motivation and the immense knowledge. Your depth of understanding issues is something I appreciate. I am very grateful for the scientific advice and the valuable inputs you provided all along from the very start of my PhD thesis up to its completion.

To my daily supervisor and co-promotor Dr. Sander Koenraadt, I am very grateful for your guidance, involvement, support and daily encouragement from the start of the project till today. Your own zeal for perfection, passion, unflinching courage and conviction has always inspired me to do more. Under your guidance I successfully overcame many difficulties and learnt a lot. I learnt that step by step and with a lot of courage you can accomplish your goal. You have shown me, by your example, what a good scientist (and person) should be. For all of these, I sincerely thank you from the bottom of my heart.

To my co-promotor Arnold van Vliet, I am grateful for your interest in both my academic and personal life. I struggled a lot at the beginning of the project, but you challenged me to think out of the box and made things easier to discuss and you provided good suggestions, which led to the completion of this thesis. You pushed me to put myself out of my comfort zone, thank you, I am extremely grateful.

To Marijn Poortvliet my co-supervisor, thank you very much for the valuable advices, constructive criticisms, positive appreciation and inputs to the chapters of this thesis. As my research was more interdisciplinary, you helped me to better understand the social aspect of it and your contribution throughout the course of my PhD has led to the successful completion of this thesis. I will not forget to thank you for keeping doors always opened for me when I had questions or needed to work at the Leeuwenborch.

My special thanks to my co-promotor Dr. Leon Mutesa, for your contribution, support, mentorship, constructive comments and for supervising the EVOCA project (Environmental Virtual Observatories for Connective Action) in Rwanda with energy and wise guidance, as local Principal Investigator, despite all the challenges. Thank you very much from the bottom of my heart.

My sincere thanks to Dr. Emmanuel Hakizimana, for your guidance, support, inputs, questions raised, and challenges that guided the path of my entire work. I gratefully acknowledge the

Vector Unit of Malaria and Other Parasitic Diseases Division - Rwanda Biomedical Center for providing me with the platform and support to carry out my research.

I had a great opportunity to take part in an interdisciplinary research programme, and I would like to thank people from different chair groups I worked with at Wageningen University. In the Knowledge Technology and Innovation Group, I would like to thank Prof. Dr. Cees Leeuwis for the patience, support and all your suggestions and inputs. A heartfelt thanks to Vera Mentzel, Inge Ruisch, Mirjam Cevat, and Meta Bakker for your administrative assistance at Wageningen University & Research. You made my stay so smooth and I felt at home thanks to you. Many thanks to Leonieke Klumpes and Jacqueline de Graaff for supporting me with my travel arrangements to the Netherlands. My special thanks to Domina Asingizwe for being an incredible project collaborator and friend. Your relevant and critical thinking and invaluable advice touched me. I learnt a lot from you and without you the journey would have been incomplete, for that I am grateful. Many thanks to all my EVOCA PhD colleagues, who have provided friendship and support, and with whom I have shared laughter, frustration and companionship. Nyamwaya Munthali, Emmanuel Nyadzi, Faith Mutavi, Shiferaw Gobena, Elias Assefa, Richard Chepkwony, Jusper Ronoh, Mariëtte McCampbell, Rebecca Sarku, Talardia Gbangou, the best of luck to you in all your endeavours. To the Laboratory of Entomology chair group at Wageningen University & Research, my deepest appreciation goes to Prof. Marcel Dicke, for hosting me in a such amazing great team. I would like to thank Pieter Rouweler, Frans van Aggelen and André Gidding for helping with rearing mosquito larvae for my laboratory experiments. To my colleagues and friends from Laboratory of Entomology group at Wageningen University & Research: Tessa, Jeanine, Marieke, Shaphan, Daan, Steve, Julien, Kay, Thibault, Helen, Julian, Charlotte, Jeroen, Rody, Margaret, Jeroen, Karen, Monicah, Filippo, Alessia, Alexander, Sandeep, Antonio, Davy, Els, Gabriela, Jessica, Bram, Julia, Karol, Katherine, Luuk, Maite, Max, Mitchel, Peter, Pieter, Simone, Sandra, Aidan, Anna, Quint, Yavanna, Qi, Hanneke, Nathan, Amelia, Wang, Sivaprakaham, Stijn, Kelly, Pauline, and Yidong; thank you for your help in various ways. Thanks for the great atmosphere you all created, for all the fun, great times, and fruitful discussions that we had during the past four years. I very much enjoyed all our coffee breaks, drinks, and parties. Special thanks to Chantal Vogels for your suggestions and inputs. I address special thanks to Jeanine Loonen and Marieke de Swart for being my paranymphs. Thanks to Luc Dinnissen for the design of the cover of my thesis. I would like also to extend my thanks to all the students that I supervised during the past four years: Deirdre Bosch and Arash Hoseni thank you for your inputs and contribution to my research work.

I extend my thanks to the team of laboratory of entomology in Rwanda: Elias Nyituma, Xavier Misago, Jackie Umufasonie, Emmanuel Rugarama, Theoneste Munyemanzi, Marie Rose Kakuze, Primitive Mukanyandwi, and Joseph Nzanywayimana, thank you for your support during my field data collection in Ruhuha, Rwanda. Many thanks to Alphonse Mutabazi for helping me out with the GIS analysis and drawing of maps of Rwanda. My sincere thanks go

to the staff member of Ruhuha health center for all facilitations they made available to me and my team, your contribution is highly appreciated. I extend my gratitude to all the community members of Ruhuha and Busoro for allowing me to work with them in their homesteads. The local leaders, community members and volunteers are acknowledged for their contribution towards this research work.

Special thanks to my colleagues at the department of Biology, College of Science and Technology, at University of Rwanda for the nice working environment and fruitful discussions and feedback. Thank you all for the inputs and interesting discussions we had.

I would like to thank the African community and the united community of African students (UCAS) groups and the Rwandan community at Wageningen University & Research, I deeply thank you for your friendship, the very memorable times we spent together, and your support whenever needed.

I would like to thank my father and my mother, you always encouraged me to stick to my education. This thesis is dedicated to your unconditional love and support. I am grateful particularly to my mum the way you looked after my daughters while I was pursuing my studies in the Netherlands. You gave me a special comfort and encouraged me to fulfill my duties. Your support during those moments was very helpful, I hope that I have made you proud. To my siblings and extended family, I express my gratitude for all the love, support and encouragement. To my friends back in Rwanda and around the world, thank you for your true friendship and encouragements.

To our daughters Priscilla et Leah, you are my inspiration to achieve greatness. Without you, I would not be where I am today. To my husband Roger thank you so much for being there for me, your great support, love, patience and encouragement are priceless. I simply could not have done this without you. I will always be grateful to you.

Finally, my profound gratitude goes to the Interdisciplinary Research and Education Fund (INREF), Wageningen University, The Netherlands and the University of Rwanda, College of Medicine and Health Sciences who made this research and my PhD possible. This experience has afforded me the opportunity to work with some of the brightest and best, and the resources for me to achieve great success. Thank you for this opportunity.

Curriculum vitae

Marilyn Milumbu Murindahabi was born on 7 March 1980 in Likasi, Democratic Republic of the Congo. After completing her primary and secondary school education, she enrolled at the National University of Rwanda where she obtained a Bachelor's degree in Biology, option Biotechnology in 2005. In 2006 she worked at the National University of Rwanda as a laboratory technician, in the faculty of Sciences, at the department of biology for two years. In 2007, Marilyn joined the International Center of AIDS Care and Treatment Programs (ICAP) Mailman School of Public Health Columbia University and Food and Agriculture Organization (FAO) Rwanda as a laboratory specialist for the Avian Influenza project at the National Veterinary Laboratory of Rwanda Animal Resources Development Authority. In 2010, she obtained a scholarship from the 'Nederlandse organisatie voor internationale samenwerking in het hoger onderwijs' (Nuffic) to pursue a two-year master programme in Life Sciences - with a specialization in Medical Biotechnology at Wageningen University and Research. During her MSc thesis, Marilyn investigated the transcriptional regulation of genes involved in immune response, fat metabolism, and oxidative stress of different *Caenorhabditis elegans* strains and the gene expression of some selected lines destined to create Recombinant Inbred Line Panel in *Caenorhabditis elegans* after exposure to different bacteria. During her MSc internship, Marilyn investigated drug resistance profiles of HIV Type 1 of infected patients under antiretroviral treatment in Rwanda using HIV Genotyping. From 2013 Marilyn worked as an assistant lecturer at the department of biology in the College of Science and Technology (CST) of the University of Rwanda. In 2016, Marilyn enrolled in a PhD programme with Wageningen University and Research, the Netherlands. Within her PhD research project, she evaluated the feasibility of a citizen science approach for malaria vector surveillance and control in Rwanda with the aim to reinforce the mosquito monitoring programmes currently in place. The results of this research are presented in this thesis. Marilyn's ambition is to continue conducting research on the development of innovative approaches and novel technologies for control of malaria vectors and other neglected tropical diseases.

List of publications

Published papers

Asingizwe, D.*, **Marilyn Milumbu Murindahabi***, Constantianus J.M Koenraadt, P. Marijn Poortvliet, Arnold J.H. van Vliet, Chantal M. Ingabire, Emmanuel Hakizimana, Leon Mutesa, Willem Takken, and Cees Leeuwis (2019). "Co-design a Citizen science Programme for Malaria Control in Rwanda" Sustainability 11 (24):1-17

Asingizwe, D., P. Marijn Poortvliet, Constantianus J.M Koenraadt, Arnold J.H. van Vliet, **Marilyn Milumbu Murindahabi**, Chantal M. Ingabire, Leon Mutesa and Peter H. Feindt. (2018). "Applying Citizen Science for Malaria Prevention in Rwanda: An Integrated Conceptual Framework". NJAS - Wageningen Journal of Life Sciences 86(87):111-122

Marilyn Milumbu Murindahabi, Domina Asingizwe, P. Marijn Poortvliet, Arnold J. H. van Vliet, Emmanuel Hakizimana, Leon Mutesa, Willem Takken, and Constantianus J. M. Koenraadt. 2018. "A Citizen Science Approach for Malaria Mosquito Surveillance and Control in Rwanda." NJAS - Wageningen Journal of Life Sciences 86–87:101–10.

**These authors contributed equally to this work*

Submitted

Marilyn Milumbu Murindahabi, Willem Takken, Xavier Misago, Elias Niyitima, Jackie Umupfasoni, Emmanuel Hakizimana, Arnold J.H. van Vliet, P. Marijn Poortvliet, Leon Mutesa, Nathalie Kayiramirwa Murindahabi and Constantianus J.M. Koenraadt. Monitoring mosquito nuisance for the development of a citizen science approach for malaria vector surveillance in Rwanda (Chapter 3 in this thesis)

In preparation

Marilyn Milumbu Murindahabi, Willem Takken, P. Marijn Poortvliet, Arnold J.H. van Vliet, Emmanuel Hakizimana, Leon Mutesa, Constantianus J.M. Koenraadt. A handmade trap for malaria mosquito surveillance by citizens in Rwanda (Chapter 5 in this thesis)

Marilyn Milumbu Murindahabi, Arash Hoseni, Corné Vreugdenhil, Arnold J.H. van Vliet, Jackie Umupfasoni, Alphonse Mutabazi, Emmanuel Hakizimana, P. Marijn Poortvliet, Leon Mutesa, Willem Takken, and Constantianus J.M. Koenraadt. Using Citizen science to evaluate the spatial and temporal dynamics of malaria vectors in Ruhuha, Rwanda in relation to environmental risk factors (Chapter 6 in this thesis)

Training and education statement

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (4.5 ECTS)

- Malaria mosquito radar as a digital citizen science platform against malaria in Rwanda: the biological and epidemiological perspective (2018)

Writing of project proposal (4.5 ECTS)

- Malaria mosquito radar as a digital citizen science platform against malaria in Rwanda: the biological and epidemiological perspective (2016)

Post-graduate courses (8.6 ECTS)

- INREF-EVOCA PhD start-up programme; WUR (2016)
- Basic statistics; WUR (2016)
- Generalized linear models; WUR (2018)
- Introduction to R for statistical analysis; WUR (2018)
- Linear models; WUR (2018)

Laboratory training and working visits (1.5 ECTS)

- Training entomological surveillance & planning; RBC, Rwanda (2017)

Deficiency, refresh, brush-up courses (5 ECTS)

- Research methodology: from topic to proposal; WUR (2016)
- GIS for society; WUR (2016)

Competence strengthening / skills courses (7.8 ECTS)

- The Essentials of Scientific Writing and Presenting (ESWP); WUR (2016)
- Project and time management; WUR (2016)
- Data management planning; WUR (2016)
- Information literacy for PhD including EndNote introduction; WUR (2016)
- Intensive workshop on efficient writing strategies (EVOCA Module); WUR (2018)
- Extreme citizen science workshop; WUR (2018)
- Companion modelling; WUR (2018)

Scientific integrity / ethics in science activity (0.4 ECTS)

- Ethics for social sciences research; WUR (2018)

PE&RC Annual meetings, seminars and the PE&RC weekend (2.4 ECTS)

- PE&RC Workshop carrousel (2016)
- PE&RC Weekend first year (2016)
- PE&RC Day (2016, 2019)
- PE&RC Weekend last year(2019)

Discussion groups / local seminars / other scientific meetings (4.75 ECTS)

- One health entomology meeting; WUR (2016)
- One health entomology meeting; WUR (2018)
- Yearly Entomology Laboratory Research & Exchange Meeting (YELREM); WUR (2018)
- One health entomology meeting; WUR (2019)

International symposia, workshops and conferences (9.7 ECTS)

- 1st International EVOCA workshop, oral presentation; WUR, NL (2016)
- SASA International conference and the 2nd Rwanda biotechnology conference; poster presentation; Kigali, Rwanda (2017)
- 2nd International EVOCA workshop, oral presentation; Accra, Ghana (2017)
- Health research & policy day; poster presentation; Kigali, Rwanda (2018)
- 3nd International EVOCA workshop, oral presentation; WUR, NL (2018)
- 4th International EVOCA workshop, poster presentation; WUR, NL (2019)
- American society of tropical medicine and hygiene meeting; poster presentation; National Harbor, USA (2019)

Lecturing / supervision of practicals / tutorials (10.8 ECTS)

- Genetics and evolution theory; College of Science and Technology, University of Rwanda (2016)
- Introduction to molecular biology and biotechnology; College of Science and Technology, University of Rwanda (2017)
- Immunology and immunogenetics; College of Science and Technology, University of Rwanda (2018, 2019)

Supervision of MSc students (3 ECTS)

- The influence of small-scale human movement on the transmission of malaria
- The spatial and temporal distribution of mosquito abundance in Ruhuha, Rwanda related to environmental factors

The research described in this thesis was undertaken as part of the collaborative interdisciplinary project of Wageningen University and partner institutions called 'Responsible life-sciences innovations for development in the digital age: Environmental Virtual Observatories for Connective Action (EVOCA)', financed by Interdisciplinary Research and Education Fund (INREF), Wageningen University & Research.

Cover design and Lay-out: Marilyn Milumbu Murindahabi and Luc Dinnissen (studio ds)
Printed by: Digiforce print and logistics ||ProefschriftMaken