A participatory approach for malaria control in southern Malawi

Effects of the environment and community on larval source management

Steven A. Gowelo

Propositions

1. Community-led initiatives can only succeed where they fall within people's priorities (this thesis)

2. Application of microbial larvicides mediates egg-laying in mosquitoes (this thesis)

3. Against the common belief, industry limits academic research

4. Only human beings are responsible for climate change

5. Democracy is never attainable where masses are poor

6. Attainment of higher education creates social discrimination in women

Propositions belonging to this thesis entitled:

"A participatory approach for malaria control in southern Malawi: Effects of the environment and community on larval source management"

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Wageningen University, June 9, 2020

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

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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 Tuesday 9 June 2020
at 4 p.m. in the Aula.

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A participatory approach for malaria control in southern Malawi: Effects of the environment and community on larval source management
156 pages.

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

ISBN: 978-94-6395-364-1
DOI: https://doi.org/10.18174/518514

Table of contents

Chapter 1

General Introduction

Introduction

Malaria in Malawi

In recent decades, significant progress has been made in the control of malaria, with *Plasmodium falciparum* infection prevalence halved between 2000 and 2015 in endemic countries in Africa (Bhatt et al. 2015). Much of this success was attributed to vector control, notably the use of insecticide-treated bed nets (ITNs) and indoor residual spraying (IRS). Further progress in the fight against malaria, however, has stalled and in some African countries malaria has even increased (WHO 2018, 2019a). As of 2012, an estimated 1.2 billion of the 3.4 billion people that were at high risk of malaria were living mostly in the African region (47%) and South-East Asia (37%) (WHO 2013a). In Malawi, malaria is endemic in more than 95% of the country (Chanda et al. 2015), and remains a huge public health challenge, with children under five years old and pregnant women being the most affected. In 2009, 40% of all hospitalizations of children under five years of age and 34% of all outpatient visits across all ages in the country were due to malaria (National Malaria Control Programme Malawi and ICF International 2012). Recent reports show that the disease accounts for 30% of all outpatient visits across all ages (2016 Health Management Information System [HMIS] data, unpublished). Entomological studies have implicated the mosquito species *Anopheles gambiae s.s., An. arabiensis* and *An. funestus* in driving the transmission of malaria in Malawi (Mzilahowa et al. 2012). The malaria parasite *Plasmodium falciparum* is responsible for 98% of the malaria cases in Malawi (PMI 2018).

Malaria vector control

The most important vectors of malaria in Africa comprise members of the *An. gambiae* species complex and the *An. funestus* group of mosquitoes because of their widespread distribution, preference for blood feeding from humans and endophilic behaviour (Annan et al. 2007). These traits result in frequent vector-human contact, allowing for efficient disease transmission (Lwetoijera et al. 2014). Current global initiatives to control malaria have included a combination of preventive and curative measures such as use of insecticide-treated bed nets, mosquito repellents, chemoprophylaxis (effective drug combinations), and effective case management (early diagnosis and treatment) (WHO 2015). Indoor residual spraying (IRS) and long-lasting insecticide treated bed nets (LLINs) have demonstrated efficacy at reducing the female mosquito's daily survival rate and human biting frequency (Irving et al. 2012). IRS has assumed much praise in terms of immediate impact on vector control and its impact has been widely proven in the elimination of malaria in many parts of the world (Karunamoorthi 2011). ITNs remain the cornerstone of malaria prevention in Africa (Lindblade et al. 2015) owing to the combination of the insecticidal and irritant effect of the pyrethroids together with the physical barrier of the bed net. Consistent use of ITNs has been demonstrated to reduce malaria transmission by up to 90% (Gimnig et al. 2003) and avert as much as 44% of all-cause mortality among children under five

(Lengeler 2004). For instance, LLINs have been successful, their scale-up contributing to avert 1.1 million deaths between 2000 and 2012 worldwide (Bhatt et al. 2015, Glunt et al. 2015).

In the past two decades, Malawi has seen massive scale up of up malaria interventions including intermittent preventive therapy in pregnancy (IPTp), ITNs, the use of RDTs for diagnosis, and ACTs for treatment. IRS has also been used in selected districts. ITNs, however, remain the backbone of malaria control in the country. Recent nationwide surveys have shown that ITN coverage (access to and use of ITNs) has increased tremendously since the early 2000s, currently estimated at above 70% across the country (National Malaria Control Programme-NMCP/Malawi ICF International 2015).

ITNs and IRS target mosquitoes that bite and rest indoors, yet some malaria vectors bite or rest outdoors, thereby escaping the effects of the interventions. A study in Kenya reported changes in species composition due to widespread use of ITNs. The study showed that over the years there has been a shift from a predominantly endophilic *An. gambiae s.s.,* to a more exophilic *An. arabiensis* dominated vector community (Bayoh et al. 2010). Such changes in vector species composition have an impact on malaria transmission dynamics and control as mosquito taxa that avoid feeding or resting indoors sustain malaria transmission as vector control interventions such ITNs and IRS are scaled up (Govella et al. 2013). A study carried out in Macha, Zambia showed that *An. arabiensis* remained highly anthropophilic despite changes in its feeding behaviour from biting indoors to outdoors and from dusk to late mornings (Fornadel et al. 2010). This highlights the vulnerability of control programmes relying solely on ITN use, and therefore calls for novel strategies that could close the existing gaps in control.

An additional problem is that insecticide resistance in malaria vectors is now widespread in many African countries, which is attributable to the extensive use of insecticides. For instance, a recent study in Kenya has shown that *An. gambiae* has acquired high resistance to pyrethroids and DDT, and patchy resistance to carbamates (Wanjala et al. 2015). Resistance to pyrethroids, the only approved class of insecticides for use on ITNs and widely used for IRS, has been documented in *An. funestus* and *An. gambiae* s.l. in many African countries, at least partially as a result of widespread distribution of ITNs (Lindblade et al. 2015). These developments have limited the options for IRS and caused concern regarding the continued effectiveness of ITNs. Resistance to some classes of insecticides is now widely spread in Malawi (Mzilahowa et al. 2016, PMI 2018). Pyrethroid resistance has been selected in Malawi in *An. gambiae, An. arabiensis* and *An. funestus,* with the highest frequency of resistance in the latter (Wondji et al. 2012, Mzilahowa et al. 2016). In Malawi, *An. funestus* from sentinel sites has developed resistance to a carbamate, bendiocarb (24–45% survival), but there is no evidence of organophosphate or organochlorine resistance (Wondji et al. 2012). Development of insecticide resistance in the malaria vectors threatens the efficacy of

current interventions and underpins the need to explore additional interventions to complement the standard strategies to further reduce transmission. Larval source management (LSM) could complement existing strategies in areas where breeding habitats are '*few, fixed,* and *findable*' (WHO 2013b) or where malaria vectors exhibit exophagic and exophilic behaviours (Okumu and Moore 2011), and in settings where insecticide resistance has emerged.

Larval source management

Mosquito larval source management (LSM) is the management of water bodies that are potential larval habitats to prevent the development of immature mosquitoes into adults (WHO 2013b). A number of studies provide evidence in support of LSM as a viable tool for malaria control (Fillinger et al. 2009, Mwangangi et al. 2011, Tusting et al. 2013, Dambach et al. 2019, Derua et al. 2019). Some of the reasons for recent recognition of LSM include opportunities to complement adulticiding with other components of integrated vector management (Fillinger et al. 2009), no reports of insecticide resistance to commonly used larvicides or harm to the environment (WHO 2013b) and cost-effectiveness (Fillinger and Lindsay 2006, Olalubi 2016). Targeting larval stages is particularly important because mosquitoes are killed indiscriminately before they disperse to human habitations and contact the insecticides used in IRS/LLINs (Mwangangi et al. 2011). Thus, LSM could supplement LLINs and IRS by further suppressing transmission via targeting of the aquatic mosquito stages hence attacking both outdoor and indoor biting vectors (Fillinger and Lindsay 2011).

Globally, LSM has been implemented against a wide range of vector species through environmental management, larvicides or larvivorous fish. Though with unclear impact on malaria transmission and adult anopheline densities, the use of larvivorous fish such as *Gambusia affinis, Tilapia* spp., *Poecilia reticulata*, and Cyprinidae in particular has been employed in mosquito control for decades (Walshe et al. 2017). Apart from chemical larvicides, microbial larvicides have also been used in the control of vector-borne diseases, including malaria. Two endotoxin-producing bacterial species, *B. thuringiensis israelensis* (*Bti*) and *B. sphaericus* (*Bs*), have been widely shown to be effective larvicides against mosquitoes, and various other nematoceran Diptera with aquatic larvae, such as Ceratopogonidae (biting midges), Chironomidae (non-biting midges) and Simuliidae (blackflies) (Walker and Lynch 2007). Habitat modification (permanent or long-lasting physical transformation of a larval habitat through draining, filling and land levelling) and habitat manipulation (planned recurrent activities aimed at producing temporary conditions unfavourable to the breeding of vectors in their habitats) have also been employed to control mosquitoes (Karunamoorthi 2011).

Inclusion of LSM in integrated malaria management in urban Dar es Salaam (Tanzania) and in Eritrea has proven its ability to reduce indoor mosquito populations but also secondary vectors that remain less affected by ITNs and IRS because of their ability to bite and/or rest outdoors (Worrall and Fillinger 2011). The strategy has been

shown to successfully reduce the density of adult vectors and consequently malaria transmission and morbidity in settings with fixed and findable larval habitats (Tusting et al. 2013, WHO 2013b).

In Malawi no research has been documented that has investigated the effects of larviciding and community engagement in malaria control. Therefore, there is no basis no basis for formulation and adoption of policy regarding LSM. Further, the categorization of larval habitats as few, fixed and findable is challenging for countries like Malawi where the landscape varies widely across the country and malaria vector larval ecology is not well studied. In some parts of rural Malawi, larval habitats are often numerous and may change over time with the seasons. It would, therefore, be ideal to involve communities in implementing LSM as the task of locating the habitats would rely on knowledge of their immediate vicinity.

Bacillus sphaericus (Bs)

Bacillus sphaericus (*Bs*) is an aerobic bacterium producing two types of proteins, crystal and Mtx toxins, which produce larvicidal effects by acting on specific receptors in the midgut of culicid larvae (Melo et al. 2009). Neither protein alone is toxic to larvae, and both are required for toxicity (Baumann et al. 1991). *Bs* is particularly advantageous because of its long residual activity observed to persist for at least 5 months in artificial pools containing waste water (Pantuwatana et al. 1989), tolerance to organic pollution and its high specificity in terms of target organisms (Mwangangi et al. 2011). However, resistance to this larvicide has been reported in some mosquito species (Poopathi and Abidha 2013) such as *Culex quinquefasciatus* (Wirth et al. 2005) and *Cx. pipiens* (Su et al. 2019).

Bacillus thuringiensis* var. *israelensis (Bti)

Bacillus thuringiensis var. *israelensis* (*Bti*) is a gram-positive, soil-dwelling and spore-forming entomopathogenic bacterium (Boyce et al. 2013) commonly used as a biological pesticide. It is highly lethal against Culicidae (mosquitoes) and Simuliidae (blackflies), and has some lethal effects against certain other Diptera, especially Chironomidae (midges). The lethal effect of *Bti* on mosquito larvae is largely due to protoxins in parasporal crystals and the sporal coat (Abdul-ghani et al. 2012). Upon ingestion by a susceptible species, the proteins in the crystals are solubilized in the midgut by a combination of alkaline pH and proteolysis (Zahiri and Mulla 2005). The larvicidal activity of *Bti* is derived from four major and at least two minor proteins referred to, respectively, as *cry4Aa*, *cry4Ba*, *cry11Aa*, *cyt1Aa*, *cry10Aa* and *cyt2Ba* (Ben-Dov 2014). The crystal toxin binds to a receptor on the midgut cell wall resulting in pore formation in the cell, which leads to cell lysis and, consequently, death of the larva (Farajollahi et al. 2013a). The bacterium is usually active for one to two weeks at most (Walker and Lynch 2007). The combination of endotoxins produced by *Bti* reduce the chance of development of resistance against the bio-insecticide. So far,

there are no reports of development of resistance to *Bti* (Varjal De Melo-Santos et al. 2001, Farajollahi et al. 2013a).

Apart from its lethal effects on larvae, *Bti* may have some negative effects on adult mosquitoes that survive exposure as larvae. The effects have been shown to range from reductions of egg rafts, low number of eggs per egg raft, and reduced hatching and survival rates in *Cx. quinquefasciatus* (Zahiri and Mulla 2005). A similar study on *An. superpictus* showed that exposure to *Bti* has adverse effects on sex ratios, and gross and net reproductive rates with the effects on the latter increasing with increasing *Bti* concentrations (Simsek et al. 2009). Exposure of *Aedes aegypti* larvae to sublethal doses of *Bti* has also been observed to significantly reduce larval and adult survival rates, and lower blood-engorgement rate and egg production at both the parental and offspring (F1) stages (Lee and Zairi 2005).

Factors affecting *Bti* activity

Despite the proven efficacy of *Bti* as a larvicide, its activity and feasibility for use as a mosquito-toxic agent is highly influenced by a number of factors: implementational, biotic and abiotic factors such as feeding behaviour of mosquito larvae which varies with age, density of larvae and habitat factors (temperature, depth of water, turbidity, presence of vegetation, etc.) (Lacey 2007). Biotic factors have been shown to affect the efficacy of *Bti*. The larvicide has been found to be less effective in habitats with a high algal content, primarily because of the inability to penetrate algal mats (Shililu et al. 2003). Strikingly, *Bs* has been found to persist in polluted aquatic environments where *Bti* rapidly loses its toxicity (Baumann et al. 1991). It has also been reported that the feeding behaviour of insects greatly influences the amount of *Bti* ingested, which in turn influences its susceptibility for the biocide. *Anopheles* spp. are less susceptible to *Bti* than *Culex* or *Aedes* as they are surface feeders unlike the two latter genera, which feed throughout the water column (Glare and O'Callaghan 1998). A number of studies have also shown that developmental stage of larvae is key in the efficacy of *Bti*. For example, increasing age of the larvae resulted in a reduced susceptibility, with 4th-instar larvae being the least susceptible larval stage (Nayar et al. 1999). Because pupae do not feed they are not vulnerable to the effects of *Bti* (Ramrez-Lepe and Ramrez-Suero 2012).

A number of abiotic factors have also been implicated in reducing the efficacy of *Bti.* Low temperature has been particularly shown to reduce the effectiveness of *Bti*. This has been attributed to reduced rate of larval feeding and hence, a reduced accumulation of lethal dosage of *Bti* (Cao et al. 2012). In *An. gambiae,* larval development ceases at temperatures below 16°C and death generally occurs at temperatures below 14°C (Minakawa et al. 2005). At lower temperatures the activity of proteolytic enzymes in the gut and the binding of *Bti* toxins to midgut epithelial cells reduce, affecting the *Bti* mode of action (Walker 1995). At higher temperatures, larval development is accelerated which makes the larvae consume more nutrients

and become more susceptible (Nayar et al. 1999). For example, in *An. gambiae* s.s, larval development increases at higher temperatures with a peak at 28°C (Bayoh and Lindsay 2003). It is thus important to consider water temperatures in the application of *Bti* in potential habitats. Greater quantities of *Bti*, therefore, need to be applied at temperatures below 8 °C (Becker et al. 1992). Sunlight has also been found to reduce the effectiveness of *Bti* by inactivating the larvicide (Becker et al. 1992). The activity of microbial larvicides is reduced by exposure to sunlight (Rydzanicz et al. 2010).

Few studies have attempted to measure the impact of water pH on *Bti* activity on anopheline larvae under field conditions. Lower pH has been observed to induce loss of efficacy of *Bti* on *Simulium decorum* larvae under laboratory conditions (Lacoursiere and Charpentier 1988). The authors propose that acidic conditions "over-stabilize" the paracrystalline structure making it less susceptible to rapid dissolution and activation. On the other hand, alkaline conditions prevalent in the insect gut dissolve the paracrystalline bodies which allows for endotoxin activation by proteolytic enzymes associated with protein digestion (Lacey et al. 1978).

The limitations posed by the factors discussed above on the activity and persistence of *Bti* indicate the need to put in place proper *Bti* spraying strategies. Knowing that *Bti* is active only for several days or a few weeks (Walker and Lynch 2007), highlights the need for repeated applications of the microbial larvicide. For instance, a study carried out in The Gambia demonstrated that weekly treatment intervals can reduce pupal production by 64–94% (Majambere et al. 2007). In a separate study in Côte d'Ivoire repeated applications of *Bti* and *Bs* caused a decline in the biting rate of both *An. funestus* and *An. gambiae* at population level (Tchicaya et al. 2010). The study further reported that the entomological inoculation rate of *An. funestus* was reduced (from 328 to 142) and this was attributed to a drop in the biting rate due to the repeated application of the larvicides. These findings suggest that successes or failure of LSM activities are partly explained by how it is implemented (WHO, 2013).

Involving communities in LSM

Until recently, LSM activities have largely been conducted by dedicated teams with relevant expertise. Though evidence for LSM as a malaria control tool is, to-date, not very convincing, success of the intervention could be further improved by involving communities in its implementation (WHO 2013b). This is largely due to high spatial heterogeneity of habitats exploited by *Anopheles* mosquitoes for breeding, which range from small temporary to very large permanent water bodies. Larval sampling from temporary habitats is challenging (Minakawa et al. 1999). This could be attributed to the laborious effort that is required to locate very small temporary water bodies as opposed to large conspicuous habitats. This presents an opportunity for community involvement in LSM activities due to knowledge of their immediate vicinity. A number of studies have documented the important role communities can play for successful LSM implementation. Involving communities in LSM activities could: (1) enable adequate coverage of targeted areas through communities' comprehensive

knowledge of mosquito larval habitats, (2) reduce costs of implementation, as human capital is locally available and (3) increase community support and ownership due to minimal requirement of technical skills. The demonstrated feasibility of LSM through community involvement in Rwanda (Ingabire et al. 2017) highlights the important role communities can play in malaria control innovations.

With lack of sufficient evidence-base regarding feasibility of community-based LSM, initiating the community's acceptability of the intervention remains one of the largest challenge. It is very important to be aware of the diverse social-cultural contexts in which individual projects can be implemented. A multidisciplinary approach involving natural and social scientists could achieve a better understanding of communities' knowledge, attitudes and practice towards malaria. Such approaches could be used to device better ways of getting communities on board. Community empowerment through a process of mindset change, leadership, vision, commitment and action has been identified as central to successful implementation of a community-based malaria control project in Malawi, The Majete Malaria Project (McCann et al. 2017, Van den Berg et al. 2018). The Majete project highlights key points for successful implementation of community-based LSM: involving communities in planning, implementation and monitoring of the project.

There is a growing realization that further reductions or elimination of malaria cannot be achieved with only the primary vector control interventions, especially due to vector behavioural plasticity and development of insecticide resistance (WHO 2017). LSM has synergistic effects on the standard vector control strategies thus it has regained renewed attention in recent years. In rural Malawi, larval habitats are either extensive or difficult to locate by inexperienced field staff, thus engaging local communities in LSM could increase coverage and also ownership of the control initiatives. In my study, we conducted laboratory and field studies to elucidate the effects of habitat ecology and community-based LSM on anopheline larval densities and feasibility of community involvement on anopheline larval population dynamics.

Research objectives

The main objective of this thesis is to assess the effects of habitat ecology and community-based LSM on anopheline larval ecology and population dynamics in Malawi. Specifically, the aims were to:

1. Characterise anopheline larval habitats in southern Malawi on the basis of habitat ecology and anopheline larval productivity. This was done to create a basis for larval control initiatives in the country (Chapter 2)
2. Assess the effect of *Bacillus thuringiensis israelensis* treatment and age on oviposition site selection in *Anopheles coluzzii* (Chapter 3)
3. Investigate the effect of larval exposure to sublethal *Bacillus thuringiensis israelensis* doses on size, oviposition and survival of adult *Anopheles coluzzii* mosquitoes (Chapter 4)

4. Investigate community factors affecting participation in LSM for malaria control in Chikhwawa District, Southern Malawi (Chapter 5)
5. Evaluate the impact of community involvement in habitat management and *Bti* treatment on anopheline larval densities (Chapter 6)

Outline of this thesis

Chapter 2 investigates the variability of aquatic habitats in their anopheline larval productivity. The underlying factors for habitat suitability for anopheline larvae are also assessed. I discuss how this knowledge can be used for targeted control of malaria vector populations, especially in resource-poor settings where adoption of LSM is hampered by concerns about implementation costs.

Chapter 3 assesses whether *Bti*-treatment of mosquito larval habitats changes the suitability of aquatic habitats either by enhancing or inhibiting oviposition by gravid females, which would modulate the effectiveness of larviciding with *Bti*. Discrimination of the treated sites by the females may reduce overall effectiveness of the intervention.

In **Chapter 4**, operational implications of anopheline larval exposure to sublethal doses of *Bti* are studied. The effects of larval exposure to sublethal doses on surviving adults' life-history parameters such as size, survival and oviposition output are evaluated. This was done to inform whether the dose rate of *Bti*, though not ideal but likely to occur under field conditions, could reduce malaria vector populations.

Chapter 5 investigates the community's knowledge and attitude towards community-driven LSM, their acceptance of the intervention and willingness to participate in associated activities. This chapter provides insight into factors that motivate community participation in disease control initiatives. Further, the chapter presents some best practices to promote community participation in disease control programmes.

Chapter 6 assesses the potential of engaging communities in malaria control via LSM. Community-executed LSM is evaluated using entomological surveys to assess its impact in control of malaria vector larvae. Sociological studies are employed to investigate factors influencing the community's actual participation in the community-led LSM. The findings demonstrate that community engagement in LSM has potential for the control of malaria vector populations.

In **Chapter 7**, I discuss the findings presented in all the chapters in this thesis. I discuss how knowledge about where anopheline mosquitoes breed, larval habitat management and community involvement in control efforts can be utilised for successful malaria control programmes. I also highlight the need to adapt programmes to local contexts for improved sense of ownership, participation and sustainability.

Acknowledgements

I thank Willem Takken, Robert McCann, Henk van den Berg, Sander Koenraadt and Lucinda Manda-Taylor for providing helpful comments on this chapter.

Chapter 2

Characterisation of anopheline larval habitats in southern Malawi

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Submitted

Abstract

Introduction: Increasing the knowledgebase of anopheline larval ecology could enable targeted deployment of malaria control efforts and consequently reduce costs of implementation. In Malawi, there exists a knowledge gap in anopheline larval ecology and, therefore, basis for targeted deployment of larval source management (LSM) for malaria control, specifically larvicides. We set out to characterize anopheline larval habitats in the Majete area of Malawi on the basis of habitat ecology and anopheline larval productivity to create a basis for larval control initiatives in the country.

Methods: Longitudinal surveys were conducted in randomly selected larval habitats over a period of fifteen months in Chikwawa district, southern Malawi. Biotic and abiotic parameters of the habitats were modelled to determine their effect on the occurrence and densities of anopheline larvae.

Results: Seventy aquatic habitats were individually visited between 1-7 times over the study period. A total of 5,123 immature mosquitoes (3,359 anophelines, 1,497 culicines and 267 pupae) were collected. Anopheline and culicine larvae were observed in sympatry in aquatic habitats. Of the ten habitat types followed, dams, freshwater marshes, ponds, borehole runoffs and drainage channels were the five most productive habitat types for anopheline mosquitoes. Anopheline densities were higher in aquatic habitats with bare soil making up part of the surrounding land cover ($p < 0.01$) and in aquatic habitats with culicine larvae ($p < 0.01$) than in those surrounded by vegetation and not occupied by culicine larvae. Anopheline densities were significantly lower in highly turbid habitats than in clearer habitats ($p < 0.01$). Presence of predators in the aquatic habitats significantly reduced the probability of anopheline larvae being present ($p = 0.04$).

Conclusions: Anopheline larval habitats are widespread in the study area. Presence of bare soil, culicine larvae, predators and the level of turbidity of water are the main determinants of anopheline larval densities in aquatic habitats in Majete, Malawi. While the most productive aquatic habitats should be prioritised, for the most effective control of vectors in the area all available aquatic habitats should be targeted, even those that are not characterized by the identified predictors. Further research is needed to determine whether targeted LSM would be cost-effective when habitat characterisation is included in cost analyses and to establish what methods would make the characterisation of habitats easier.

Keywords: Malaria mosquito, Larval ecology, Habitat characterization

Introduction

Larval source management (LSM) is designed to control mosquito densities by targeting the immature, aquatic stages of the mosquito (WHO 2013b).. It is thus considered a viable complimentary tool for malaria control next to long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) (WHO 2013b). Implementation of LSM has shown to reduce adult vector populations (Tusting et al. 2013) and hence reduce malaria burden in communities already using LLINs (Fillinger et al. 2009). However, LSM is most likely to be successful in settings where potential mosquito larval habitats are few, fixed and findable (WHO 2013b). Implementation of LSM could, thus, be operationally challenging in many parts of rural Malawi where these sites are extensive, numerous and difficult to access. In such cases, knowing which sites are most productive could enable targeted deployment of LSM in these selected sites.

The presence of mosquito larvae is dependent on unique ecological factors prevalent in each aquatic habitat. These factors should be thoroughly understood before LSM is executed. For example, smaller habitats, due to their transient nature, are less diverse in terms of species hosted and they support lower densities of mosquito immatures than larger habitats (Sunahara et al. 2002, Koenraadt et al. 2004, Minakawa, et al. 2005, Mala and Irungu 2011). Other abiotic factors have also been observed to influence differential productivity of larval habitats. For instance, water temperature determines the rate at which feeding and metabolism occur which affects the larval development rate (Clements 1992, Nayar et al. 1999, Bayoh and Lindsay 2003). Water turbidity and pH also influence mosquito diversity in aquatic habitats. Culicines have been observed to thrive in more turbid water than anophelines (Bukhari et al. 2011, Dida et al. 2018). Generally, in all mosquito species water pH below 4.5 or above 10 is associated with higher larval mortalities (Emidi et al. 2017). Biotic factors such as presence of larval competitors and predators, and vegetation play important roles in determining the suitability of larval habitats. For example, gravid mosquitoes avoid habitats occupied by their competitors (Impoinvil et al. 2008) and predators (Blaustein et al. 2004, Sumba et al. 2004). The avoidance of predator-infested habitats is attributed to the ability of gravid females to detect predator kairomones (Roberts 2014, Silberbush and Blaustein 2018). The role played by the presence of vegetation within or around larval habitats in influencing both larval diversity and density is well documented (Minakawa et al. 2005, Wamae et al. 2010). Besides altering the organic content of water through falling plant material (Muturi et al. 2008) thus influencing mosquito species composition in larval habitats, presence of vegetation also serves as either a larval food source (Mutuku et al. 2006) or shelter from predators and physical disturbances.

Understanding how the different habitat-associated ecological factors influence mosquito occurrence, abundance and diversity could assist in the development and deployment of effective larval control strategies (Stein et al. 2011). Mosquito larval

habitat ecology has been understudied in Africa (Dida et al. 2018) and no such research has been documented for Malawi. This has operational implications for the deployment of LSM for malaria control. The present study was, therefore, undertaken to characterize potential anopheline larval habitats on basis of their ecology and larval productivity in Malawi.

Material & Methods

Study area

The study was undertaken in eight months split between 2017 and 2018 in six villages which were participating in a community-led malaria control project known as the Majete Malaria Project (MMP) in Chikwawa district (16° 1' S; 34° 47' E), Malawi (Van Den Berg et al. 2018). These six villages were not included in the LSM arm of the MMP trial and hence were unaffected by the interventions. All study villages under MMP were divided into three regions referred to as focal areas A, B and C (McCann et al. 2017). The six study villages in the current study were evenly divided between focal areas B and C. Chikwawa district is an area of high malaria transmission in southern Malawi (Bennett et al. 2013). The area is hot and dry from September to December, hot and rainy from January to May, and cool and dry from June to August (Mzilahowa et al. 2012, Joshua et al. 2016). The higher temperatures and presence of water bodies create more humid environments, which further promote mosquito proliferation. The study areas are situated in river valleys, such that the terrain is generally flat but receives surface water runoff from the surrounding hilly watershed. The Shire River, the largest river in Malawi and only outlet of Lake Malawi, flows through Chikwawa District, including focal area B. This creates numerous breeding opportunities for mosquitoes. The smaller Mwanza River flows through focal area C. When the river dries, shallow wells are created for irrigation. Diverse potential mosquito larval habitats, including cattle hoof prints, brick-pits, wells, rice paddies and stream beds, are present in the area (Fig. 1). The principal malaria vectors in this area are *Anopheles gambiae s.s.*, *An. arabiensis* and *An. funestus* (Mzilahowa et al. 2012). Most inhabitants of the villages engage in millet cultivation and maize production. Furthermore, the majority of people keep livestock, with cattle and goats being the predominant animals.

Selection of study villages and larval habitats

The study villages were selected using simple random sampling. Names of all villages per focal area not participating in the LSM arm of the larger project were written on cards and placed in a dish before an independent research assistant blindly selected three cards for each of the two focal areas (B and C). Within the confines of each of the six selected villages and in a 500 m radius outside the boundary of each village, all potential mosquito larval habitats were geo-referenced using the Global Positioning System (GPS). A set of ten habitats was selected from the list of all mapped habitats in each selected village using simple spatially inhibitory random sampling. Here the minimum distance between the randomly selected habitats was set at 50 m. Because

larval habitats can dry up over time, any selected habitat containing no water during the monitoring of larval habitats was replaced with the nearest neighbouring habitat that contained water regardless of habitat type. This effectively increased the total number of habitats visited from 60 to 70 as initially proposed. If no habitats with water were identified, habitats were selected from outside the 500 m buffer zone as long as there was no LSM activity ongoing in the area. In case of habitat flushing, which was a likely event in the rainy season, the habitats were visited when the water had stabilised or stopped overflowing.

Collection of ecological data

Based on their origin, their permanence, presence of vegetation and source of water, the potential larval habitats were classified into one of the following 11 categories: (1) Brick pits: water-filled pits resulting from brick-making, (2) Dam: artificial barrier constructed to hold water, (3) Drainage channel: artificial channel constructed to allow water passage, (4) Hoof print: an outline or indentation left by a hoof on the ground, (5) Pond: a naturally formed, permanent water body, (6) Rice field: an irrigated or flooded field where rice is grown, (7) Borehole runoff: a body of standing water resulting from overland flow of water from a borehole, (8) Freshwater marsh : an area of low-lying land with heavily water-saturated soil and dominated by plants, (9) Stream bed: a water body found in a natural water channel, (10) Well: a hole or pit created for purposes of exposing ground water and (11) Tyre tracks: an outline left by a tyre on the ground. All these habitat types fell into one of three main classes: natural, human-made/artificial and modified-natural. Following this classification, the following habitat-level biotic and abiotic parameters were recorded during each visit: geo-location, depth and area covered by water body, water turbidity, estimated duration of habitat exposure to sunlight per day, presence or absence of vegetation, substrate coverage, water surface temperature and pH, and presence of larval mosquito predators. The land use-land cover (LULC) profile of each habitat's surroundings was also recorded, using the Braun Blanquet scale (Wikum and Shanholtzer 1978) to assign classes based on their percentage coverage: 0%, <5%, 6-10%, 11-25%, 26-50%, 51-75% and 76-100%. Water bodies that had dried up during the long dry season were not sampled until they contained water again.

Larval sampling

For each aquatic habitat, larvae were sampled from within an area sampler at one to three sampling points, which were equally distributed around the habitat perimeter. Collections were made between 9 am and 4 pm. The number of sampling points was based on the perimeter length of the habitat. For smaller habitats with perimeters equal or less than 10 m, one sampling point was selected. For habitats with perimeters larger than 10 m but less than 30 m, two sampling points were selected. Three samples were selected for all habitats with perimeters larger than 30 m. For each sample, an area sampler was used to mark the boundary of sampling and to prevent any

mosquito larvae and predators from escaping sampling (Fig. 1C). The area sampler was made of aluminium measuring 45 cm high with 27 cm diameter openings on both ends. The bottom lip of the sampler was serrated. Area samplers enable accurate estimation of larval density (Service 1993), and are more reliable than standard dippers in habitats with low larval densities (Fillinger et al. 2009). Standard 300 ml dippers, fish nets and pipettes were used to collect all mosquito larvae and pupae, and predators, from within the area sampler until all larvae were depleted. Reference to existing literature was basis for determining which of collected organisms were predacious (Shaalan and Canyon 2009, Sivagnaname 2009, Ohba et al. 2010, Kweka et al. 2011, Kundu et al. 2014, Dida et al. 2015, Benelli et al. 2016, Udayanga et al. 2019). All invertebrates were collected and separated into different orders such as Coleoptera, Odonata, Ephemeroptera and Hemiptera. Vertebrate predators such as fish and tadpoles were also recorded. All mosquito larvae were sorted by subfamily, anopheline or culicine, and separated by larval instar or pupal stage, and counted for entry into an *Open Data Kit* (ODK) form uploaded on a tablet. The number of anopheline larvae collected per area sampler yielded anopheline larval density per sampler. Per habitat, the anopheline larval density was calculated as sum of all anopheline larvae collected per area sampler divided by the number of samples taken for the habitat on the same day. A random sample of the collected anopheline larvae pooled from all habitat types was taken to a laboratory at the field station and reared to adults for further identification by microscopy using the keys of Gillies and Coetzee (1987) (Gillies and Coetzee 1987). Species identification within the *Anopheles gambiae* species complex and *An. funestus* group of mosquitoes were subsequently carried out by polymerase chain reaction (PCR) (Scott et al. 1993, Koekemoer et al. 2002).

Data analysis

Generalised linear mixed models were employed to quantify the effect of environmental variables on the density of *Anopheles* larvae. We first conducted bivariate tests to explore the variables that were significantly associated with the anopheline larval density. Non-parametric Mann-Whitney U and Kruskal Wallis tests were used to select which categorical variables go into the models. The associations between the response variable and continuous covariates were explored using Spearman's correlation coefficient. The level of significance was set at 0.05. The significant covariates were then included in multivariable regressions to model density of *Anopheles* larvae, while adjusting for other covariates and also accounting for potential confounders. These regression models were fitted as zero-inflated negative binomial (ZINB) models, which included components to account for both over-dispersion and the high number of zeros in the data. The negative binomial component was fitted with a log link, while the zero-inflated component was fitted with a logit link. From the full model with all the covariates identified from the bivariate analyses, we employed a backward variable selection algorithm. The threshold was set as 0.25 so as not to discard variables which could be important in determining the anopheline larval density under actual field conditions. Thereafter,

we fitted a ZINB mixed model using the covariates identified in the ZINB model in the preceding step. In addition to these covariates, this model added habitat as a random effect term in order to account for the repeated measurements at each habitat. All the analyses were performed using statistical package R version 3.6.1.

Results

Weather patterns

Weather conditions during the period of the study were recorded (Fig. 2). June and July were the coldest months with minimum temperatures reaching 10°C. During warm months, September, October and November, maximum temperatures of over 40°C were observed. The highest total rainfall of around 300 mm was observed during the month of March in 2017. Drought conditions were prevalent in the study area two years prior to and during the period of the study. During the hot season, most potential larval habitats dried up, thus limiting mosquito breeding to larger permanent water bodies and small man-made wells dug for irrigation and domestic use in the dry season.

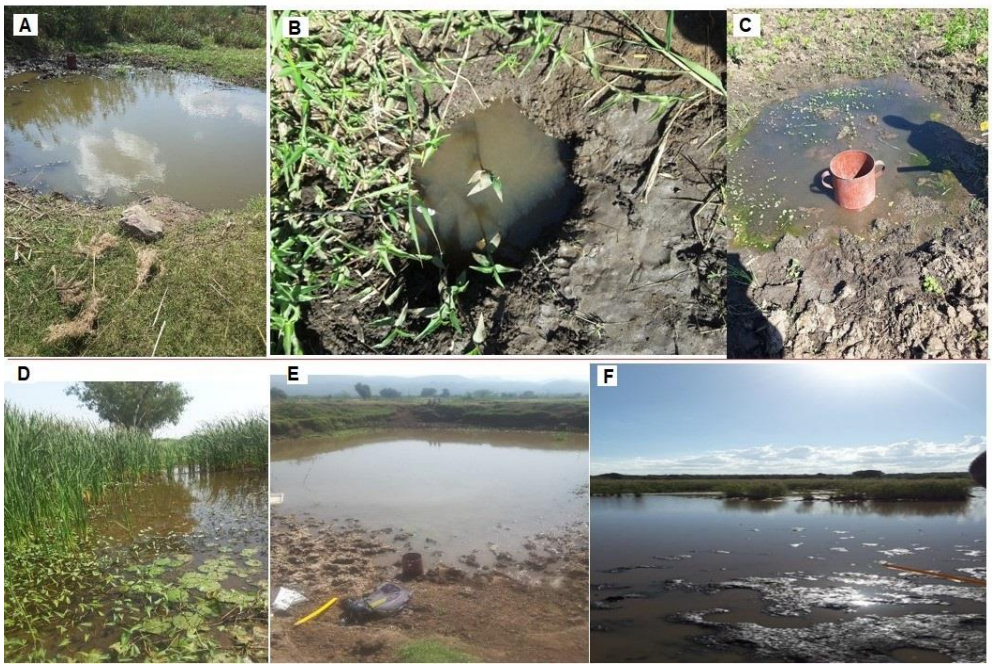

Fig. 1: Examples of mosquito larval habitat types in the study area: (A) Pond, (B) Well, (C) Borehole run-off, (D) Freshwater marsh, (E) Dam and (F) Streambed

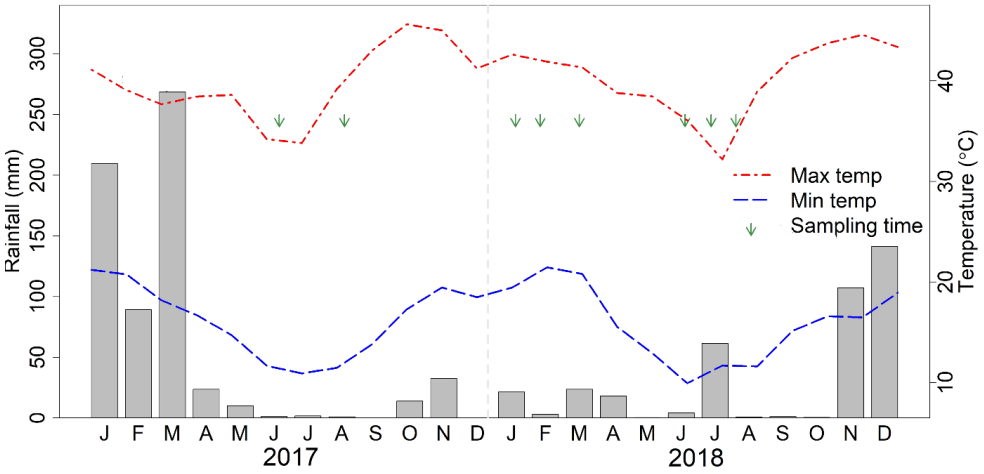

Fig. 2: Total monthly rainfall (mm; grey bars) and average, maximum and minimum temperatures (°C) over the study period. The data collection times are shown with green arrows.

Mosquito larval densities and diversity

Prior to commencement of data collection, a total of 140 potential habitats were mapped in all the six study villages. Ten habitats were randomly selected per village which resulted in 60 selected habitats. However, due to droughts that hit Malawi in 2016 and 2017 some of the selected habitats dried up in the course of the study and were replaced with nearby habitats in the same village. Effectively, 70 potential mosquito larval habitats were visited during the study, each between 1 to 7 times, for a total of 170 visits. Of the visited habitats, 46 (65.7%) were colonised by mosquito larvae during at least one visit. A total of 5,123 immature mosquitoes were observed: 3,359 anophelines, 1,497 culicines and 267 pupae in 39, 33 and 28 habitats, respectively (Table 1). Detailed taxonomic analysis by PCR was done on 330 anopheline larvae collected from positive habitats and reared to adult stage. Of these, 258 (78.2%) were *An. arabiensis* while 11 (3.3%) and 6 (1.8%) were *An. quadriannulatus* and *An. gambiae s.s.*, respectively. Fifty-five of the anophelines initially identified as *An. funestus* s.l. based on morphological features were all confirmed to be *An. funestus s.s by PCR*. All anopheline species were found sympatrically across habitat types. *An. arabiensis* were collected from all observed habitat types.

Table 1: The number of anopheline and culicine larvae and pupae collected (n = the number of water bodies in which the larvae were observed on at least one visit)

	Instar 1	**Instar 2**	**Instar 3**	**Instar 4**	**Total (n)**
Anophelines	1262 (n=34)	1150 (n=39)	666 (n=34)	281 (n=24)	3359 (39)
Culicines	451 (n=30)	512 (n=33)	323 (n=32)	211 (n=28)	1497 (33)
Pupae					267 (28)

Productivity of larval habitat types

Ranked in terms of their contribution to the numbers of collected larvae per visit, dams, freshwater marshes, ponds, borehole runoffs and drainage channels were the five most productive habitat types. Collectively, these habitat types contributed 81.4% and 65.9% of the anopheline and culicine larvae observed, respectively (Table 2). Co-colonisation of habitats by the two mosquito subfamilies was observed in 39.5% (34/86) of all positive visits.

Table 2: Contribution of the different habitat types to mosquito immatures.

Habitat type	No. sites	No. times sampled* (range)	Sites with immatures observed on ≥ 1 visit			Total immatures observed			Mean number of immatures per visit		
			Anophelines	Culicines	Pupae	Anophelines	Culicines	Pupae	Anophelines	Culicines	Pupae
Dam	4	10 (1~4)	3	2	2	301	121	30	30.1	12.1	3
Freshwater marsh	10	34 (1~6)	8	6	3	1020	417	32	30	12.3	0.9
Pond	4	14 (1~7)	4	3	0	380	177	22	27.1	12.6	1.6
Borehole runoff	12	32 (1~6)	6	5	5	866	245	70	27.1	7.7	2.2
Drain-channel	4	7 (1~2)	2	1	0	168	27	0	24	3.9	0
Rice field	8	18 (1~6)	7	7	1	271	287	10	15.1	15.9	0.6
Well	8	16 1~3)	5	6	2	152	116	54	9.5	7.3	3.4
Streambed	11	18 (1~2)	5	6	3	153	107	17	8.5	5.9	0.9
Brick pit	9	21 (1~4)	3	2	2	48	5	32	2.3	0.2	1.5

*The number of times sampled is totaled across all sites while the range is per site.

Presence of mosquito larval predators

A diverse range of predators were collected from the potential larval habitats (Table S1). The predators included copepods and members of orders Odonata, *Ephemeroptera,* Hemiptera and Coleoptera. Vertebrate predators, amphibians and fish, were also found. The predators were collected in 75.3% (128/170) of all visits. Backswimmers and mayfly larvae were collected in 73% (93/128) and 55% (71/128) of the positive visits, respectively (Table S2). Amphibians and members of order Odonata were found in 26% of the positive visits. Water striders and fish were the least collected predators both in only 2% of the positive visits. Sympatry was observed in the types of habitats colonised by the predators. Backswimmers and mayfly larvae were collected in all habitat types while amphibians, water bugs, water scavenger beetles and, dragonfly and damselfly larvae were collected in eight of the nine habitat types. Copepods and water scorpions were both collected in five of the habitat types.

Temperature and pH of positive habitats

The habitats positive for the anopheline and culicine larvae had overlapping ranges of physiochemical properties (Table 3). The average water pH and temperature for all habitats in the study were 6.8 ± 0.1 and 28.6°C ± 0.3, respectively. The habitats colonised by anophelines had 6.71±0.2 and 28.4°C ± 0.4 as average water pH and temperature values. When the temperature range for all habitats visited was categorised into two: 19.4°C to 32°C and > 32°C to 40.8°C, more anopheline larvae were collected in the lower 19.4°C to 32°C temperature range (85.4%, 2809/3359) than in the upper (14.6%, 490/3359). The average pH and temperature values recorded in culicine habitats were 6.5 ± 0.2 and 27.8°C ± 0.4, respectively. Like with the anopheline larvae, more culicine larvae were collected in the lower 19.4°C to 32°C temperature range (88.6%, 1326/1497) than in the upper range (11.4%, 171/1497).

Table 3: Range of physiochemical variables in habitats with anopheline and culicine larval presence

Physiochemical variable	**Subfamily**			
	Anophelines		**Culicines**	
	Range	Mean ± SE	Range	Mean ±SE
pH	3.58 - 8.95	6.71±0.2	3.4 - 8.95	6.5±02
Temperature (°C)	21.6 - 37.8	28.4 ± 0.4	19.4 - 40.8	27.8±0.4

Effects of habitat and terrestrial factors

Of the 33 variables collected in the study, 10 variables were significantly associated with anopheline larval density ($p < 0.05$; Table S3). These variables were all included in the initial ZINB model before backward selection. Water temperature ($p = 0.071$)

was also included in the ZINB model because temperature can have a strong impact on mosquito development and survival (Paaijmans et al. 2008). Soil cover, turbidity of the water and the presence of both culicine larvae and predators were significant factors in the final ZINB model (Table 5). Based on the final ZINB model, anopheline densities were higher in aquatic habitats with bare soil making up part of the surrounding land cover ($p < 0.01$) and in aquatic habitats with culicine larvae ($p < 0.01$). The densities were significantly lower in highly turbid habitats ($p < 0.01$) than in the least turbid habitats. The presence of predators in the aquatic habitats significantly reduced the probability of anopheline larvae being present ($p = 0.04$).

Table 4: Description and results of univariate tests conducted for all variables in the habitat characterization study. Kruskal Wallis, Spearman's correlation coefficient and Mann-Whitney U tests were the statistical tests undertaken prior to the model fitting. The variables with p-values expressed in bold were used in the initial ZINB model before backward selection of the final variables.

Variable	Description	Statistical test	P-value
Habitat type	Nine habitat types: Brick pits; ponds; wells; borehole-runoffs; freshwater marshes; dams; rice fields; drainage channels and stream beds	Kruskal Wallis	**0.01**
Water flow	Flow of the water is categorised into two classes: standing and moving	Mann-Whitney U	**0.03**
Habitat size	Surface area of water body (length x width) categorised into two classes: ≤500m2 and >500m2	Mann-Whitney U	0.09
Sunlight	Estimation in hours of the amount of sunlight that reaches the water body	Spearman's rank correlation	0.78
Water temperature	Average temperature of water at centre of habitat and within area samplers categorised into two classes: 18-32^{o}C and >32^{o}C	Mann-Whitney U	**0.07**
Water pH	pH value	Spearman's rank correlation	0.92
Water turbidity	Turbidity of the water measured as clear, medium and turbid	Kruskal Wallis	**<0.01**
Substrate	Substrate at bottom of water body: clay; silt; sand and rock	Kruskal Wallis	0.55

Algae coverage	Coverage of algae classfied based on Braun Blanquet scale. Categories collapsed to two levels: presence and absence	Mann-Whitney U	0.9
Aquatic film	Coverage of aquatic film classfied based on Braun Blanquet scale: Categories collapsed to two levels: presence and absence	Mann-Whitney U	0.86
Emerging vegetation	Coverage of emerging vegetation based on Braun Blanquet scale: Categories collapsed to two levels: presence and absence	Mann-Whitney U	0.52
Aquatic leaves	Coverage of aquatic leaves classfied based on Braun Blanquet scale: Categories collapsed to two levels: presence and absence	Mann-Whitney U	0.33
Submerged vegetation	Coverage of submerged vegetation classfied based on Braun Blanquet scale: Categories collapsed to two levels: presence and absence	Mann-Whitney U	0.61
Floating vegetation	Coverage of floating vegetation: Categories collapsed to two levels: presence and absence	Mann-Whitney U	0.94
Presence of culicine larvae	Presence of culicine larvae (yes or no)	Mann-Whitney U	**<0.01**
Presence of predators	Presence of predators (yes or no)	Mann-Whitney U	**<0.01**
Presence of mosquito pupae	Presence of pupae (yes or no)	Mann-Whitney U	**<0.01**

Main-land cover	Seven main land uses (20 m radius): Bare soil; grass; herbaceous; agriculture; shrubs; trees and rock	Kruskal Wallis	0.27
Coverage of soil <1m	Coverage of soil within habitat's 1m radius classfied based on Braun Blanquet scale: Categories collapsed to two levels: presence and absence	Mann-Whitney U	0.83
Coverage of trampled soil ≤1m	Coverage of trampled soil within habitat's 1m radius classfied based on Braun Blanquet scale: Categories collapsed to two levels: presence and absence	Mann-Whitney U	0.99
Coverage of herbs ≤1m	Coverage of herbs within habitat's 1m radius classfied based on Braun Blanquet scale: Categories collapsed to two levels: presence and absence	Mann-Whitney U	0.26
*Coverage of agriculture ≤1m	Coverage of agriculture within habitat's 1m radius classfied based on Braun Blanquet scale: Categories collapsed to two levels: presence and absence	Mann-Whitney U	**0.01**
Coverage of shrubs ≤1m	Coverage of shrubs within habitat's 1m radius classfied based on Braun Blanquet scale: Categories collapsed to two levels: presence and absence	Mann-Whitney U	0.52
Coverage of trees ≤1m	Coverage of trees within habitat's 1m radius classfied based on Braun Blanquet scale: Categories collapsed to two levels: presence and absence	Mann-Whitney U	0.65
Coverage of rocks ≤1m	Coverage of rocks within habitat's 1m radius classified based on Braun Blanquet scale: Categories collapsed to two levels: presence and absence	Mann-Whitney U	0.81
Coverage of grass ≤1m	Coverage of grass within habitat's 1m radius classfied based on Braun Blanquet scale: Categories collapsed to two levels: presence and absence	Mann-Whitney U	0.33

Coverage of herbs ≤20m	Coverage of herbs within habitat's 20m radius classfied based on Braun Blanquet scale: Categories collapsed to two levels: presence and absence	Mann-Whitney U	0.53
Coverage of shrubs ≤20m	Coverage of shrubs within habitat's 20m radius classfied based on Braun Blanquet scale: Categories collapsed to two levels: presence and absence	Mann-Whitney U	0.81
Coverage of trees ≤20m	Coverage of trees within habitat's 20m radius classfied based on Braun Blanquet scale: Categories collapsed to two levels: presence and absence	Mann-Whitney U	0.88
Coverage of rocks ≤20m	Coverage of rocks within habitat's 20m radius classfied based on Braun Blanquet scale: Categories collapsed to two levels: presence and absence	Mann-Whitney U	0.35
Coverage of grass ≤20m	Coverage of grass within habitat's 20m radius classfied based on Braun Blanquet scale: Categories collapsed to two levels: presence and absence	Mann-Whitney U	**0.03**
Coverage of agriculture ≤20m	Coverage of agriculture within habitat's 20m radius classfied based on Braun Blanquet scale: Categories collapsed to two levels: presence and absence	Mann-Whitney U	**0.04**
Coverage of soil ≤20m	Coverage of soil within habitat's 20m radius classfied based on Braun Blanquet scale: Categories collapsed to two levels: presence and absence	Mann-Whitney U	**0.04**

*Agriculture: any type of land use with farming activity, including irrigation.

Table 5: Results of the ZINB mixed model (with log link and logit link functions) that examined the effects of aquatic and terrestrial variables on anopheline larval densities

Variable	Coefficient	SE	Z-value	P-value
Count model coefficients (negbin with log link)				
(Intercept)	0.81	0.5382	1.505	0.13
Soil cover ≤20m	1.94	0.5145	3.767	**<0.01**
Medium turbidity	-0.34	0.4469	-0.766	0.44
High turbidity	-4.34	1.0943	-3.964	**<0.01**
Presence of culicine larvae	1.27	0.4195	3.025	**<0.01**
Zero-inflation model coefficients (negbin with logit link)				
(Intercept)	1.28	0.5276	2.42	0.02
Presence of predator	-1.33	0.6469	-2.054	**0.04**

In Table 5, the variable levels recorded using the Braun Blanquet scale (assigned 7 classes: 0%, <5%, 6-10%, 11-25%, 26-50%, 51-75% and 76-100%) were reduced to 2 levels (presence and absence)

Discussion

Habitat factors determine both mosquito larval densities and diversity, and consequently malaria transmission. We characterised anopheline larval habitats on the basis of their ecology and larval productivity. The results showed that all the habitat types prevalent in the study area contributed to the production of anopheline larvae but with differing densities. *Anopheles arabiensis* was the most abundant anopheline species and was collected in all types of the habitats examined. Higher anopheline larval densities were associated with presence of bare soil around the habitat and the presence of culicine larvae. Habitats with high turbidity and those with predators were associated with lower anopheline densities.

Nine larval habitat types with varied contribution to anopheline larval densities were identified in the study area. The number of habitat types observed was lower than would be expected in a normal year when enough rains fall. For example, due to the drought during the study period, hoofprints could not be counted as stand-alone habitats as they were only found to contain water when they existed within other more permanent habitat types such as dams, freshwater marshes and ponds. Dams, freshwater marshes, ponds, borehole runoffs and drainage channels were the five most productive habitat types for anopheline mosquitoes. The contribution of most of these habitat types could be associated with their relatively larger sizes and also

permanence as compared to the smaller, less stable habitat types also visited during the study. The larger-sized permanent larval habitats are likely to host more mosquito larvae at a time thus contributing more to larval productivity. However, by being more stable the larger habitats also accommodate larger numbers of competitor and predator species than smaller temporary habitats (Minakawa et al. 2004). Though not supporting as many larvae as larger habitats at a given time, the smaller habitats may contribute more to adult densities over time due to reduced loss of larvae from predation (Mwangangi et al. 2007). Further, the lower depth of smaller habitats allows efficient absorption of sunlight in the shallow water column which promotes photosynthetic processes enabling availability of food and also increasing the water temperature hence larval development (Muturi et al. 2008).

In this study, we found that presence of bare soil within a 20m radius from larval habitats was signicantly associated with higher anopheline larval densities. Indeed, *An. gambiae* s.l. have been shown to utilize shallow temporary puddles over bare soil as larval habitats (Gimnig et al. 2001, Minakawa et al. 2005, Huang et al. 2006, Fillinger et al. 2009, Ndenga et al. 2011). This finding has implications on the seasonality of anopheline larvae in our study area where larval habitats are predominantly surrounded by short-lived seasonal vegetation types. Death of these seasonal vegetation types, in the dry season, would create more bare ground thus promoting selection of the formerly vegetation-surrounded habitats by gravid anophelines as the vegetation dies.

Presence of culicine larvae was associated with higher anopheline larval densities. Anopheline and culicine larvae have been observed in sympatry in aquatic habitats elsewhere (Majambere et al. 2007). Three plausible mechanisms would explain this phenomenon. First, presence of culicine larvae in the habitats might have served as an alternative prey to predators thus reducing predation on the anophelines. Second, the presence of cues emanating from culicine larvae in the habitats could signal both safe and resource-rich sites for oviposition by gravid anophelines. Co-occurrence of anophelines and culicines is possibly caused by cues emitted by either species such as oviposition pheromones (Mwingira et al. 2019). Third, both species may be using the same habitat information to select the habitats. Stable coexistence in different mosquito species is possible due to their ability to exploit different niches within the same water bodies (Gilbreath et al. 2013). However, occupying the same habitat could potentially lead to competitive interaction for either resources and space (Carrieri et al. 2003, Kweka et al. 2012) which may have detrimental effects on both larval development and survival (Blaustein and Margalit 2018). This may induce discrimination of habitats occupied by other species by gravid females. For example, in a study in Kenya higher densities of anopheline and culicine immatures were observed when they occurred individually and not simultaneously (Impoinvil et al. 2008).

In the current study increasing turbidity was associated with reduced anopheline larval densities. Significantly larger densities were observed in the least turbid water than in highly turbid water. This finding is consistent with observations made on anopheline mosquitoes where their numbers were positively associated with clean water (Bukhari et al. 2011, Dida et al. 2018). Increasing turbidity levels reduce light penetration into the water which reduces food resources via reduced photosynthetic processes (Chirebvu and Chimbari 2015) and microbial growth (Muturi et al. 2008). Other studies, however, have recorded higher anopheline numbers with increasing turbidity (Gimnig et al. 2001, Fillinger et al. 2009, Mereta et al. 2013). Turbidity is caused by particles such as clay and silt, finely divided organic matter, plankton and microorganisms (Paaijmans et al. 2008). Therefore, whether turbidity influences mosquito larval presence likely depends on the absolute level (rather than the relative level) and the particles responsible for it. Habitats with moderate turbidity caused by edible particles are suitable for mosquito larvae (Sattler et al. 2005). Excessively turbid water, regardless of causative particles, reduces larval densities in *An. gambiae* s.l. (Ye-Ebiyo et al. 2003), as also confirmed by our results. Turbidity is considered an important index in larval monitoring of mosquito larvae (Chirebvu and Chimbari 2015).

The presence of predators was associated with reduced anopheline larval densities in the aquatic larval habitats. In this study a wide range of predators was recorded, both invertebrate and vertebrate. Direct predation of the larvae by the predators and avoidance by gravid mosquitoes to oviposit in predator infested habitats are likely the main explanations for reduced larval densities in such habitats. Gravid mosquitoes are known to detect cues emanating from predators thus avoid habitats from which the cues are coming (Blaustein et al. 2004, Munga et al. 2006). This was further confirmed by a dual choice study (Munga et al. 2006) in which *An. gambiae s.s.* provided with water conditioned with backswimmers and tadpoles or control non-conditioned water showed reduced oviposition output in the former compared to the latter. These phenomena have been observed in mosquitoes against many other species of predators (Munga et al. 2006, Roberts 2014). Since smaller habitats do not support large predator densities (Collins et al. 2019), predation rates in such habitats are low (Sunahara et al. 2002) hence they are more preferred by some anopheline species (Minakawa et al. 2004).

Our findings suggest that for more efficient anopheline larval control, lesser turbid habitats surrounded by bare soils and colonised by culicine larvae should be prioritised. Based on the findings, dams, freshwater marshes, ponds, borehole runoffs and drainage channels were the five most productive habitat types and should be prioritised by larval control efforts. However, all water bodies could be potential contributors to the mosquito populations and should be addressed if logistics, manpower and resources allow. Moreover, treatment of all available habitats has been shown to achieve high mosquito reductions than selective habitat treatment (Dambach et al. 2019). Though observed to be lesser costly (Dambach et al. 2016),

probably due to fewer habitats targeted for treatment, selective treatment of habitats could be more costly in terms of labour and time requirements if habitat characterisation to determine the most productive habitats is factored into the analyses.

The current study had some limitations. First, many habitats were of a temporal nature, which resulted in fewer repeated samples. Some sites were found to have water only once. Although this could not be avoided due to the highly seasonal occurrence of rainfall in the study area, this made investigation of effects of temporal changes on anopheline larval densities difficult for such sites. For this reason, all habitats that dried up during the course of the study were replaced with nearby habitats. Second, the lack of a significant influence of water temperature in determining anopheline larval densities could be attributed to limitations in our study design to account for the effect of hourly changes in water temperature. It is likely that at some time points, especially the early afternoon when the solar radiation is highest, larval densities are highly impacted by the higher temperatures which reach thermal death points (Paaijmans et al. 2008). Although logistically challenging, collecting larvae within the same, relatively small time frame, would reduce the range of surface water temperature, and we expect that temperature would then become a significant variable in predicting the presence of anopheline larvae.

The current study has shown that the presence of bare soil, culicine larvae, predators and the level of turbidity of water are the main factors determining anopheline larval densities in aquatic habitats in Majete, Malawi. These determinants provide basic associations between ecological variables and anopheline larval density hence could guide deployment of targeted larval control. However, for the most effective control of vectors in the area all available aquatic habitats should be targeted, even those that are not characterized by the determined predictors. Further research is needed to determine whether targeted LSM would be cost-effective when habitat characterisation is included in cost analyses and to establish what methods would make the characterisation of habitats easier.

Acknowledgements

The study was generously supported by the Dioraphte Foundation, The Netherlands, through The Majete Malaria Project. The content is solely the responsibility of the authors and does not necessarily represent the official views of the funders. RSM is supported by an NIH-funded postdoctoral fellowship (T32AI007524). We thank Kim Mphwatiwa, Spencer Nchingula, Davies Kazembe and Happy Chongwe for the significant contributions they made in data collection. We also thank Asante Kadama, Richard Nkhata and Tinashe Tizifa for logistical support.

Table S1: List of predators identified from the mosquito aquatic habitats in the study

Predator	Phylum	Subphylum	Class	Order	Suborder	Family
Dragonfly larva	Arthropoda		Insecta	Odonata	Anisoptera	
Damselfly larva	Arthropoda		Insecta	Odonata	Zygoptera	
Water strider	Arthropoda		Insecta	Hemiptera	Heteroptera	Gerridae
Mayfly larva	Arthropoda		Insecta	Ephemeroptera		
Backswimmer	Arthropoda		Insecta	Hemiptera	Heteroptera	Notonectidae
Water bug	Arthropoda		Insecta	Hemiptera	Heteroptera	Naucoridae
Water scorpion	Arthropoda		Insecta	Hemiptera		Nepidae
Water scavenger beetle	Arthropoda		Insecta	Coleoptera		Hydrophilidae
Copepod	Arthropoda	Crustacea	Hexanauplia			
Frog (Tadpole)	Chordata		Amphibia	Anura		
Fish	Chordata	Vertebrata				

Table S2: Number of habitats colonised and percentage positive visits for predators in predator-infested habitats.

Predator	No. visits with predators present	% visits (out of 128) with predators present	No. habitat types colonised (out of 9) (%)
Gerridae	2	1.6	2 (22)
Fish	3	2.3	3 (33)
Nepidae	12	9.4	5 (55)
Copepoda	14	10.9	5 (55)
Naucoridae	22	17.2	8 (88)
Odonata	33	25.8	8 (88)
Amphibian	33	25.8	8 (88)
Coleoptera	51	39.8	8 (88)
Ephemeroptera	71	55.5	9 (100)
Notonectidae	93	72.7	9 (100)

Chapter 3

Effect of application of *Bacillus thuringiensis* var. *israelensis* on oviposition site selection in *Anopheles coluzzii* under laboratory conditions

Steven Gowelo, James Chirombo, Jeroen Spitzen, Constantianus J.M. Koenraadt, Themba Mzilahowa, Henk van den Berg, Willem Takken, Robert McCann

Abstract

Background: Limited progress in malaria control in many African countries underscores the need to add new interventions with synergistic effects to the standard insecticide-based interventions, such as bednets and indoor residual spraying. Use of *Bacillus thuringiensis israelensis (Bti)* as a component of larval source management (LSM) is recognized as an effective complementary tool. However, treatment with *Bti* may enhance or inhibit oviposition by gravid female mosquitoes, which would modulate the effectiveness of larviciding with *Bti*. In this study we examined the effect of *Bti* on oviposition by *Anopheles coluzzii* under laboratory conditions.

Methods: Dual choice experiments were carried out with gravid *Anopheles coluzzii* females. In control cages, two cups containing demineralised water were provided as oviposition sites. In the treatment cages one cup contained demineralised water while the other contained water that had been treated with *Bti* either 0 days, 4 days, or 8 days before the experiment. Eight replicates of the experiments were conducted under laboratory conditions.

Results: Total egg-laying across both available oviposition cups was higher in 'Day 8 *Bti*' than control cages (95% CI 1.01–4.34, $p = 0.05$). Among the treatment cages no differences were observed in the mean number of eggs laid. Within the treatment cages, the mean number of eggs laid were significantly different only in cages containing 'Day 4 *Bti*' ($p = 0.049$) where more eggs were laid in the cup containing *Bti* than in control cups.

Conclusions: Treatment of water with *Bti* 8 days earlier increases oviposition output in gravid *An. coluzzii* females when compared to settings with only demineralised water available, but the ovipositing females do not apparently have a preference between the *Bti*-treated water and demineralised water. Fresh *Bti* has neither attractant nor repellent properties on the gravid females at close range. This neutral effect of *Bti* on egg-laying renders the larvicide effective in malaria control, as the vector would still oviposit eggs that will subsequently hatch and be exposed to a lethal dose of *Bti*. For maximum effectiveness of *Bti*, all available larval habitats must be treated with *Bti*, because the mosquitoes do not distinguish between treated and untreated sites.

Key words: *Bacillus thuringiensis israelensis*, Oviposition, *Anopheles coluzzii*

Introduction

Insecticide-based interventions play a major role in malaria vector control (Bhatt et al. 2015, Kleinschmidt et al. 2018). However, widespread use of the insecticides has selected for insecticide-resistant malaria vectors (Ilboudo-sanogo et al. 2013, Tokponnon et al. 2019). Insecticide resistance is now widespread in the most important malaria vector species in Africa, and changes in the biting and resting patterns of these species have also been reported (Moiroux et al. 2012, Guyant et al. 2015, Killeen et al. 2016, Mzilahowa et al. 2016). These developments have been implicated in driving residual malaria transmission (WHO 2014), underpinning the need for additional vector control interventions. Larval source management (LSM) is recognized as a complementary control strategy for malaria vectors (Fillinger and Lindsay 2011). Different forms of LSM have been implemented against a wide range of vector species. For decades, larvivorous fish have been used in mosquito control (Asimeng and Mutinga 1993, Kumar et al. 1998, Walker and Lynch 2007, Chandra et al. 2008). Habitat modification through draining, filling and land levelling, and manipulation aimed at creating conditions less favourable for mosquito larvae have also been employed (Karunamoorthi 2011). In many parts of the world, microbial larvicides are used for larval control. Two endotoxin-producing bacterial species, *Bacillus thuringiensis* var. *israelensis (Bti)* and *Bacillus sphaericus* (*Bs*), are effective larvicides against mosquitoes (Walker and Lynch 2007). The use of *Bti* has demonstrated no harm to non-target organisms, which can be attributed to the specificity of its endotoxins (Gwal et al. 2015), as well as no harm to the environment due to its low persistence under field conditions (Davis and Peterson 2008, Tetreau et al. 2012, Fayolle et al. 2016).

Numerous studies have reported the effectiveness of *Bti* on a wide range of vector species under laboratory and field conditions. For example, a study conducted in Cambodia showed significant interruption in dengue transmission in areas with temephos resistant *Aedes aegypti* populations (Setha et al. 2016). Other studies demonstrated that the larvicide was effective in controlling another dengue vector, *Ae. albopictus* , under laboratory (Farajollahi et al. 2013b) and field conditions (Lee et al. 2008, Jacups et al. 2013). The effects of *Bti* can also extend beyond the direct mortality of larvae that ingest *Bti*. Non-larvicidal effects of *Bti* include reducing oviposition and adult survival in *Culex quinquefasciatus* (Zahiri and Mulla 2005). For malaria control, *Bti* application in some regions of Peru and Ecuador resulted in over 50% reductions of adult *Anopheles* populations (Kroeger et al. 1995). Though not desirable, application of sublethal concentrations of *Bti* reduce fitness parameters and consequently vectorial capacities of different mosquito vectors (Flores et al. 2004, Wang and Jaal 2005, Simsek et al. 2009).

In Africa, there is a growing realization that *Bti* can be used in malaria control. Studies from many countries have shown that *An. gambiae* s.l. larvae are highly susceptible to *Bti*, with field applications reducing larval densities by up to 95% (Fillinger et al. 2003,

Shililu et al. 2003, Fillinger and Lindsay 2006, Majambere et al. 2010, Dambach et al. 2014, Djènontin et al. 2014). In urban and peri-urban Malindi in Kenya, larviciding using *Bti* reduced both anopheline and culicine larval densities (Mwangangi et al. 2011). Similarly, rice paddy treatment with a mixture of *Bti* and fertilizer reduced *Anopheles gambiae* s.l. densities in a semi-field study conducted in Tanzania (Mazigo et al. 2019). In a study conducted in western Kenya where the larvicide was used in addition to existing control strategies, reductions in malaria burden were reported (Fillinger et al. 2009). As a synergistic strategy, larviciding using *Bti* can contribute to reductions in residual malaria transmission by targeting both insecticide-resistant (Guyant et al. 2015) and behaviourally plastic vectors (Durnez and Coosemans 2013).

However, treatment with *Bti* may either enhance or inhibit oviposition in treated water by gravid female mosquitoes, which would modulate the effectiveness of larviciding with *Bti*. Gravid mosquitoes use a wide range of stimuli in selection of oviposition sites (Nazni et al. 2009). Environmental stimuli are likely to attract or repel mosquitoes to an oviposition site (Day 2016). Volatile chemicals emanating from aquatic habitats have been reported to attract gravid mosquitoes to the sites (Lindh et al. 2008). Bacteria and bacterial associated volatiles mediate selection of oviposition sites (Ponnusamy et al. 2008). The presence of volatiles released by *Bti*, the presence of co-formulated substances in *Bti* products or molecules derived from their degradation has been shown to attract *Ae. albopictus* to a site (Carrieri et al. 2009). Selection of suitable oviposition sites is crucial in mosquito survival and population dynamics (Navarro-Silva et al. 2009) as it affects production and size of adults (Wong et al. 2012). *Anopheles coluzzii* is an important malaria vector which uses chemical signals emanating from microbial (Lindh et al. 2008) and predator communities (Chobu et al. 2015) for selection of oviposition sites.

Despite the widespread use of *Bti* for mosquito control, little is known about its effects on oviposition site selection in anopheline mosquitoes. Under laboratory conditions *Bti* significantly enhances attraction of *Ae. albopictus* females to treated ovitraps (Stoops 2005). To our knowledge, no studies are known that have investigated the effect of *Bti* application on oviposition site selection in anopheline mosquitoes. Degradation of *Bti* under field conditions after application could alter concentrations of bacteria and/or associated volatiles, which would modulate any effects that fresh *Bti* may have on oviposition. The current study investigated the potential effects of *Bti* application and decomposition on oviposition output and choice in the malaria vector, *Anopheles coluzzii*, under laboratory conditions.

Material and methods

This study was conducted at the Laboratory of Entomology, Wageningen University, The Netherlands, using *Anopheles coluzzii* mosquitoes from a colony maintained at the laboratory (Spitzen et al. 2013). All adult mosquitoes used in the experiments were newly emerged from the colony and transferred to 30cm x 30cm x 30cm cages, and kept at 27 ± 1°C. Humidity in the insectary was maintained at 70 ± 5% using a humidifier. Six percent glucose solution was given to the mosquitoes *ad libitum*.

On days five and six post-emergence, presumably after successful mating, the female mosquitoes were fed human blood using a membrane feeding apparatus (Hemotek, Discovery workshops, UK). Two days after blood feeding, twenty-four randomly selected gravid females were individually placed in numbered cups using a mouth aspirator. Four of these females were randomly selected and assigned to four new 30 x 30 x 30 cm cages for a replicate of the oviposition dual choice tests. The other 20 gravid females were returned to the communal cage. A new batch of gravid females taken from a new starter cage was used for each replicate. Females in all experimental cages were provided with 6% sugar solution *ad libtum*.

Each cage used in the oviposition dual choice tests had two oviposition cups set on opposite corners of the cage according to four treatments: (1) Control: contained two cups both filled with demineralised water, (2) 'Day 0 *Bti*': contained one cup with demineralised water and another with fresh *Bti* solution made on the day of the experiment, (3) 'Day 4 *Bti*': contained one cup with demineralised water and another with four day old *Bti* solution and (4) 'Day 8 *Bti*': contained one cup with demineralised water and another with eight day old *Bti* solution. In the control cages, the two control cups containing demineralised water were assigned letters A and B. In all the experiments a water-dispersible granule (WDG) formulation of *Bti* (VectoBac® WDG, Valent BioSciences) was used. In each treatment cage, the treatment cups contained 100 mL of water treated with 0.4 mg/L of *Bti* prepared on different days as described above. To remove possible effects of contrast differences between the treatments, a double cup system was used where the experimental plastic cup was placed inside a larger paper cup and filter papers were placed on top of the cups so that visual cues were similar for all treatments. The filter papers were folded into funnel-like shape and the lower tip touched the water column to keep the papers moist. The different cups were rotated daily within the cages to remove effects due to position of cup in the cage. The numbers of eggs laid on filter papers placed on each cup were counted daily. The sum of eggs laid in the two oviposition cups within each cage was referred to as the number of eggs laid per cage. The observations were made with the same gravid female mosquito for seven consecutive days. Eight replicates of the experiment were conducted, each consisting of four individual mosquitoes in four different cages.

Data analysis

Descriptive statistics with boxplots were used to compare the median number of eggs laid by the gravid females in the different cages and cups. Statistical tests of significance were carried out to investigate differences in the number of eggs laid. A generalized linear model (GLM) assuming a Poisson distribution and log link, with treatment as fixed effects, was used to investigate the effect of treatment on the number of eggs. Non-parametric Wilcoxon tests were performed to investigate statistical differences in the numbers of eggs laid in the two cups within cages. All data analyses were done using the statistical package R version 3.6.1.

Results

Across the eight replicates, the thirty-two *An. coluzzii* females laid a total of 2802 eggs. The mean number of eggs laid by the *An. coluzzii* females per cage increased with increasing age of *Bti* solution (Fig.1). When the number of eggs laid in the two cups in each cage were combined, cages with Day 8 *Bti* recorded the highest number of eggs while the lowest number was recorded in the control cages containing the two water cups. The Poisson GLM with confirmed the positive relationship between the combined number of eggs laid in the two cups per cage and the treatment applied.

On average, 63% and 67% more eggs were laid in cages containing a control cup plus a Day 0 or Day 4 *Bti* cup, respectively, than in the control cages containing the two demineralised water cups. These differences were not statistically significant. A two-fold, significant increase in the numbers of eggs laid was observed in the cages with Day 8 *Bti* and a control cup than in cages containing the two water controls ($p = 0.05$) (Table S1).

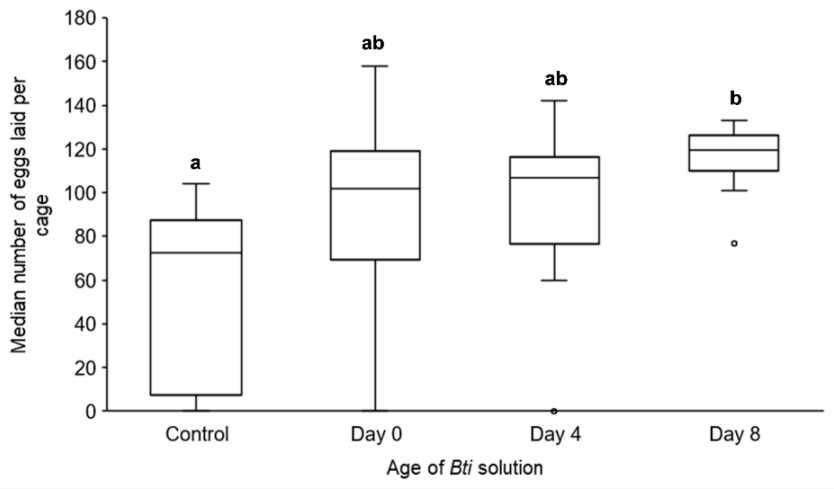

Fig. 1: Boxplots showing the median number of eggs laid by *An. coluzzii* females per treatment by cage. Dot on Day 8 is an outlier. Boxplots with different letters are significantly different: $p < 0.05$. Table S1 presents details of the statistical analysis.

Within each treatment cage, the effect of *Bti* differed based on the age of the *Bti* (Fig. 2). In cages containing Day 4 *Bti* and untreated-water cups, significantly more eggs were laid in the *Bti*-treated cups ($p = 0.049$) (Fig. 2). For cages containing Day 0 and 8 *Bti* and untreated-water cups, no statistical differences were observed in the number of eggs laid between the *Bti*-treated cups and the untreated-water cups. The number of eggs laid in the control cages did not differ between the two control cups ($p = 0.37$).

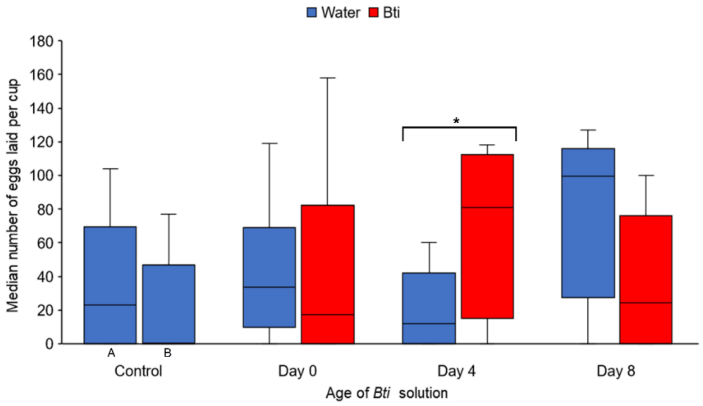

Fig. 2: Boxplots showing the mean numbers of eggs laid by *An. coluzzii* females per treatment by cup measured over the eight replicates. *$p = 0.049$ between the indicated treatments.

Discussion

Whether *Bti* remains effective depends on how gravid females respond to the changes due to treatment with the larvicide. We assessed the effects of treatment and ageing of *Bti* on oviposition site selection in gravid *An. coluzzii* under laboratory conditions. We observed that the mosquitoes preferred cups that had been treated with *Bti* four days prior to the experiment. Further, presence of cups with *Bti*-treated water in cages enhanced overall egg-laying by the gravid females. Thus, our study shows that application of *Bti* can successfully control malaria vectors as it does not induce discrimination of the treated sites by oviposition-site seeking gravid females.

Since the degradation of *Bti* or its coformulant substances releases volatiles (Carrieri et al. 2009), the moderately decomposed Day 4 *Bti* likely attracts the gravid females. Our results suggest that fresh Day 0 and more aged Day 8 *Bti* release lower concentrations of the volatiles, and thus are less attractive to the gravid females. Similarly, *Bti* spores yield volatiles that promote oviposition behaviour in *Aedes albopictus* (Nazni et al. 2009). A number of factors are likely to influence activity of *Bti* under natural conditions, some in a positive way. For example, exposure to sunlight causes the *Bti* spores to release attractant volatiles that promote oviposition (Nazni et al. 2009). Under natural conditions in aquatic habitats, mosquitoes detect a range of volatile and non-volatile chemical cues from both biotic and abiotic sources. Chemical cues released by predators play an important role in influencing the

behaviour of prey species (Hay 2009). In *An. gambiae*, selection of aquatic habitats has been observed against backswimmers (Blaustein et al. 2004, Munga et al. 2006) and tadpoles (Munga et al. 2006). Based on our results, application of *Bti* under natural conditions could lure mosquitoes to oviposit even in predator-infested habitats thus enhancing predation of their larvae and consequently contributing towards vector control.

In the current study, age of *Bti* solution did not have any apparent repellent effect on oviposition in gravid *An. coluzzii*. The gravid females were observed to indiscriminately lay eggs in the fresh, moderate and more-aged *Bti.* This finding has operational implications in the continued use of *Bti* as a complimentary tool in malaria control as mosquitoes which are unable to discriminate against treated sites would expose their offspring to the larvicide resulting in reduced population sizes. Similar response was observed in wild *Ae. albopictus* where ovitraps treated with *Bti* remained toxic for at least 14 days but did not prevent the mosquitoes from ovipositing in the traps (Carrieri et al. 2009). In contrast with our study, *Bti* treatment of sites under laboratory and semi-field conditions had repellent effects on *Culex pipiens* oviposition (Akiner and Eksi 2015). As mosquitoes can detect volatile chemicals emanating from *Bti*-treated water, it would be interesting to identify the chemicals that cause this attraction. For the most effective use of *Bti*, infusion of the product with mosquito attractants should be considered, so that an effective lure-and-kill strategy can be developed.

The sample size in our studyt was small hence the findings can not be generalised. Also, the cages in which the dual-choice tests were conducted were small, 30cm x 30cm x 30cm, which reduced the distance between treated and untreated cups, and may have prevented gravid females from distinguishing between cups with *Bti* and the controls. For more conclusive results further experiments need to be undertaken under field conditions, in which the treated and untreated sites are placed at a greater distance from each other.

Conclusions

The results of this study showed that treatment with and decomposition of *Bti* enhance egg laying in gravid *An. coluzzii* females. The results also showed that fresh *Bti* has neither attractant nor repellent properties on gravid *An. coluzzii* at close range. This neutral effect of *Bti* on egg-laying confirms the effectiveness of the larvicide in malaria control, as the vector would still oviposit eggs that will subsequently hatch and be exposed to a lethal dose of *Bti*. For maximum effectiveness of *Bti*, all available larval habitats must be treated with *Bti*, because *An. gambiae* mosquitoes did not distinguish between treated and untreated sites. Development of *Bti* formulations with greater residual effect should also be considered for cost-effective and efficient vector control. Additionally, the effectiveness of *Bti* could be increased if the larvicide were infused with attractants that could lure vectors to treated sites.

Acknowledgements

We are grateful to Pieter Rouweler, Joeri Groot, Seppe Salari, Frans K.M van Aggelen and André J. Gidding for mosquito rearing. This work was supported by a grant from the Dioraphte Foundation.

Funding

This work was supported by the Dioraphte Foundation, The Netherlands. The content is solely the responsibility of the authors and does not necessarily represent the official views of the funders.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Table S1: Effect of *Bti* decay and replication on the mean of the total number of eggs laid by *An. coluzzii* females in each cage, combined for the two cups in each cage

		95% CI		
Variable	**Estimate**	**Lower**	**Upper**	**p-value**
Intercept	24.85	10.68	57.86	<0.001
Day 0	1.63	0.74	3.62	0.23
Day 4	1.67	0.78	3.56	0.18
Day 8	2.09	1.01	4.34	**0.05**

Chapter 4

Effects of larval exposure to sublethal doses of *Bacillus thuringiensis* var. *israelensis* on body size, oviposition and survival of adult *Anopheles coluzzii* mosquitoes

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Published in Parasites & Vectors (2020)

DOI: 10.1186/s13071-020-04132-z

Abstract

Introduction: Application of the larvicide *Bacillus thuringiensis* var. *israelensis* (*Bti*) is a viable complementary strategy for malaria control. Efficacy of *Bti* is dose-dependent. There is a knowledge gap on the effects of larval exposure to sublethal *Bti* doses on emerging adult mosquitoes. The present study examined the effect of larval exposure to sublethal doses of *Bti* on the survival, body size and oviposition rate in adult *Anopheles coluzzii*.

Methods: Third-instar *An. coluzzii* larvae were exposed to control and sublethal *Bti* concentrations at LC_{20}, LC_{50} and LC_{70} for 48 h. Surviving larvae were reared to adults under standard colony conditions. Thirty randomly selected females from each treatment were placed in separate cages and allowed to blood feed. Twenty-five gravid females from the blood-feeding cages were randomly selected and transferred into new cages where they were provided with oviposition cups. Numbers of eggs laid in each cage and mortality of all adult mosquitoes were recorded daily. Wing lengths were measured of 570 mosquitoes as a proxy for body size.

Results: Exposure to LC_{70} *Bti* doses for 48 h as third-instar larvae reduced longevity of adult *An. coluzzii* mosquitoes. Time to death was 2.58 times shorter in females exposed to LC_{70} *Bti* when compared to the control females. Estimated mortality hazard rates were also higher in females exposed to the LC_{50} and LC_{20} treatments, but these differences were not statistically significant. The females exposed to LC_{70} concentrations had 12% longer wings than the control group ($p < 0.01$). No differences in oviposition rate of the gravid females were observed between the treatments.

Conclusions: Exposure of *An. coluzzii* larvae to sublethal *Bti* doses reduces longevity of resultant adults and is associated with larger adult size and unclear effect on oviposition. These findings suggest that anopheline larval exposure to sublethal *Bti* doses, though not recommended, could reduce vectorial capacity for malaria vector populations by increasing mortality of resultant adults.

Keywords: *Bacillus thuringiensis* var. *israelensis*, Sublethal dose, Larval source management, Mosquito, Vector control

Introduction

Malaria remains a major public health problem in the world, especially in Africa where the incidence rate has remained stable, and in some cases even increased, over the past few years (WHO 2018) despite widespread use of control methods (Yé et al. 2017). Widespread insecticide resistance has been reported in the important malaria vectors across all classes of insecticides used on insecticide-treated bed nets and indoor residual spraying (Ranson et al. 2011, Karaağaç 2012, Hemingway 2014, Guyant et al. 2015). Further, outdoor feeding and resting by malaria vectors, whether in response to use of insecticide-treated bed nets (Moiroux et al. 2012, Durnez and Coosemans 2013, Killeen 2014, Killeen et al. 2016) or as a natural behaviour in some vector populations, expose people to residual malaria transmission. This has led to global advocacy for additional vector control tools to eliminate residual malaria transmission by targeting outdoor resting and feeding, as well as insecticide resistant vectors (Durnez and Coosemans 2013). Larval source management (LSM) indiscriminately kills malaria vectors before they emerge as adult mosquitoes (Mwangangi et al. 2011) and relies on separate modes of action from those used in insecticide-treated bed nets and indoor residual spraying. Thus, LSM can contribute to malaria control where the vectors exhibit exophagic and exophilic behaviours, and in settings where insecticide resistance has emerged.

Strains of the bacteria *Bacillus thuringiensis* var. *israelensis* (*Bti*) are widely used as active ingredients in larvicides because they do not cause harm to non-target organisms or the environment (Kandyata et al. 2012, Aïssaoui and Boudjelida 2014). The bacteria produce parasporal crystalline protein inclusions (Cry and Cyt) which are lethal only to specific insect taxa (Crickmore et al. 1998). In mosquitoes, the protein crystals bind to specific receptors exposed on the surface of the plasma membrane and then insert into the membrane, creating lytic pores in microvilli of apical membranes (Aronson and Shai 2001) that disturb the cell's osmotic balance, resulting in cell lysis and consequently death of the larvae (De Maagd et al. 2001, Bravo et al. 2007). The low persistence of *Bti* toxins under field conditions (Tetreau et al. 2012) makes *Bti* an eco-friendly larvicide even when used in repeated treatments from three to seven years (Fayolle et al. 2016). This rapid degradation, however, necessitates repeated applications of the larvicide for effective control of target organisms.

Environmental conditions experienced by mosquitoes during larval growth and development affect adult fitness in a number of ways (Muturi et al. 2011, Barreaux et al. 2016, Sneha and Preet 2016). Nutritional and larvicidal stresses can reduce adult size, survival and fecundity in different mosquito species (Flores et al. 2004, Alto and Lord 2016, Shapiro et al. 2016, Vantaux et al. 2016). One such larvicidal stressor could be sublethal doses of *Bti*. Sublethal *Bti* concentrations reduce adult mosquito survival rates, lower blood-engorgement rate and egg production, increase development time from egg to adult, and decrease offspring sex ratio in *Aedes aegypti* (Wang and Jaal 2005). Similarly, sublethal doses of *Bti* may cause adverse effects on life parameters of

exposed *Ae. aegypti* and their unexposed first-generation progeny (Flores et al. 2004). Prolonged development time, reduced longevity and reduced reproductive rates were observed in *Anopheles superpictus* exposed to sublethal doses of *Bti* (Simsek et al. 2009).

Mosquito larvae may be exposed to sublethal concentrations of *Bti* under field conditions. This could result from some of or all of these factors: (i) anthropological factors such as poor measurement of *Bti* in relation to habitat size and poor calibration of instruments used in weighing the product, (ii) biotic factors such as growth of vegetation in habitats that may trap the product and reduce larva-product contact, and (iii) abiotic factors such as water pH, turbidity and temperature, which may degrade the product or modulate product activity within the mosquito. These doses may result directly or indirectly in biological changes in the surviving larvae and consequently impact adult fitness. Though not ideal, sublethal *Bti* doses could impact vector populations and malaria parasite transmission, and should thus be well understood. The purpose of this study was to understand how these doses affect the survival, body size and oviposition rate of *Anopheles coluzzii*, an important human malaria vector in Africa.

Materials and methods

Anopheles coluzzii colony

The mosquitoes used in all experiments came from an *An. coluzzii* colony maintained at the insectary of the Laboratory of Entomology of Wageningen University, The Netherlands. Standard colony rearing conditions for the immature stages consisted of plastic larval trays (10 × 25 × 8 cm) filled with salt-treated demineralised water (0.008 g/ml) to reduce the potential for larval pathogen infections. In each start-up tray, salt-treated water containing approximately 200 first-instar larvae was pipetted. Larvae were provided 0.1 mg/larva Tetramin fish food (Tetrawerke, Melle, Germany) for the first instars and 0.3 mg/larva for the other larval stages. All pupae were removed from the trays and placed in salt-treated demineralised water in 100 ml plastic cups in 30 × 30 × 30 cm cages. Emerging adults were fed 6% sugar solution *ad libitum*. From day 3–4 post-emergence, adult females were offered human blood for 2–3 h per day for 11 days. A membrane feeding system (Discovery Workshops, Acrington, Lancs, UK) (Spitzen et al. 2014) was used for the blood-feeding.

Sublethal *Bti* concentration determination

The protocol of Becker & Rettich (1994) was adopted for preparing a *Bti* solution. Fifty milligrams of a water-dispersible granule (WDG) formulation of *Bti* (VectoBac® WDG, Valent BioSciences) were added to 10 ml distilled water. The mixture was homogenised at 700 *rpm* for 10 min and vortexed for 15 min. Then 1 ml of the homogenised suspension was added to 49 ml distilled water to make a stock solution of 100 mg/l. The suspension was vortexed for 5 s at maximum speed. Aliquots of the suspension ranging from 0 to 1200 µl were pipetted into plastic cups containing 100

ml salt-treated demineralised water to produce final experimental concentrations ranging from 0 to 0.4 mg/l (Additional file 1: Table S1).

Over six different days, larvae were exposed to three treatment cups per concentration and three control cups (salt-treated demineralised water). Twenty-five third-instar larvae were placed in each cup, and mortality was recorded after 24 and 48 h. If pupation occurred, the pupae were removed, and their numbers were excluded from calculations. Based on the proportion of larval mortality observed at each *Bti* concentration after 48 h, concentrations producing about 20% mortality (LC_{20}), 50% mortality (LC_{50}) and 70% mortality (LC_{70}) were fixed and used throughout the subsequent experiments.

Tests of sublethal doses on fitness parameters

To determine the effects of sublethal exposure to *Bti* during larval development, trays with third-instar larvae were obtained from the insectary and were exposed to experimental conditions for 48 h. These experiments were replicated six times. For each replicate, we used three control trays containing the salt-treated demineralised water and three trays for each of the three sublethal *Bti* concentrations (LC_{20}, LC_{50} and LC_{70}). During the 48-h exposure period, each tray was provided 20 mg fish food per day. Air temperature in the climate chamber was maintained at 27 ± 1°C. All dead and moribund larvae were counted after 24 and 48 h. After the 48-h exposure period for each replicate, all surviving larvae from the same treatment were pooled and placed in new trays with fresh salt-treated water only (i.e. no *Bti*). Sixty milligrams of fish food were given to the larvae in each tray daily. All emerging pupae were placed in plastic cups (100 × 50 mm diameter), which were placed in 30 × 30 × 30 cm cages, separated by treatment and replicate. The emerging adults were fed 6% sugar solution *ad libitum*. On days 5 and 6 post-emergence, 30 females from each treatment were indiscriminately removed using a mouth aspirator and placed in new cages, still separated by treatment and replicate. The females in each cage were given a chance to feed on human blood *via* arm feeding for 10 min per day for two days. The same person was used for all the blood-feeding. The mosquitoes were also provided with 6% sugar solution in the new cages when not blood-feeding. At 24 h after blood-feeding, oviposition cups were introduced in the cages and 25 randomly selected gravid females were kept in the cage. The other five females were taken back to the non-oviposition cages which contained mosquitoes which were not given blood meals. All dead mosquitoes from day of emergence in both oviposition and non-oviposition cages were counted daily until 37 days post-emergence. The records were separated by treatment, replicate and sex. The number of eggs laid was counted daily for seven days and separated by treatment and replicate. Wing lengths were measured of 570 mosquitoes as the distance between the alula and the wing tip, excluding fringe scales, using CMEX DC 5000 binocular microscope (Euromex, The Netherlands) and Image Focus Version 3 software. The wing measurements were separated by treatment, replicate and sex of the mosquitoes.

Data analysis

To determine the impact of *Bti* concentration on wing length, we fitted a linear mixed model to account for the effect of treatment (as a fixed effect) and replicate (as a random effect). We fitted a random intercept model where the effect of replicate was allowed to deviate from the overall, to investigate if replicate as a covariate contributed to the overall variation in the wing length. Kaplan Meier curves were plotted to visualize mosquito survival patterns over time. To estimate the hazard rate of mortality for each level of *Bti* concentration, we fitted a multivariate Cox proportional hazards model with treatment and replicate as covariates. A Poisson generalized linear model with treatment and wing length as covariates was fitted to investigate their effect on mean number of eggs laid by gravid females. The level of significance was set at 0.05. All statistical analyses were carried out in R version 3.6.1.

Results

Determination of sublethal concentrations of *Bti* on *An. coluzzii* larvae

Mortality of *An. coluzzii* larvae exposed as third instars to *Bti* for 48 h increased with increasing *Bti* concentrations (Fig. 1). Based on these larval mortality rates, we selected 0.03 mg/l, 0.12 mg/l and 0.28 mg/l as *Bti* concentrations for LC_{20}, LC_{50} and LC_{70} respectively (Additional file 1: Table S1).

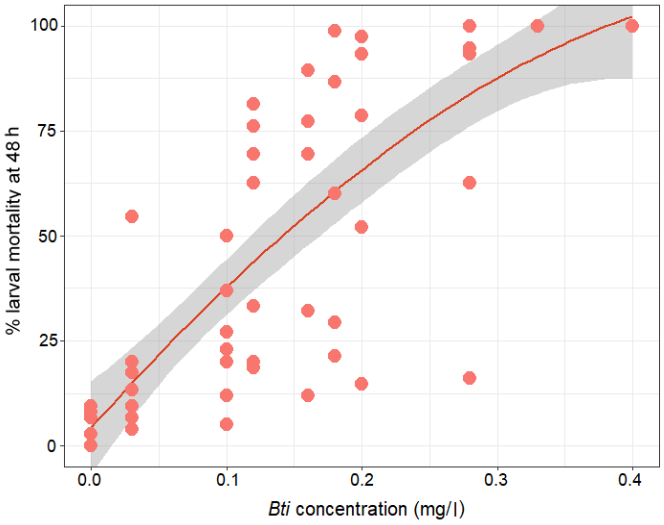

Fig. 1 The relationship between *Bti* concentration and mortality of *Anopheles coluzzii* larvae after 48 h. Each point represents the *Bti* concentration and corresponding larval mortality in each cup per replicate. Overlapping points indicate the same values for multiple cups. The red line is the weighted mean larval mortality due to the varied *Bti* concentrations and the grey area represents the 95% confidence interval. See Additional file 1: Table S1 for the number of replicates run for each *Bti* concentration

Table S1: Concentrations of *Bti* used in the bioassay determination experiments, and the observed mortality of *An. coluzzii* larvae at each concentration after starting with 75 larvae across 3 cups per treatment per replicate. Preparation of the stock solution is explained in the methods section. *across replicates after 48hrs

Volume (ul) *Bti* 1ml:49ml stock solution	Concentration of *Bti* (mg/L)	Number of replicates	Mean number of dead larvae per tray* ± SE	Mean % mortality*
0	0	7	3.2 ± 2.1	4.3
90	0.03	7	13.4 ± 8.6	17.9
300	0.1	7	32.4 ± 4.5	24.9
360	0.12	7	38.7 ± 3.1	51.7
480	0.16	6	30 ± 3.7	56
540	0.18	6	44.4 ± 4.4	59.2
600	0.2	6	50.4 ± 3.9	67.2
840	0.28	6	55 ± 3.7	73.3
1000	0.33	6	75 ± 0	100
1200	0.4	6	75 ± 0	100

Mortality was observed of *An. coluzzii* larvae at each concentration after starting with 75 larvae across 3 cups per treatment per replicate. Preparation of the stock solution is explained in the methods section. *across replicates after 48hrs.

Effect of larval exposure to sublethal *Bti* on post larval stage counts in *An. coluzzii*

A total of 2902 adult mosquitoes emerged from larvae from the control and treatment trays. The number of mosquitoes surviving to the adult stage decreased with increasing *Bti* concentrations (Table 1). In the control groups, there were 679 (69.9%) females and 293 (30.1%) males. For the treatment groups, 524 (60.8%) and 337 (39.1%), 423 (66.7%) and 211 (33.3%), and 315 (72.4%) and 120 (27.6%) were females and males for LC_{20}, $LC5_0$ and LC_{70}, respectively. Treatment did not have an effect on the sex ratio of mosquitoes surviving to the adult stage ($\chi^2 = 0$, $df = 3$, $p = 1$).

Table 1: Effect of exposure to sublethal *Bti* concentrations during larval stages on the number of *An. coluzzii* pupae and adults and corresponding wing lengths.

Treatment	Mean number of pupae ± SE	Mean number of adults ± SE	Mean wing length (mm) of adult males ± SE (N)	Mean wing length (mm) of adult females ± SE (N)
Control	162 ± 9.02	159 ± 7.33	2.39 ± 0.02 (57)	2.36 ± 0.02 (160)
LC_{20}	143.5 ± 13.68	137 ± 9.04	2.36 ± 0.03 (40)	2.41 ± 0.02 (133)
LC_{50}	105.7 ± 13.63	93 ± 10.23	2.42 ± 0.04 (37)	2.50 ± 0.02 (63)
LC_{70}	72.5 ± 7.98	64 ± 5.32	2.48 ± 0.02* (34)	2.58 ± 0.05* (46)

*Indicates statistical significance of the treatment in relation to the control ($p < 0.01$). See Additional file 1: Tables S2 and S3 for the effect sizes of larval exposure to the sublethal *Bti* concentrations on wing lengths of adult males and females, respectively

Effect of larval exposure to sublethal *Bti* concentrations on wing length

Five hundred and seventy wings were measured and separated by sex: 402 and 168 for female and male mosquitoes, respectively. For both sexes, increasing *Bti* concentration was associated with an increase in wing length (Table 1), but the difference was only significant between the control and LC_{70}. The mean wing length of the adult female *An. coluzzii* exposed to LC_{70} *Bti* concentrations increased by 12% compared to the control group (Additional file 1: Table S2). Similarly, adult *An. coluzzii* males exposed to LC_{70} concentrations had wings that were 20% longer than the control group (Additional file 1: Table S3).

Survival of adult *An. coluzzii* after exposure to sublethal *Bti* concentrations as larvae

Survival of adult *An. coluzzii* decreased with increasing *Bti* concentration exposure as larvae (Fig. 2). The highest cumulative survival probabilities for both female and male mosquitoes were observed in the control and LC_{20} concentrations. The survival probabilities dropped more rapidly in males compared to females.

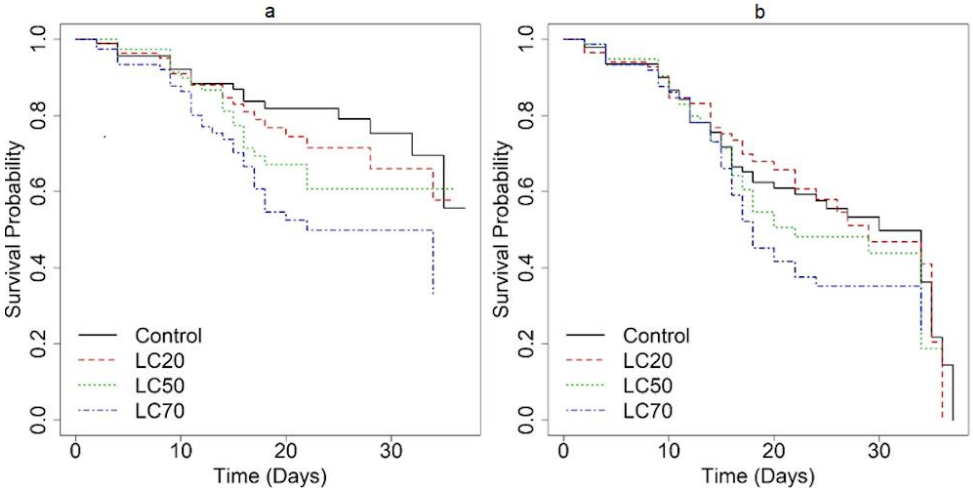

Fig. 2 Survival curves for *Anopheles coluzzii* adults exposed to different concentrations of *Bti* as larvae. **a** Survival curves for adult female *An. coluzzii.* **b** Survival curves for adult male *An. coluzzii*

An increasing trend of hazard ratios (HR) with increasing *Bti* concentrations was observed in adult females compared with the control group (Table 2). When exposed to *Bti* LC_{70} as larvae, the proportional hazard rate for mortality as adult females was about three times higher than the rate from the control group (HR = 2.58, CI: 1.44–4.53, $p < 0.01$). The mortality hazard ratio for adult males was also significantly increased when exposed to *Bti* LC70 as larvae, (HR =1.54, CI: 0.99–2.38, $p = 0.049$; Table 2).

Table 2: Parameter estimates of the Cox Proportional hazards model for mortality of adult female and male *An. coluzzii* exposed to sublethal *Bti* concentrations as larvae

Variable	Time to death (females)			Time to death (males)		
	HR	**95% CI**	**P-value**	**HR**	**95% CI**	**P-value**
LC_{20}	1.25	0.65–2.38	0.5	0.95	0.6–1.51	0.83
LC_{50}	1.62	0.86–3.05	0.14	1.25	0.8–1.96	0.33
LC_{70}	2.58	1.44–4.63	**0.001**	1.54	0.99–2.38	**0.049**

Bold values indicate statistical significance of the treatment in relation to the control ($p < 0.05$).

Effect of sublethal *Bti* concentrations on number of eggs laid by adult *An. coluzzii*

There was an apparent trend of decreasing number of eggs laid per cage of 25 females with increasing concentration of *Bti* exposure as larvae (Fig. 3). However, these differences were not statistically significant (Table 3). Further, the mean wing length of gravid females per cage did not have an effect on the number of eggs laid (Table 3).

Table 3: Effect of *Bti* treatment and wing length on number of eggs laid by gravid *An. coluzzii* females.

Variable	Estimate	95% CI	P-value
Intercept	1.16	0.59–2.26	0.667
LC_{20}	0.72	0.41–1.26	0.55
LC_{50}	0.58	0.27–1.24	0.32
LC_{70}	0.52	0.23–1.13	0.25
Mean wing length	0.75	0.06–5.45	0.72

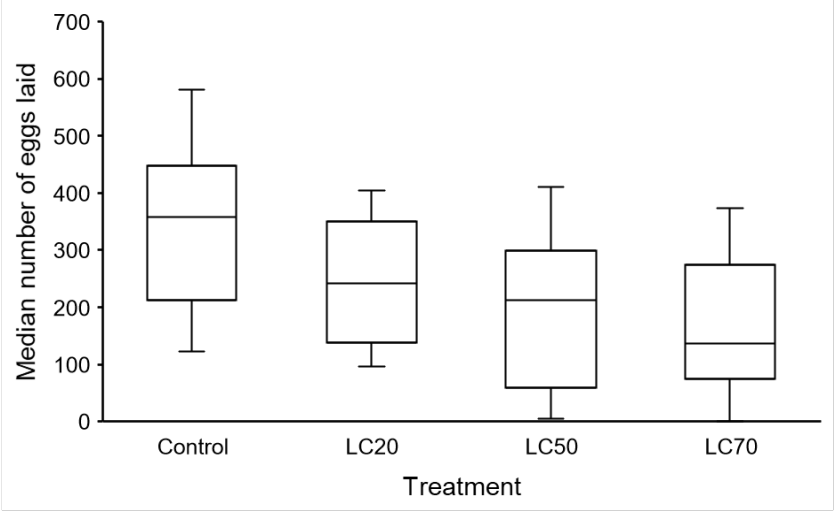

Fig. 3. Effect of sublethal *Bti* doses on the median number of eggs laid by a group of 25 gravid females. The 25-female egg count was repeated 6 times within each treatment.

Discussion

This study assessed potential effects of larval exposure to sublethal doses of *Bti* on fitness parameters of adult *An. coluzzii.* We observed that larval exposure to sublethal *Bti* doses reduced survival of adult *An. coluzzii* mosquitoes. For both male and female *An. coluzzii*, the lowest survival probability was recorded at the highest *Bti* concentration. It is not well understood how the larvicide reduces longevity of the adults that survive exposure as larvae. However, there is evidence that a *Bti* toxin, Cry1C, has a toxic effect to brain cells of larvae of a lepidopteran, *Lymantria dispar,* in vitro (Cerstiaens et al. 2001). This suggests that *Bti* may cause similar damage to the adult mosquitoes surviving larval exposure to sublethal doses which may reduce their life spans. Similar results have been reported for *Culex quinquefasciatus* exposed to sublethal doses of cypermethrin as both larvae and adults (Sunday et al. 2016). The authors attributed their findings to physiological damage caused to the nervous system and associated aberrations due to abnormal hormone release and dehydration as a result of exposure to cypermethrin. Malaria parasites require 8–35 days to develop in their anopheline hosts (Ndoen et al. 2012), and therefore reduced adult longevity is widely recognized to reduce the vectorial capacity of a vector population (Smith and McKenzie 2004). Under highly controlled laboratory conditions, 10% and 37% female mortalities were observed by day 15 in the control and LC_{70} groups, respectively, suggesting that the sublethal doses can potentially contribute to reductions in malaria parasite transmission by reducing the longevity of adults.

Wing length in adult *An. coluzzii* mosquitoes increased with larval exposure to increasing sublethal *Bti* concentrations. Similar findings were observed in adult female *Ae. aegypti* mosquitoes exposed to sublethal concentrations of a naturally derived insecticide, spinosad (Antonio et al. 2009). The larger mosquitoes emerging from larval development at higher sublethal *Bti* concentrations may have at least two explanations. First, larger mosquito larvae may be more capable of coping with any stress induced by *Bti* exposure, and therefore survived exposure to concentrations that smaller larvae could not. Secondly, *Bti* treatment reduced larval densities and, thus, competition over food and other resources following *Bti* exposure. Reduced resource competition due to lower larval densities has previously been associated with larger mosquito size (Gimnig et al. 2002, Scott and Takken 2012). Wing length is used as a standard indicator of body size in mosquitoes (Siegel et al. 1992, Jirakanjanakit et al. 2007) as the two measures are positively correlated. Larger females typically produce more eggs because they can take larger bloodmeals, which means they contribute more offspring to the population (Briegel 1990, Takken et al. 1998). Larger size has also been related with better ability to disperse in *Culex pipiens* (Alcalay et al. 2018). The potential increase in oviposition rates and dispersal ability for larger mosquitoes may increase their contribution to malaria transmission. Additionally, larger mosquitoes may exhibit reduced susceptibility to the synthetic insecticides used in current vector control tools (Oliver and Brooke 2013, Owusu et al. 2017) although it is unclear how

this effect might interact with the reduced survival of *An. coluzzii* exposed to sublethal concentrations of *Bti* observed in our study. It is also known that smaller female mosquitoes require multiple blood meals before they can reproduce, thus increasing their contact with hosts and effectively becoming more efficient vectors (Scott and Takken 2012). Therefore, the impact of larger adult *An. coluzzii* mosquitoes on malaria parasite transmission due to sublethal *Bti* concentrations remains unclear.

We observed no associations between the mean number of eggs laid and *Bti* treatment. Similar findings have been observed in another malaria vector, *An. superpictus*, also exposed to sublethal concentrations of *Bti* as larvae under laboratory conditions (Simsek et al. 2009). Also, the results agree with observations made on *Ae. aegypti* under similar conditions (Flores et al. 2004). Our study might have been limited by clustering of the 25 gravid females in an oviposition cage containing only one oviposition cup. Clustering of females in a cage might have led to egg retention in some of the females as a way of avoiding competition for oviposition space. Evidence of egg retention has been reported in gravid female mosquitoes in absence of suitable oviposition sites (Seenivasagan et al. 2015). The mean wing length of gravid females also was not associated with the number of eggs laid per cage. The averaging of wing lengths of gravid females might have masked small but meaningful variations in wing sizes due to treatments. Despite not significantly explaining differences in the egg counts between control and treatment groups, we observed a declining trend in median number of eggs laid with increasing *Bti* concentrations. This effect deserves further study, as a reduction in the number of eggs with larval exposure to *Bti* would directly reduce vector population size.

Conclusions

Exposure of *An. coluzzii* larvae to sublethal *Bti* doses reduced longevity of adult *An. coluzzii* and was associated with larger adult size. Whether the increased size is mechanistically linked to *Bti* toxins or decreased larval density is unclear. There was not a clear effect of larval exposure to *Bti* on oviposition. It remains important to apply the recommended dosage when applying *Bti* for malaria vector control, as concentrations high enough to kill larvae before they emerge as adults provide the most effective control against malaria parasite transmission. Still, the effect of sublethal *Bti* exposure could lead to a reduction in vectorial capacity for malaria vector populations by increasing mortality of adults that survived exposure to *Bti* in their larval stage.

Acknowledgements

We are grateful to Pieter Rouweler, Joeri Groot, Seppe Salari, Frans K.M van Aggelen and André J. Gidding for mosquito rearing. This work was supported by a grant from the Dioraphte Foundation.

Funding

This work was supported by the Dioraphte Foundation, The Netherlands. The content is solely the responsibility of the authors and does not necessarily represent the official views of the funders.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Ethics approval and consent to participate

Human blood used to rear the main mosquito colony was obtained from the blood bank (Sanquin, The Netherlands) where donors filled out an informed consent. The female mosquitoes emerging from larvae that survived exposure to sublethal *Bti* doses were fed on human blood *via* arm feeding. The Medical Research Ethics Committee of Wageningen University concluded that the experimental procedure did not require ethical approval according to the Dutch 'Medical Research Involving Human Subjects Act'.

Table S2: Effect of larval exposure to sublethal *Bti* concentrations on wing lengths of adult female *An. coluzzii* mosquitoes.

Variable	Estimate	SE	P-value
Intercept	2.42	0.032	**<0.01**
LC20	-0.02	0.034	0.62
LC50	0.04	0.035	0.24
LC70	0.12	0.035	**<0.01**

Bold values indicate statistical significance of the treatment in relation to the control ($p < 0.01$).

Table S3: Effect of exposure to sublethal *Bti* concentrations as larvae on wing lengths of adult male *An. coluzzii* mosquitoes.

Variable	Estimate	SE	P-value
Intercept	2.36	0.03	**<0.01**
LC20	0	0.05	0.97
LC50	0.05	0.04	0.29
LC70	0.2	0.05	**<0.01**

Bold values indicate statistical significance of the treatment in relation to the control ($p < 0.01$).

Chapter 5

Community factors affecting participation in larval source management for malaria control in Chikwawa District, southern Malawi

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Submitted

Abstract

Introduction: For further reductions in the burden of malaria, larval source management (LSM) is proposed as a complementary strategy to the existing strategies. The complementary effects of LSM could include the ability to control insecticide resistant, outdoor biting and outdoor resting vectors. Concerns about costs and operational feasibility of implementation of LSM at large scale are among the reasons the strategy is not part of malaria control programs in many African countries. Involving communities in LSM has the potential to increase intervention coverage, reduce costs of implementation and improve sustainability of operations. Community acceptance and participation in community-led LSM depends on a number of factors. We explored these factors under The Majete Malaria Project in Chikwawa district, southern Malawi.

Methods: We conducted separate focus group discussions (FGDs): with members from the general community (n=3); with health animators (HAs) (n=3); and with LSM committee members (n=3). In-depth interviews (IDIs) were conducted with community members. Framework analysis was employed to determine the factors contributing to community acceptance and participation in the locally-driven intervention.

Results: Nine FGDs and 24 IDIs were held, involving 87 members of the community. Widespread knowledge of malaria as a health problem, its mode of transmission, mosquito larval habitats and mosquito control was recorded. High awareness of an association between creation of larval habitats and malaria transmission was reported. Perception of LSM as a tool for malaria control was high. The use of a microbial larvicide as a form of LSM was perceived as both safe and effective. However, actual participation in LSM by the different interviewee groups varied. Factors that contributed to lower participation included labour intensity and time requirements of activities, lack of financial incentives, and concern about health risks when wading in water bodies.

Conclusions: Community involvement in LSM as an additional tool for malaria control increased local awareness of malaria as a health problem, its risk factors and control strategies. However, community participation varied among the respondent groups, with labour and time demands of the activities, and lack of incentives, among the reasons cited for reduced participation. Employing innovative tools with the potential to reduce labour and time demands could improve community participation in the activities. Further studies are required to investigate the forms and modes of delivery of incentives in operational community-driven LSM interventions.

Key words: Malaria, Larval source management, *Bacillus thuringiensis israelensis*, community, Malawi

Introduction

In the last decade, remarkable progress has been achieved in the fight against malaria (WHO 2018). This is largely attributed to a combination of preventive and curative measures including insecticide-treated bed nets and effective case management (Bhatt et al. 2015, WHO 2018). Long-lasting insecticide treated bed nets (LLINs) and indoor residual spraying (IRS) as vector control interventions have made major contributions towards the recent gains (Irving et al. 2012, Lindblade et al. 2015). Despite these gains, malaria still remains a major public health problem in Africa as reported by stable or increasing incidence rates over the past few years in many African countries (WHO 2018). Development of resistance to drugs (Dondorp et al. 2017) and insecticides (Ranson and Lissenden 2016, Riveron et al. 2019) in the malaria parasites and vectors, respectively, and vector behavioural plasticity such as outdoor feeding and resting (Killeen et al. 2016) threaten the efficacy of available interventions to reduce the malaria burden.

The shortfalls of the current malaria interventions suggest a need for new strategies that can further reduce malaria transmission. Larval source management (LSM), which controls malaria vector populations through reduced suitability of mosquito larval habitats, is recognised as an effective supplementary tool for malaria control under specific conditions (Fillinger et al. 2009, WHO 2019). As a complementary malaria control strategy, LSM could be ideal for situations where vector aquatic habitats are few, fixed and findable (WHO 2013b). Other factors cited for adoption of LSM as a complimentary tool include cost-effectiveness when compared with other tools (Fillinger and Lindsay 2006, Mzilahowa et al. 2012) and its ability to control vector populations that avoid contact with insecticide-based tools (Killeen et al. 2002). Further, the microbial larvicides under advocacy for use in LSM have not, to date, been shown to cause any signs of resistance in vector populations or harmful effects on non-targeted organisms (WHO 2013b). In Kenya, the deployment of LSM as a complementary measure to communities already using LLINs was shown to significantly improve malaria control compared to the situation with LLINs used as a stand-alone method (Fillinger et al. 2009). A number of other studies have reported similar results showing the contribution of LSM to malaria reduction in Africa (Shililu et al. 2003, Fillinger and Lindsay 2006, Mwangangi et al. 2011, Imbahale et al. 2012, Djènontin et al. 2014, Mazigo et al. 2019).

In Malawi, like in many other African countries, LSM has not yet been introduced or evaluated for malaria control. This is due to a number of factors including a lack of data on local larval mosquito vector ecology (Worrall and Fillinger 2011), lack of local evidence for LSM in malaria control, and concerns about the cost of implementation on a large scale. One potential method of managing implementation costs and intervention coverage is to closely involve communities in the application of LSM. This approach could enable adequate coverage of targeted areas through education and skills development of communities about LSM, reduce costs of implementation

as human capital is locally available, and increase community acceptance and ownership (Dongus et al. 2007).

The Majete Malaria Project (MMP) was a community-led malaria control project undertaken in villages along the perimeter of the Majete Wildlife Reserve in Chikwawa district in Southern Malawi (McCann et al. 2017). Local communities were involved in the development and implementation of the LSM activities as part of MMP (Van Den Berg et al. 2018). In this study, conducted two years after commencement of community involvement in the LSM activities, we assess the factors influencing implementation and acceptability of LSM for malaria control using a community-driven approach. An understanding of these factors could inform the best practices for future development and deployment of community-based interventions.

Methods

Study Area

Larval source management was implemented in 26 villages as part of MMP from May 2016 through April 2018 as part of a cluster randomized trial described in detail elsewhere (McCann et al. 2017, Van Den Berg et al. 2018). All 26 villages assigned the LSM arm of the randomized trial were included in the current study. All villages were located along the Majete Wildlife Reserve perimeter in Chikwawa district (16º 1′ S; 34º 47′E), southern Malawi. Chikwawa is hot and dry from September to December, hot and rainy from January to April, and mild and dry from June to August. The district is generally dry with typical Savannah type of vegetation, though agricultural land use is common in the landscape. The majority of people in the study villages keep livestock with cattle, goats and pigs being the predominant animals. Most of the households practice subsistence farming with maize, millet and beans as staple food. The study villages were divided into three sub-regions, called focal areas, spaced roughly evenly around the wildlife reserve and covering a total population of about 25,000 people in 65 villages (Fig. 1) (Kabaghe et al. 2017).

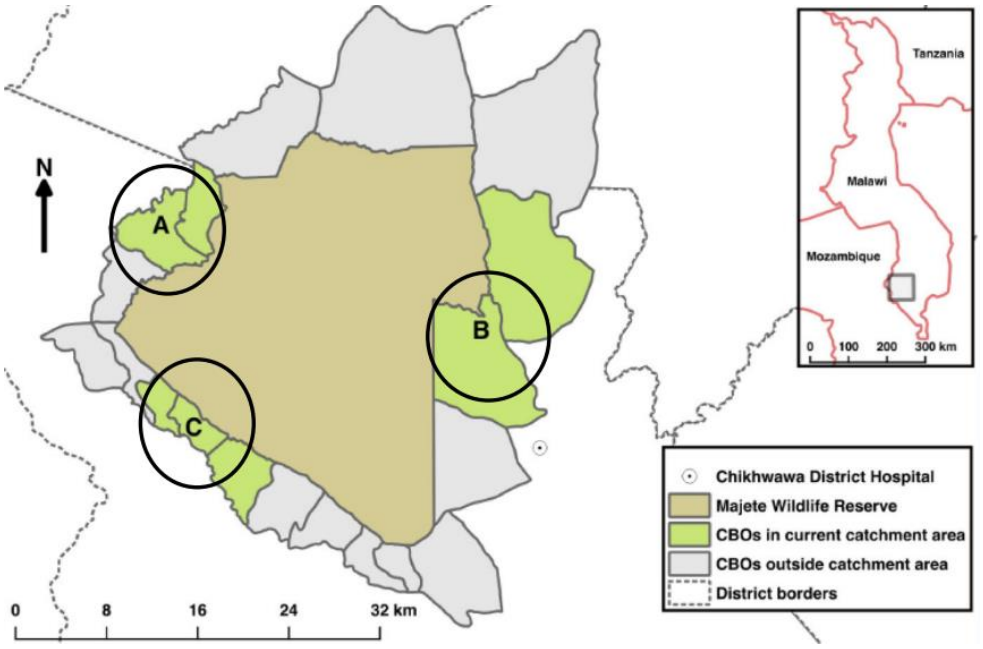

Fig. 1. Map of Majete Wildlife Reserve and the Majete Perimeter showing the three focal areas (Kabaghe et al. 2017).

Study population

The study was undertaken with community members from the 26 villages spread across the three focal areas, assigned 'A', 'B' and 'C'. Three different groups of respondents were identified: 1) Health Animators (HAs), 2) LSM committee members, and 3) members from the broader community. The HAs and LSM committee members received formal joint training from MMP and The Hunger Project-Malawi (THP) staff on malaria topics such as vector biology, parasite transmission, and vector control. After training, the HAs were tasked with organising and conducting village workshops in their respective villages to share knowledge on the malaria topics. They were also responsible for fostering malaria discussions, facilitating community-based implementation of larval habitat draining and filling as part of community-based LSM and coordinating all malaria control activities at village level. The LSM committees were comprised of 10 to 12 individuals from the respective village selected by members of each village at community meetings. These LSM committees were formed to carry out LSM activities in each selected village, and they were tasked with quarterly mapping of potential mosquito larval habitats, lobbying for and coordinating community participation in larval habitat draining and filling, and *Bti* application. Community members were then tasked with larval habitat draining and filling.

Data collection

Survey instruments comprised of focus group discussions (FGDs) and in-depth interviews (IDIs) that were developed based on points stemming from quantitative surveys conducted by the first author prior to the qualitative study. Prior to commencement of data collection, data collectors were trained and the data collection tools were piloted. This was done in order to acquaint the data collectors with the purpose of the study, interview guides and consent forms, and the consenting process, and data collection using voice recorders. Tables S1 and S2 provide summaries of the interview guides. Questions for the different interview sessions included perception of malaria as a problem, its symptoms, mode of transmission, risk factors and control, and recommendations for effective community involvement in control initiatives. Questions related to the perception of malaria as a problem and knowledge about malaria transmission were restricted to IDIs and FGDs involving the general community.

Twenty-four IDIs were conducted with members from the general community in the study villages. Selection of the IDI participants was based on overall village-level motivation and participation in the LSM activities. This was based on results of the quantitative surveys conducted *a priori*. To rank the villages, proportions of participants per village who indicated both motivation and participation in the activities were compared with the proportion of those who indicated no or little motivation and participation. Then the villages were divided into two groups: 1) Above average motivation and participation and 2) Below average motivation and participation. Twelve IDIs were conducted with participants from villages with above average motivation and participation, and the other twelve from the villages with below average motivation and participation.

Nine mixed-village FGDs were undertaken with community members, HAs and LSM committee members drawn from different LSM villages. These did not include participants of the IDIs. Like in the IDI sessions, selection of villages from which participants would come was based on how each village ranked on the scale described for the IDIs. Thus, for each mixed-village FGD session the participants came from villages with above average motivation and participation and below average motivation and participation. The FGDs were conducted in each of the three focal areas, such that one FGD for each of the three target groups was conducted in each focal area. To stimulate discussion and ensure contribution of all members the number of participants in the FDGs was between six and eight.

Data analysis

The IDIs and FGDs were conducted in the local language *Chichewa*. All data were audio-recorded, transcribed and translated into English. Data was analysed thematically. The first author familiarized himself with the whole data set and the last

author coded four transcripts. A common coding framework was developed through discussion. A codebook was developed using inductive and deductive coding methods. The inductive approach allowed generation of new themes emerging from the data while the deductive approach was based on a pre-developed codebook, which guided the coding process. The translated excerpts were coded using NVivo 12 (QSL international, Victoria, Australia). The first and last author identified key themes.

Ethical consideration

The University of Malawi's College of Medicine Research and Ethics Committee granted ethical approval (COMREC protocol number P.12/17/2222). Permission to collect data in the study villages was provided by the Chikwawa District Heath Office (DHO). Prior to recruitment of participants, communication about the study was sent to the community through local village heads in liason with HAs. Written informed consent was obtained from all participants during data collection. All the participants were men and women aged above 18 years. Literate participants provided a signature on the consent form and illiterate participants provided a thumbprint. Interviews were conducted in a private space, and participants were assured that their personal details would be omitted from transcripts and no personal details would be divulged to ensure confidentiality. Finally, participants were informed that their involvement in the research was voluntary and that withdrawal was permitted at any time and without personal consequence.

Results

Respondent characteristics

A total of 87 respondents participated in the 33 interview sessions: 24 IDIs and 9 FGDs (Table 1). All the IDIs were conducted with the community members that were not HAs or LSM committee members. Three FGDs were conducted per focal area: one with community members; one with HAs; and one with LSM committee members. Most of the participants were in the age group 18 to 24 (71.3%) and reported primary education as their highest level of formal education (51.7%). More males (57.5%) than females (42.5%) participated in the interviews.

The study results were grouped into five main themes drawn through the inductive and deductive methods (Table 2). Theme 1 covered topics that were only asked to the participants from the general community and not to the other groups (HAs and LSM committee members).

Table 1: Characteristics of study participants.

Characteristic	Focal Area (n)			Respondents [N, (%)]
	Focal area A (30)	Focal area B (26)	Focal area C (31)	
Sex				
Female	11	13	13	37 (42. 5)
Male	19	13	18	50 (57.5)
Age				
18 – 24	7	11	5	23 (26.4)
25 – 44	23	15	24	62 (71.3)
≥45			2	2 (2.3)
Education				
None	16	8	2	26 (29.9)
Primary	10	11	24	45 (51.7)
Secondary	4	7	4	15 (17.2)
Tertiary			1	1 (1.2)
Session				
FGD	3	3	3	9 (27.3)
IDI	8	8	8	24 (72.7)

Table 2: Main themes drawn from the qualitative study

	Theme
1.	Community perception of malaria as a health problem
2.	Community knowledge about malaria transmission
3.	Community trust, support and acceptance of microbial larviciding
4.	Community participation in LSM: enabling and hindering factors
5.	Recommendations for scale-up and future community-led LSM

Community perception of malaria as a problem

Our results showed widespread perception of malaria as a health problem among members from the broader community. Unlike the HAs and LSM committee members, the broader community received minimal formal training on the malaria topic. Much of their knowledge came from their interactions with the HAs and LSM committee members who received tailored training from the larger project, MMP. The community members mentioned that their own malaria related illness, and/or illness of those close to them, reduced their performance of income generating activities and increased financial expenses via treatment and treatment-seeking activities.

> *"When one suffers from malaria they need money to access treatment while at the same time all economic activities that would be undertaken to improve their livelihood are halted"* (IDI, Community participant, Kandeu 2).

> *"I find malaria to be particularily burdensome because it is very hard to find medicines at the local health centres hence we are forced to buy from pharmacies at higher prices"* (IDI, Community participant, Kabwatika)

Although malaria was identified as a problem that affects everyone, most participants reported that pregnant women and children are most vulnerable to the disease.

> *"Much as everyone is at risk of malaria, young children and pregnant women are the most vulnerable"* (IDI, Community participant, Kampaundi).

Knowledge of malaria transmission

There was widespread knowledge among all respondents about the mode of transmission of malaria parasites and the type of environment conducive for breeding and development of mosquitoes. Almost all respondents reiterated that bites from infected mosquitoes drive malaria transmission. Interestingly, a member from the community was even able to mention the sex and genus of the mosquito responsible for the transmission of malaria.

> *"When a female Anopheles mosquito bites a person with malaria and then another person without the disease, the malaria parasite is transmitted to the latter"* (IDI, Community participant, Chipula).

When asked where mosquito larvae could be found, participants provided varying responses. Some participants identified natural and human-made sites and objects as potential larval habitats. Particular mention was made on the duration of water storage, the nature of the water (stagnant, dirty or clean), including the container or vessel and the contents in it, as factors that contribute to where mosquito larvae are most likely to be found. The term "dirty water" was in these cases synonymous with foul water.

> *"Mosquitoes breed in standing water or in water that has been stored or has not been used for a long time"* (FGD, LSM Committee, FA-B)

> *"There are some mosquito aquatic habitats which are natural such as streambeds while many are man-made"* (FGD, HA, FA-A)

> *"Anopheline larvae are found only in clean water while Culicine larvae are found in dirty water"* (FGD, HA, FA-A)

Most participants implicated human activities with creation of the potential mosquito larval habitats. The responses provided were categorised based on purpose: 1) domestic: washing and drinking, 2) agriculture: irrigation, fish farming and watering points for livestock, 3) and construction: brick-making and mud. Brick-making purposes were the most mentioned reason for creation of larval habitat sites. However, the issue of eliminating these water bodies revealed community perspectives on conflicts between economic activities and malaria control.

As one community member said,

> *"These larval habitats came into existence due to development activities being conducted in our communities such as school building initiatives which demand us to make bricks. We are caught in a situation where one development activity affects another"* (IDI, Community participant, Mkangeni)

An LSM committee member who was responsible for carrying out larviciding activities supported this opinion.

> *"Much as we know that these are the very places where mosquitoes driving malaria transmission breed, some of these places are very important to us as we use them to irrigate crops, drinking points for our livestock and also to soak bamboos for making traditional mats"* (FGD, LSM committee, FA-A).

Despite the perceived conflict between community developments and malaria control, participants displayed an understanding of the role of these places as refuge for immature stages of mosquitoes. This enabled some of the participants to suggest solutions for malaria control.

> *"Mosquitoes breed in standing water bodies which are readily available in our villages. Removing these potential aquatic habitats is the only sure way forward''* (IDI, Community participant, Jana)

> *"If we are not careful, discharging water anyhow into these freshwater marshes creates suitable environments for mosquito proliferation, a thing which can increase malaria prevalence in the area"* (FGD, LSM committee, FAC).

Community trust, support and acceptance of microbial larviciding

Most of the participants agreed on the effectiveness of *Bti* for mosquito control. However, it was observed that some community members did not want to work with the larvicide for fear of a health risk for themselves or their livestock, especially at the onset of the project. Lack of evidence of the product's activity and safety was the major reason for the skepticism and lack of trust in the product by the community.

A community member highlights this perception:

> *"I do not really know how Bti works but I think it can cause cancer. Because no livestock has died due to the larvicide does not mean I should not be concerned"* (IDI, Community participant, Mkangeni)

Additionally, some participants were initially sceptical about the product, because LSM committee members used mouth masks during application of *Bti*.

> *"The use of masks by members of LSM committees during Bti application made some people suspicious of the product"* (FGD, HA, FA-A)

The initial concerns were on the safety of livestock, crops and human life, but as time passed the community members could see that *Bti* did not have harmful effects on their crops, livestock and their personal health. Increased engagement with LSM committees and HAs increased community trust, support and acceptance of the larvicide.

> *"Initially we had a lot of fears about Bti as we thought it would be harmful to those using treated water sources but we have neither seen nor heard of any harm due to the larvicide. We are beyond convinced that this product only kills mosquito larvae"* (IDI, Community participant, Kampaundi).

> *"We did not allow LSM committees to apply Bti in water bodies, especially those used for irrigations purposes because we had fears the larvicide would cause damage. Now we have realised that our fears were unfounded. We are very willing and ready to have the habitats sprayed with the product"* (FGD, Community members, FA-C)

In some cases, field-based workshops were held with the community where *Bti* was actually applied on habitats infested with mosquito larvae. At these sites the activity of *Bti* on the larvae and other aquatic organisms was co-investigated with the community members.

> *"When the intervention just started, people had concerns about harmful effects of Bti on crops, livestock and people. To prove to them that the larvicide was very safe we conducted sensitization meetings in our communities. The communities are now aware that spraying Bti does not introduce any risks to crops, humans and livestock"* (FGD, LSM committee, FA-C)

The LSM committees believed that it was only those people who did not attend community workshops who had negative concerns about the product.

> *"The people who complained were those who never attended village workshops so they did not know the benefits of Bti. Once they come to understand they will never protest again"* (FGD, LSM Committee, FA-B)

Factors enabling participation in LSM activities

Under this theme we explored factors that motivated community participants in carrying out LSM activities. Enabling factors included involvement of local leaders in the initiative and the knowledge gained through workshops about malaria control and implementing control measures. Most LSM committee members felt that the knowledge they attained about mosquito larval control made them aware of their role in the fight against malaria.

> *"We have gained a lot of knowledge about the malaria topic from the numerous trainings we have gone through. This knowledge motivates us to participate in the malaria control activities"* (FGD, LSM Committee, FA-B)

> *"Our village heads contribute to the cause by organizing community meetings where they encourage us to actively participate in the LSM activities"* (FGD, Community members, FA-B).

The community members perceived a visible decline in malaria cases in their communities, which they attributed to their work. They indicated that such achievements encouraged them to work towards more reductions in the malaria burden. They also cited problems faced to access treatment for malaria as a factor driving their actions towards malaria control.

> *"We have had the worst experiences with malaria. We live very far from health facilities hence have problems to access health care services. This initiative is our lifeline hence our great zeal to participate"* (IDI, Community participant, Kampaundi)

> *"I am motivated to participate in the activities because our community has been very disadvantaged in terms of access to health care services. We live very far from the nearest health facility, which is also a paying facility. I fully understand the challenges faced to access medical help at the facility. So when we were told about what we are supposed to do to reduce the malaria burden I decided to participate"* (IDI, Community participant, Kandeu 2)

There was a general feeling among the community members that HAs and LSM committee members were more motivated to participate in the LSM activities than the rest of the community. However, the community members expressed mixed sentiments as to why HAs and LSM committees seemed more motivated to participate in the activities. Some community members felt that the knowledge the two groups gained during the course of their duties enticed them to participate in the control initiative. Another section of the community felt that the money given to the two groups by MMP to meet logistical requirements for trainings outside their focal areas incentivised them.

> *"These people work hard because they understand that the intervention would be beneficial to their communities"* (IDI, Community participant, Kandeu 2)

> *"LSM committee members work hard because they are taken to trainings where they are given money. If there were no such incentives none of them would be as active"* (IDI, Community participant, Kampaundi IDI)

Factors hindering participation in LSM activities

When asked what they felt were the limiting factors for community implementation of the LSM activities, the respondents cited a number of issues. One of the major factors cited by LSM committee members was the high amount of labour and time required to carry out *Bti* application activities. Weekly applications of *Bti* were necessary for optimum effectiveness of the *Bti* because of its short residual activity. However, LSM committee members reported that much of their time was spent

carrying out the LSM activities, which reduced their time to participate in income generating activities for their households.

> *"The work is too laborious. We do Bti pre- and post-spray surveys every week, and we spray Bti after every seven days. This means we spend much of our time working in LSM at the expense of our families' well-being"* (FGD, LSM Committee, FA-C)

They also mentioned the long walking distances to the sites where they applied *Bti* and the continued creation of potential mosquito larval habitats.

> *"The major problem is distance, when we go to spray Bti, we travel long distances because some water bodies are very far. Sometimes we plan to spray more aquatic habitats per day but fail to realise the plan because we have to travel long distances hence end up spraying in very few. This makes us work for more days than expected"* (FGD, LSM Committee, FA-C)

> *"This work is very tiresome as we are required to continuously fill and drain, and spray Bti every week in the potential mosquito breeding sites. From the look of things we will continue to create these sites as we do not have alternatives to bricks [the excavation of which creates breeding sites]"* (FGD, LSM committee, FA-A)

Some respondents indicated that provision of no monetary incentives was a major factor influencing lack of participation in the activities. While this feeling was widespread, it was not true for some villages.

> *"Some members are discouraged because they want outright benefits. Of course, in my area there have never been such cases, but I know this happens in other villages"* (FGD, LSM Committee, FA-C)

Lack of gumboots as protection from water-borne infections, for example to protect against schistosomiasis, for each committee member was the most cited challenge. While acknowledging the provision of several pairs of gumboots by the project, they noted that these were not sufficient for all committee members. They also indicated their reluctance to share boots due to risk of contracting foot-borne fungal infections.

> *"We do not have enough gumboots for all members of the committee. We were told to be sharing the few we have but we cannot do that for fear of athlete's foot"* (FGD, LSM committee, FA-A)

Some LSM committee members cited the indifference of some community members towards LSM as a demotivating factor. Respondents noted that some community members did not attach value to the work of committee members and demeaned their volunteerism. This indifference left some LSM committee members frustrated, and in some cases led to dropping out from the committees.

> *"We are often discouraged by poor remarks from some communities members despising our volunteerism"* (FGD, LSM committee, FA-B)

"We are called stupid and time wasters by some community members for volunteering to work in this project" (FGD, HA, FA-C)

Recommendations for scale-up and future community-led LSM

There was a widespread perception among the respondents that village heads were not fully involved in the on-going LSM activities. The respondents suggested that for increased community participation in the activities the village heads needed to receive training and be tasked with specific roles. Some participants recommended that for future or for scale-up of existing community-led initiatives, groups comprised of village heads should be created to monitor the activities locally.

"A team of village heads should be instituted which should be tasked with monitoring LSM activities at village level. They should receive the same training as LSM committees. These people are highly respected by communities, which could ensure high community participation in the LSM activities. This team should be constantly updated by HAs and LSM committees" (FGD, HA, FA-C).

Some participants also recommended restructuring LSM committees by removing non-active members to improve group performance, adding more members to existing committees to reduce member work-load, or by making the selected committees work for a fixed period after which new committees take over.

"I think the LSM committees are burdened by the too large amount of work they are doing. It would be a better idea to bring in more people into the committees so the committee can do more sensitization meetings and cover more habitats" (FGD, Community members, FA-C)

"I think LSM committees should work for a maximum of one year and a new one be selected. Currently, some members have lost interest in the activities hence need to keep replacing them with new members willing to take over" (IDI, Community participant, Mkangeni)

The participants also recommended need for constant feedback on how the intervention is progressing. They felt this could encourage their participation in the activities.

"The community should be given feedback on how the intervention is performing. This could motivate them" (FGD, HA, FA-A)

Lastly, continued community sensitization was reported to be paramount if buying-in and participation in the LSM activities were to be successful.

"There is need for continued sensitization meetings. It is through repeated messages that some people change their attitude" (FGD, HA, FA-A)

Discussion

Our findings show that community involvement in LSM increased awareness of malaria as a health problem, its risk factors and control strategies. Lack of incentives as observed in other research paradigms in Malawi (Mfutso-Bengo et al. 2015) reduced participation of members from the broader community in the activities. Support from community leaders was a very critical factor for community participation in the activities. Labour intensiveness, the time-demanding nature of the activities, and fears about health risks associated with working in water bodies, created barriers to successful implementation of the intervention by the LSM committees. These results suggest that a wide range of factors must be considered for optimum effectiveness of community-driven malaria interventions.

Participants in our study perceived malaria as a health problem prevalent in their communities and recognized children and pregnant women as groups most vulnerable to the disease. Participants were aware or the role of mosquitoes in transmitting the malaria parasite and had knowledge of potential mosquito larval habitats. This knowledge is attributable to the malaria workshops conducted by the HAs in each village. Previous studies have suggested that community awareness of malaria as a burden has the potential to trigger positive action towards malaria control (Yasuoka et al. 2006, Castro et al. 2009).

Our findings suggest that the communities understood the association between mosquito larval habitats and malaria. However, some water bodies served a specific function in the community and were deemed useful by the respondents. This presents potential limitations in the adoption of habitat draining and filling for malaria control. Similar observations were made in Kenya where perceived importance by the community of some water bodies limited their willingness to remove such sites (Imbahale et al. 2010). Where habitat draining and filling are not feasible, application of larvicides is a viable alternative (Fillinger et al. 2009), and this was widely practiced by the communities in this study. The use of other LSM strategies such as predatory fish or shading of the aquatic habitats with plants such as Napier grass or coco-yams to make such sites less suitable for malaria vector mosquitoes has also been suggested (Takken and Knols 2009).

Community perception of *Bti* as a mosquito control tool improved with increased engagements with HAs and LSM committees, and interaction with the product. Initially, the communities reported skepticism about the product over potential harmful effects to humans, livestock and crops. The lack of a befitting synonym for the word "pesticide" when referring to *Bti* in the participants' vernacular, *Chichewa*, confounded their fears of the product. In *Chichewa*, the word "pesticide" is loosely interpreted as "poison" which denotes an inherent element of side effects. Through community workshops and handling of the product in the field, the community learned about the product's activity and specificity, which resulted in improved acceptance of the product by the community. Similar observations were made in

Rwanda where acceptance of *Bti* was observed to improve with increased interaction with the product by rice farmers tasked with its application (Ingabire et al. 2017). The findings suggest that for meaningful acceptance of control strategies, community training should focus on approaches that build trust by demonstrating the safety of the products to non-target organisms.

The HAs and LSM committees were more motivated to participate in the LSM activities than the members from the community at large. According to the HAs and LSM committees, attainment of knowledge of malaria and its control, and their sense of 'duty' motivated their participation in the LSM activities. For the both groups, the status received in the community for their role made them feel valued and motivated. However, some members from the broader community felt that the motivation of the HAs and LSM committees was a result of the "monetary incentives" they received during their trainings. This could be justified by the frequent calls made by the LSM committees for refresher trainings. This could potentially pose a barrier in community participation in the intervention as observed in another study conducted in Malawi where receipt of incentives by some groups demotivated other groups (Kok et al. 2017). Similarly, in a sub-study conducted under MMP in the same area as the current study "monetary incentives'' received by the HAs during their trainings were feared to have weakened the sustainability of the Health Animator approach (Kaunda-Khangamwa et al. 2019). Indeed, the forms and modes of delivery of incentives in volunteer-based initiatives are critical but they remain less studied (Ikeoluwapo, Ajayi et al. 2012). In Kenya, adaptation of a malaria control intervention (odour-baited mosquito traps) to local context by providing a source of solar energy to householders increased community acceptance and uptake of the intervention (Oria et al. 2018). Based on these findings, incentives have a role in influencing acceptability, uptake and sustainability of community-led interventions. To increase interest of a community and motivation to participate, we propose that the intervention agenda be developed in light of the local contexts, with enhanced attention for the community's needs.

Participants considered LSM activities to be labour intensive and time consuming, especially larviciding with *Bti*, which required weekly application. Some LSM committee members felt that the demands of the activities prevented them from actively engaging in income generating activities for the betterment of their livelihoods. The findings underscore the need to incorporate technical solutions that increase intervention coverage and quality while reducing labour demands. These technical solutions include powered sprayers, drones, and remote-sensing based risk maps (Dambach et al. 2012, Knapp et al. 2015).

In our study it was evident that local leadership was needed for effective implementation of the community-led LSM activities. A hierarchical structure with village heads, HAs and LSM committees as leaders was regarded as supportive by most of the respondents. This finding suggests that local authorities should not be engaged for administrative purposes only but also in both planning and

implementation of community-led initiatives. The findings also suggest that the village heads should work closely with LSM committees and HAs, with the latter groups only addressing the operational aspects and not the village politics such as calling for community workshops. Importantly, interventions should capitalize on the existing traditional structures present in each community. Rural communities have strong social structures resulting from their communal living (Imbahale et al. 2010) which, if exploited, could make community engagements attainable.

Conclusions

Community involvement in LSM as an additional tool for malaria control increased local awareness of malaria as a health problem, its risk factors and control strategies. However, community participation varied among the respondent groups, with labour and time demands of the activities, and lack of financial incentives, among the reasons cited for reducing participation. Employing innovative tools with potential to reduce labour and time demands could improve community participation in the activities. Further studies are required to investigate the forms and modes of delivery of incentives in operational community-driven LSM interventions.

Acknowledgements

De Vos Fund, The Netherlands, generously supported the study. We also thank the Dioraphte foundation for some financial support. The content is solely the responsibility of the authors and does not necessarily represent the official views of the funders. RSM is supported by an NIH-funded postdoctoral fellowship (T32AI007524). We thank all the research assistants for their support in data collection. Davies Kazembe, Happy Chongwe, Asante Kadama, Richard Nkhata and Tinashe Tizifa are thanked for logistical support. We also thank the study population for their willingness to participate in the study.

Supplementary material

Table S1: Interview guide (FGD) for Community members, HAs and LSM committees.

1. How would you describe your roles as a community member, member of an LSM committee or HA in malaria control?
2. What do you know about mosquito larval control?
3. Who do you think should take the lead in mosquito larval control? Why?
4. What are the common mosquito aquatic habitats in your area? Probe: How did these water bodies come into existence? Of what importance are they to communities? How would you relate presence of these water bodies with malaria transmission?
5. What do you know about mosquito larval control using *Bti*? Probe: where would you recommend use of *Bti* as opposed to other larval control initiatives i.e. draining and filling?
6. How are the LSM activities implemented in your village? Probe: Do you think the activities are too laborious or not? If yes, what factors make the activities demanding? How would you suggest the activities be approached?
7. What challenges do you encounter as you carry out the activities? How do you deal with each challenge?
8. What factors make some members of the committee not to actively participate in the LSM activities?
9. For those actively participating in the activities, what reasons could be attributed to their motivation?
10. What is the community's perceptions about *Bti*? What are the comments generally made about the intervention?
11. What changes, if any, have you seen in terms of malaria cases since the Majete Malaria Project came into your community?
12. What do you think should be done to improve community participation in community-led malaria control?

Table S2: Interview guide (IDI) for Community members

1. What are the most common health problems in this community?
2. Is malaria considered a serious health problem in this community? Why?
3. Who do you perceive to be the most susceptible to malaria?
4. In your opinion how do people get malaria?
5. Do you think it is possible to control mosquitoes? Explain your response
6. What kind of things do people in this community usually do to protect themselves from malaria?
7. Do you practice (some of) these preventive measures? Which measures do you practice?
8. What do you know about mosquito larval control?

9. Who do you think should take the lead in mosquito larval control? Why?
10. How would you describe your roles as a community member in malaria control?
11. What are the common mosquito aquatic habitats in your area? Probe: How did these water bodies come into existence? Of what importance are they to communities? How would you relate presence of these water bodies with malaria transmission?
12. What do you know about mosquito larval control using *Bti*? Probe: where would you recommend use of *Bti* as opposed to other larval control initiatives i.e. draining and filling?
13. How are the LSM activities implemented in your village? Probe: Do you think the activities are too laborious or not? If yes, what factors make the activities demanding? How would you suggest the activities be approached?
14. What challenges do you encounter as you carry out the activities? How do you deal with each challenge?
15. What factors make some members of the committee not to actively participate in the LSM activities?
16. For those actively participating in the activities, what reasons could be attributed to their motivation?
17. What is the community's perceptions about *Bti*? What are the comments generally made about the intervention?
18. What changes, if any, have you seen in terms of malaria cases since the Majete Malaria Project came into your community?
19. What do you think should be done to improve community participation in community-led malaria control?

Chapter 6

Community participation in habitat management and larviciding for the control of malaria vectors

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To be submitted

Abstract

Background: Larval source management (LSM) has the potential to reduce the malaria burden when executed in addition to standard vector control strategies. Involving communities in LSM activities could increase intervention coverage and reduce operational costs. However, it is not clear whether involving communities in LSM is both feasible and effective. This study investigated the impact of community involvement in LSM on anopheline larval densities in Majete, southern Malawi.
Methods: Communities in 26 LSM villages participated in larval control of malaria vectors through habitat draining and filling, as well as larviciding using *Bacillus thuringiensis* var. *israelensis* (*Bti*). The densities of immature stages of *Anopheles* within habitats in intervention villages and non-intervention villages were compared to assess the impact of the intervention. Sociological surveys involving 502 respondents were undertaken in the LSM villages only to investigate community motivation and participation, and factors influencing these two outcomes.
Results: The findings show that community monitoring of the LSM activities was highly irregular. Participation in habitat draining and filling was low and did not reflect the impressive knowledge respondents exhibited about malaria control. In contrast, community-executed *Bti* treatment was done as planned. *Bti* larviciding was associated with reduced anopheline larval densities in the LSM villages ($W = 24681$, $p = 0.004$). The comparison between intervention and non-intervention villages did not show a difference in larval densities. Attainment of knowledge about vector biology and control, and their role in the LSM activities motivated the LSM committees to participate in the malaria vector control programme.
Conclusions: Community-led LSM, particularly *Bti* treatment, was effective in reducing larval densities of malaria vectors in villages where the intervention was applied. Knowledge about mosquito aquatic habitats and *Bti* as a mosquito control tool, and ability to recognise mosquito larvae were the main factors that influenced both motivation and participation in the LSM activities. The effects of community-led LSM may have contributed to vector reductions in nearby villages not participating in the intervention. Concentrating efforts in making communities more aware of their risk of malaria and their role in malaria proliferation through habitat creation could potentially reduce risk factors, suppress creation of larval habitats, and promote ownership and participation in control efforts.

Keywords: Malaria**,** Larval source management, *Bacillus thuringiensis* var *israelensis (Bti)*, Community knowledge, Acceptance, Participation

Introduction

In recent years there has been renewed interest for larval source management (LSM) as a complementary tool for malaria control in Africa (Fillinger and Lindsay 2011, Derua et al. 2019), where the vast majority of malaria-related morbidity and mortality occurs (WHO 2018). LSM has contributed to reductions in adult vector populations (Tusting et al. 2013) and malaria burden, especially where it has been integrated with other vector control tools (Fillinger et al. 2009).

The two most common types of larval source management are 1) habitat modification, which includes physical transformation of a larval habitat through draining, filling and land levelling (Karunamoorthi 2011) and 2) larviciding, commonly using an endotoxin-producing bacterial larvicide, *Bacillus thuringiensis* var. *israelensis* (*Bti*) (Zahiri and Mulla 2005, Walker and Lynch 2007, Boyce et al. 2013, WHO 2013b). Wherever these methods have been implemented they have mostly been carried out by dedicated control teams involving targeted groups of the community without involvement of all members of the community. For example, in Sri Lanka, rice farmers were involved in integrated pest and vector management to manage vector-borne diseases and to improve rice yields using the "farmer field school" approach (Van Den Berg et al. 2007). Also, in Dar es Salaam, Tanzania, appointed members of the community known as Community Owned Resource Persons (CORPs) were involved in malaria control via identification and treatment of anopheline larval habitats (Vanek et al. 2006, Chaki et al. 2014). Though the approach involving selected members of the community has been effective in malaria vector control, co-opting all members of the community would be more beneficial as it stands to: 1) improve intervention coverage due to the community's knowledge of the location of larval habitats, 2) reduce costs of implementation owing to locally available human capital and 3) increase community support and ownership of the intervention.

There is, currently, lack of knowledge on whether co-opting the community-at-large in community-led larval control initiatives would successfully control malaria similar to expert-led initiatives involving only selected members of the community. To our knowledge there have been only two studies in Africa which involved the community-at-large as opposed to selected members who received special training in community-led larval control. One was carried out in Rwanda (Ingabire et al. 2017) and another in Malawi (McCann et al. 2017, Van Den Berg et al. 2018). The lack of involvement of the wider or whole community has implications in the formulation of policy around the adoption and sustainability of community-based LSM for malaria control. This study was set up to investigate whether involving the community-at-large in habitat management and specially trained members from the community in *Bti* treatment could reduce anopheline larval densities, and hence malaria risk.

Methods

Study Area

The study was conducted in 39 villages along the perimeter of Majete Wildlife Reserve in Chikwawa district (16º 1′ S; 34º 47′E), southern Malawi, as part of a community-based malaria control research trial, Majete Malaria Project (MMP). MMP was conducted in 65 villages along the perimeter of the wildlife reserve (McCann et al. 2017, Van Den Berg et al. 2018). All the study villages under MMP were divided into three sub-regions called focal areas A, B and C **(Fig. 1**) (Kabaghe et al. 2017). The initial plan was to conduct the current sub-study in 46 villages under MMP and spread across the three focal areas: 26 LSM and 20 non-LSM villages (**Table 1**). However, a community mapping exercise (**Fig. 2**) revealed scarcity of water-containing larval habitats in some villages and hence the study was effectively conducted in 39 villages: 26 LSM and 13 non-LSM villages.

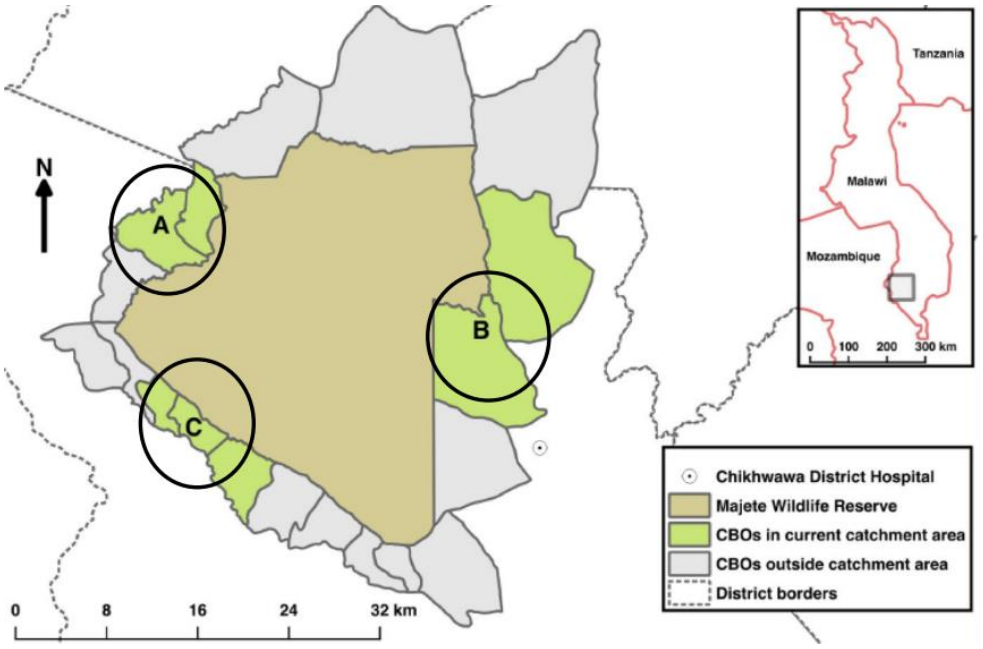

Fig. 1. Map of Majete Wildlife Reserve and the Majete Perimeter showing the three focal areas (Kabaghe et al. 2017).

Malaria is endemic in Chikwawa district (Bennett et al. 2013). The district is generally hot and dry from September to December, hot and rainy from January to April, and mild and dry from June to August. Situated in river valleys, the terrain in the study area is flat and receives surface water from highland areas North and West of the study area. The majority of people in the study areas raises livestock, including cattle,

goats and pigs, and practices subsistence farming, predominated by maize and millet cultivation. The availability of water, especially in the rainy season, and prolonged high temperatures create favourable humid conditions for mosquitoes in the area. The presence of rivers contributes to potential mosquito larval habitats in the area. In the dry season, small human-made water bodies are created for irrigation and as watering points for livestock. *Anopheles gambiae s.s., An. arabiensis* and *An. funestus* are the main vectors driving malaria transmission in Chikwawa district (Mzilahowa et al. 2012).

Description of community-led activities

In this study, a set of LSM activities were conducted by local communities. Per village, one or two volunteers (based on size of the village) called Health Animators (HAs) were selected by village heads and members of the community to coordinate the local malaria control initiatives (Van Den Berg et al. 2018). Prior to commencement of their work, the HAs received training from the MMP research team in collaboration with The Hunger Project-Malawi, Ministry of Health (Chikwawa District Health Office) and the African Parks on the topic of malaria through a tailored curriculum. In liaison with village heads and members of the general community, the HAs facilitated the selection of groups of between 10 to 12 volunteers per LSM village termed "LSM committees". These groups were tasked with coordinating all the LSM activities including mapping of all potential mosquito larval habitats in their villages, facilitating draining and filling of habitats, planning and conducting weekly *Bti*-treatment of all potential aquatic habitats holding water in their villages, and reporting to both the MMP research team and their communities via written reports and village workshops, respectively. It was the role of the general community to actually carry out larval habitat management through draining or filling of potential larval habitats. In May 2016, prior to the LSM activities, the LSM committees received trainings which focused on: (1) the role of mosquitoes in malaria transmission, (2) recognizing mosquito larvae, (3) the biology of mosquitoes, (4) breeding and mosquito larval habitats, (5) control of mosquitoes via disruption of larval habitats, (6) habitat draining, filling and larviciding as control tools, (7) activity of *Bti* as a larvicide, (8) operation of spraying machines, (9) *Bti*-water measurements and spraying, (10) tracking longitudinal changes on numbers and sizes of habitats containing water over time (11) intervention evaluation, (12) activity reporting and (13) planning. The training sessions incorporated many practical aspects where the participants were first introduced to the parts of a sprayer and its assemblage followed by preparation of *Bti*-water mixtures following guidelines by the larvicide manufacturers. The training sessions also involved actual spraying of mosquito larvae-infested habitats with predetermined amounts of *Bti* based on the area covered by water. Assessment of the efficacy of the spraying activities and reporting to the research team and their communities were the last sessions of the trainings. To effectively manage the activities and cover all potential mosquito larval habitats, the LSM committees in liaison with community members and HAs developed work plans and drew maps of

their villages detailing all the potential habitats **(Fig. 2).** Forms were developed to guide the committees in tracking habitat presence and size, and also to assess efficacy of the spraying activities on larval densities. After completion by the LSM committees, copies of the habitat-tracking forms were sent to a THP project officer who later forwarded them to the MMP field supervisor.

Evaluation of community executed larviciding

To assess the effect of community-implemented *Bti*-treatment on anopheline larval densities the MMP research team conducted independent larval density sampling surveys in *Bti*-treated water bodies in LSM villages, as well as in water bodies in non-LSM villages. In these surveys three water bodies per village were selected using a "spin-the-bottle" method **(Appendix A)** every two to three months (round). Because of dry spells prevalent during the study period, no or fewer than three habitats were effectively visited in some villages. This effectively reduced the number of villages visited from the planned 46 to 39. Five rounds of anopheline larval density surveys over a period of 13 months (April 2017 – May 2018) were undertaken in LSM villages while four rounds over a period of 11 months (July 2017 – May 2018) were conducted in the non-LSM villages. In round one, the larval density sampling was conducted in LSM villages only while rounds two to five were undertaken in both LSM and non-LSM villages.

To assess the effect of *Bti*-treatment on anopheline larval densities, the LSM committees conducted weekly surveys on three selected habitats per village before and after *Bti* application. A different set of three habitats per village was selected weekly for the assessment. *Bti* application was conducted every seven days in all aquatic habitats, regardless of presence or absence of anopheline larvae, to synchronize with mosquito larval development. Unlike in the LSM villages, one weekly survey was done per habitat in non-LSM villages owing to no *Bti* treatment. We assumed that consistent application of *Bti* would suppress anopheline larval populations over time in LSM villages hence comparisons between pre-spray larval densities in LSM villages and larval densities in non-LSM villages could be made. Every selected larval habitat in the LSM villages had a pre-spray survey done at least four days after the previous *Bti* application and either 1, 2, or 3 days before the next *Bti* application, and a post-spray survey done two or three days after the last application of *Bti.* The two surveys would respectively establish whether any larvae were present in the habitat before *Bti* was applied and determine whether the *Bti* effectively killed the larvae in the habitat. The number of larval sampling points at each habitat was dependent on the habitat's perimeter. One sampling point was selected for habitats with perimeters equal or smaller than 10m. Two and three samples were done at habitats with perimeters larger than 10m but less than 30m and larger than 30m, respectively.

For each sample, a circular aluminum tin, open on both ends, of 27 cm diameter and 45cm high was used. This 'area sampler' has been shown to be effective for sampling of larvae (Service 1993). Mosquito larvae and pupae were collected from within the area sampler using a 300ml dipper, fish net and pipette. The collected larvae were sorted into subfamilies. All anopheline larvae were further sorted into separate instar stages. Each habitat was geo-referenced during sampling. For each larval habitat, data were collected on water depth, permanence, and presence of aquatic vegetation. Water depth was reported as an average from three measurements performed at indiscriminately selected random positions along the edges and in the middle of the larval habitat. All the data were recorded on an Open Data Kit (ODK) form uploaded on a tablet.

Knowledge, attitude and practices survey of communities involved in larval control of malaria vectors

To understand the factors enhancing or hampering community participation in the LSM activities a Knowledge, Atttitude and Practice (KAP) survey was undertaken with community members from the 26 LSM trial villages. This survey was not conducted in the non-LSM villages. Data were collected through a standard structured questionnaire developed in English and uploaded on a Samsung® tablet **(Appendix B).** Two different groups of respondents were enrolled in the study: 1) HAs and LSM committee members, who had all received training directly from the project; and 2) members from the general community. For each LSM village, participants were systematically selected from a randomized list of household heads. Any member of the household present at the time who was older than 18 years was asked to participate. If eligible participants were not available or present in a selected household, an eligible participant was sought from the nearest neighboring house. To ensure sufficient representation of the HAs and LSM committee members in the study, five LSM committee members and all HAs from each LSM village were included in the interviews. The tablet-based question guides were administered by trained research assistants in the local language, Chichewa, and entered in English. The question guides included questions on demographic features, knowledge on malaria, mode of transmission, symptoms, possibility of vector control and methods, and motivation and participation in LSM activities. Prior to commencement of data collection, one day of training was conducted for research assistants to familiarize them with questions in the questionnaire. Following this, a one-day field pilot was organized to practice the questionnaires in a real-life setting and to adjust the questions accordingly.

Ethical Statement

The KAP survey was carried out in conformity with the principles of human subjects' protection. Ethical approval was obtained from the College of Medicine Research and Ethics Committee (COMREC; protocol number P.12/17/2222). Before commencement

of data collection activities, key gatekeepers were sensitized and informed on the purpose of the study and permission was sought from chiefs for entry into the community. The participants were clearly informed on the purpose of the study, as well as on potential risks and benefits of participating in the study. The participants were further informed about their rights to participate in the research, including the right to refuse, participate or withdraw from participation without negative consequences. Written informed consent was obtained from all participants during data collection.

Data analysis

Mann-Whitney U tests were used to compare the larval densities between non-LSM and LSM villages during pre-*Bti* spray surveys in rounds 2 to 5. To compare larval densities during pre- and post *Bti* spray surveys in LSM villages, non-parametric Wilcoxon rank sum tests were employed. To investigate whether there were differences in larval densities among the different rounds, a non-parametric Kruskal Wallis Test was employed. For the KAP survey data, the responses to open questions of the questionnaire were coded after completion of the survey. Chi-square tests (χ^2) were used to examine whether the distribution of individuals among the categories of one variable were independent of their distribution among the categories of another. Multivariate binary logistic regression analyses of participants' responses and characteristics were used in a backward stepwise approach to explain variations in respondents' motivation and participation in the community-led LSM. All data were analysed using SPSS version 20.

Results

Community management of potential mosquito larval habitats

The monitoring of larval habitats by the larger community based on reports by the LSM committees was highly irregular in many of the villages **(Fig. 3)**. Based on weekly site visits by the research team during evaluation of community executed larviciding, *Bti* application by the trained LSM committees remained uninterrupted in most villages than the other LSM components executed by the general community.

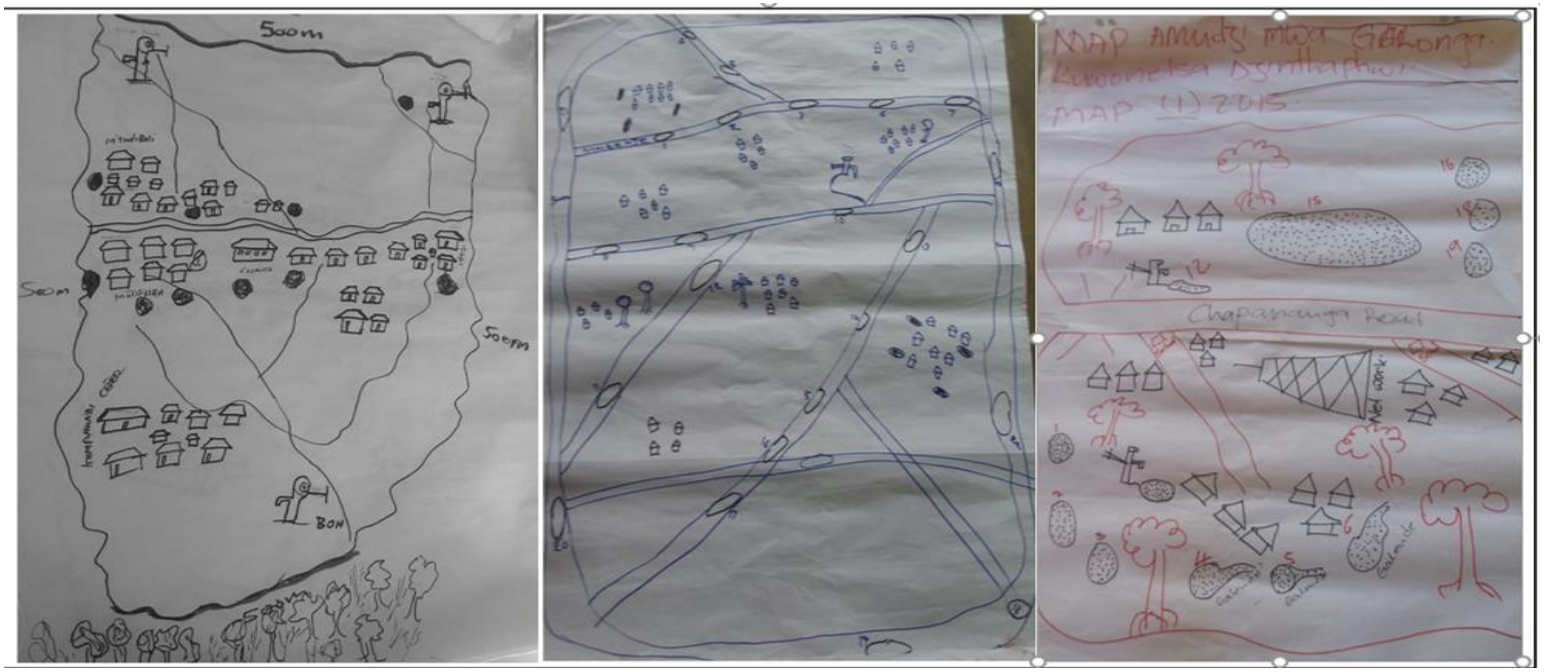

Fig. 2: Community participatory mapping of mosquito breeding sites

Village	2016							2017												2018	
	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2
Sauti	*			*			*			*			*			*	*				
Kampaundi	*			*						*			*			*			*		
Simoni (Kabwatika)	*			*			*			*			*			*					
Zabuka	*			*			*			*						*			*		
Mwenje	*																				
Esinele		*																			
Kandeu 2	*	*	*					*	*												
Kandeu 1	*	*	*	*		*											*	*	*		
Kuzambo	*							*	*							*					
Liwonde	*			*																	
Maganga 2	*		*			*		*										*			
Chipula/Tsekera	*																				
Galonga	*																				
Chamdire	*						*	*		*	*	*	*	*	*	*	*	*	*		
Kalinjala	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		
Jana	*							*		*			*			*					
Mkangeni	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*		
Mkadana 1	*												*								
Mkadana 2	*			*				*	*				*	*		*				*	
Chigwata 1/Dominic	*			*			*			*			*	*		*					
Mikola	*							*					*								
Mtayamanja	*												*								*
Chatenga/Tsiku	*										*		*								
Bzyakulimalima	*		*							*		*	*	*	*	*					
Novu/Alimoso/Theka/Felo	*			*					*							*					*
Ngirazi/Harrison	*			*			*			*		*	*			*			*		

Fig. 3: Frequency of larval habitat tracking based on self-reporting by the LSM committees. The habitat tracking reports listed each habitat in the village along with the size of the habitat and whether it had water or vegetation. The numbers below year columns correspond to months of a 12 months year. * indicates that habitats were tracked in that month. The weekly *Bti* treatment continued throughtout but is not shown in this figure.

Table 1: Distribution of study villages across the study area

Study village	Focal area		
	A	**B**	**C**
LSM	5	7	14
Non LSM	3	5	5

Many communities frequently reported longitudinal changes in the number and area covered by aquatic habitats **(Table 2),** and these data reflected the seasonality of rainfall and differences in reporting by LSM committees. For example, the mean area covered by the aquatic habitats was higher in the 2016 and 2017 rainy months of January to March than in the same years' dry months of June to September, 45,334m^2 and 24,321m^2, respectively. Almost all reports about the presence of habitats in June 2016 and the vast majority of surface area came from a single village in March 2016, which suggested that there were differences in reporting by LSM committees.

Table 2: Community tracking of potential mosquito breeding sites, expressed as surface area of water covered

LSM village	No. habitats	Longitudinal changes in number and size (m2) of *Bti*- treated habitats																					
		2015		2016										2017									2018
		Dec		Jan		March		June		Sept		Dec		Jan		March		June		Sept		Dec		Jan	
		No	Area	No	Area	No	Area	No	Area	No	Area	No	Area	No	Area	No	Area	No	Area	No	Area	No	Area	No	Area
Kabwatika	7	7	103			7	103	4	83	2	51					4	84	3	83						
Zabuka	8	6	254			4	116	5	61	4	153	8	468			5	176					8	404		
Sauti	6					6	12	5	12	3	12	2	12			6	12	0	NA	0	NA				
Kampaundi	3							2	53	0	NA	0	NA			3	25	0	NA	1	4	1	4		
Mwenje	2							2	8																
Kandeu 1	29							17	1875	14	1872									0	NA				
Kandeu 2	34			18	472	18	412	15	249	10	241														
Esinele	18					18	215	18	453	14	246														
Kuzambo	25							18	1607					9	209	8	193			10	325	12	244		
Liwonde	33							18	2044																
Maganga 2	96							8	79	8	83			15	143							5	34		
Chipula	26							11	10336																
Jana	25							18	1567							25	2560	9	3133	2	90				
Mkangeni	24					3	147			3	2346	2	246			6	3040	2	246						
Mkadana 1	12					11	4869	7	1664			12	3423	8	3545	9	3824	6	4486						
Mkadana 2	11					6	26224			3	18			10	31485									11	32214
Kalinjala	23					9	1057	11	1084	6	902	8	1070			8	1074	8	1061	8	1050	8	1100		
Chamdire	9			9	186									9	302			5	110	5	164	9	301		
Galonga	53							10	3551																
Bzyakulimalima	10							10	1552			2	220	2	75										
Mikola	24							3	280	5	760			3	520			2	145	2	139				
Chatenga	37																	2	220						
Mtayamanja	25					6	678	0	NA									3	362	2	37			13	579
Ngirazi	38							0	NA	3	196	3	207									9	1386		
Novu	5	0	NA					5	2060	3	760									0	NA			2	328
Chigwata2	20					17	8909	0	NA	0	NA	20	9006					10	678	3	51				

In Table 2 blank cells indicate that the habitats were not visited or visits were not reported.

Anopheline larval surveillance

Between April 2017 and May 2018, a total of 561 visits were made to 39 villages to monitor anopheline larval densities. **Fig. 4** gives details of the anopheline larval densities during the pre- and post *Bti* spray surveys across the five rounds in LSM villages. Significant reductions (W = 24681, $p = 0.004$) in larval densities were observed in post-spray surveys across the five rounds of sampling (**Fig. 5-A)**. The median value of larval densities per round was zero.

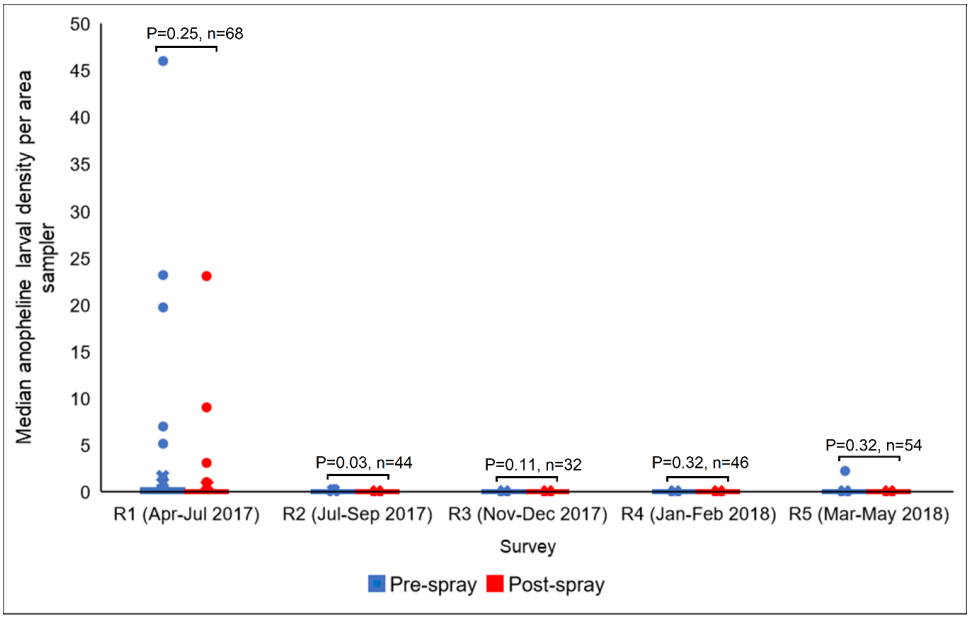

Fig. 4: Anopheline larval densities during pre- and post-*Bti* spray surveys across the five rounds of anopheline larval sampling in the villages that participated in the LSM trial arm. No anopheline larvae were found during post spray surveys in rounds 2-5. Dots in the figure are outliers.

No larvae were found during post-spray surveys in rounds 2 to 5, hence no differences in densities were found between the rounds. Differences in larval densities were observed among the different rounds only when round one was included in the analysis ($p = 0.003$). A Bonferroni Post-Hoc test showed that the pre-spray larval densities in round one were significantly higher than those of the other individual four rounds: $p = 0.032$, $p = 0.048$, $p = 0.033$ and $p = 0.021$ for rounds 2 to 5, respectively. No differences in pre-spray larval densities were found among the other rounds.

A Mann-Whitney U test showed that there were no significant differences in anopheline larval densities between the non-LSM and LSM villages during the pre-spray surveys in rounds 2 to 5 (U = 8636, $p = 0.554$) **(Fig. 5B).** Per round, the median value of larval densities was zero.

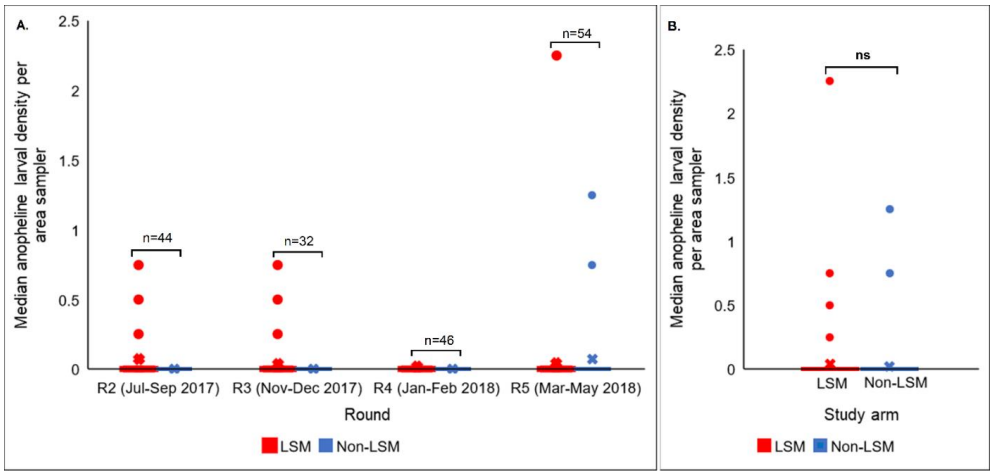

Fig. 5: Anopheline larval densities during pre-spray surveys in LSM and non-LSM villages across rounds 2-5: (A) larval densities per round and (B) larval densities across the rounds during pre-spray surveys in LSM villages and non LSM villages. Dots in the figure are outliers.

Community knowledge, attitudes and practice in malaria control via LSM

Demographic characteristics

Table 3 describes the characteristics of the participants of the sociological study conducted in LSM villages only. The majority of participants, 44.2%, belonged to the age group 26 to 40 years. Most of the participants were females (60.8%), had primary school education (55.8%) and engaged in subsistence farming as their main occupation (67.5%). Not surprisingly, the significant differences between the two groups suggest that HA and LSM committee members were not representative for the general community, as they were younger, higher educated and less unemployed (Table 3).

Table 3. Summary of socio-demographic characteristics of the study participants. The chi-square tests indicate (significant) differences between the participants from the general community and Health Animators+LSM committee members.

Characteristics	Participants from the general community	Health Animators+LSM committee members
Total participants	328	174
Gender ($\chi^2 = 3.483$; df = 3; $p = 0.062$)		
Male	119 (36.3)	78 (44.8)
Female	209 (63.7)	96 (55.2)
Age ($\chi^2 = 18.261$; df = 3; $p < 0.001$)		
18-25	94 (28.7)	47 (27.0)
26-40	126 (38.4)	96 (55.2)
41-64	81 (24.7)	27 (15.5)
65+	27 (8.2)	4 (2.3)
Education (Fisher's exact = 13.062; p =0.002)		
None	99 (30.2)	28 (16.1)
Primary	171 (52.1)	109 (62.6)
Secondary	57 (17.4)	37 (21.3)
Tertiary	1 (0.3)	0 (0.0)
Occupation (Fisher's exact = 9.958; p = 0.039)		
None	19 (5.8)	3 (1.7)
Manual labor	51 (15.5)	23 (13.2)
Farmer	214 (65.2)	125 (71.8)
Business	33 (10.1)	22 (12.6)
Formal employment	11 (3.4)	1 (0.6)

Perceived susceptibility of malaria

Based on responses about who they perceived to be most at risk of malaria, 59.6% and 55.6% of participants from the general community and HAs+LSM committee members, respectively, cited children aged under five years (children < 5) to be the most at risk **(Table 4).** Pregnant women were considered the second most susceptible group with 22% and 28% of participants from the general community and HAs+LSM committee members, respectively, mentioning this risk group.

Table 4. People perceived to be the most at risk of malaria. The chi-square tests indicate significant differences among participants from the general community and Health Animators+LSM committee members.

Characteristic	Frequency of responses (%)	
	Participants from the general community	Health Animators+LSM committee members
Total number of responses	403	257
Children <5 ($\chi^2 = 5.107$; df = 1; $p = 0.024$)	240 (59.6)	143 (55.6)
Youth < 15 ($\chi^2 = 0.209$; df = 1; $p = 0.648$)	1 (0.2)	1 (0.4)
Women ($\chi^2 = 2.244$; df = 1; $p = 0.134$)	8 (2.0)	1 (0.4)
Pregnant women ($\chi^2 = 20.240$; df = 1; $p < 0.001$)	73 (18.1)	72 (28.0)
Adults ($\chi^2 = 0.209$; df = 1; $p = 0.648$)	1 (0.2)	1 (0.4)
Elderly ($\chi^2 = 0.003$; df = 1; $p = 0.956$)	11 (2.7)	6 (2.3)
Farmers ($\chi^2 = 1.432$; df = 1; $p = 0.231$)	2 (0.5)	3 (1.2)
Those not using preventive measures ($\chi^2 = 1.615$; df = 1; $p = 0.204$)	15 (3.7)	4 (1.6)
Those with a weak immune system or AIDS ($\chi^2 = 0.419$; df = 1; $p = 0.517$)	2 (0.5)	2 (0.8)
Those living close to mosquito aquatic habitats ($\chi^2 = 1.065$; df = 1; $p = 0.302$)	2 (0.5)	0 (0.0)
Non-residents ($\chi^2 = 25.157$; df = 1; $p < 0.001$)	0 (0.0)	13 (5.1)
Anyone ($\chi^2 = 7.407$; df = 1; $p = 0.006$).	45 (11.2)	10 (3.9)
No idea ($\chi^2 = 0.166$; df = 1; $p = 0.648$)	3 (0.7)	1 (0.4)

Knowledge of mode of spread of malaria

Mosquito bites were mentioned by 98% of all respondents as the mode through which malaria is spread. Fly bites, soaking in rain and witchcraft, and no idea were incorrect responses from the remaining 2% of respondents. Statistically, responses by the two respondent groups were not different, χ^2=0.626, df=1, p = 0.429.

Of the 98% respondents who mentioned mosquito bites as the mode of spread of malaria, 58% correctly mentioned female anophelines as the vector. Differences in this knowledge were observed between the two respondent groups (χ^2 = 99.129; df = 2; p < 0.001) with more HAs+LSM committee members (86.8%) than participants from the general community (43.3%) having that knowledge.

Vector control

Ninety-six percent of all respondents felt that mosquitoes can be controlled. Of those participants who believed otherwise, 5% and 0.6% were participants from the general community and HAs+LSM committee members, respectively. These differences were significant (χ^2 = 6.984; df = 1; p = 0.008). Among the reason as to why some participants did not believe that mosquitoes can be controlled were: (1) it is difficult to locate all mosquito larval habitats, (2) there are insufficient preventive measures available, (3) preventive measures are inefficient and (4) the level of community involvement in vector control initiatives is never adequate.

Knowledge of mosquito control methods

With regard to knowledge or experience with mosquito control methods, the responses of participants from the general community differed from those of HAs+LSM committee members (χ^2 = 41.043; df = 4; p < 0.001). For example, 4.9% of the participants from the general community listed incorrect methods such as cleaning the house and clearing bushes. No HA+LSM committee member listed an incorrect method.

Knowledge of mosquito larvae

Significantly more HAs+LSM committee members mentioned their ability to recognise mosquito larvae (95.9%) than participants from the general community (31%) (χ^2 = 194.117; df = 1; p < 0.001). Further, significantly more HAs+LSM committee members (90.2%) than members from the general community (0%) mentioned to be able to distinguish culicine from anopheline larvae: χ^2=431.838, df=1, p < 0.001.

Knowledge of mosquito larval habitats

When asked where mosquito breeding takes place in their communities, a range of potential larval habitat types were cited **(Table 5).** Interestingly, human-made habitats such as borehole run-offs, dams, brick pits, wells and pit-latrines were the most mentioned larval habitats. Ordered in terms of frequency of responses, borehole run-

offs and dams were most often mentioned. Wells and pit latrines were the second and third most mentioned habitat types even though the latter are not anopheline breeding sites. The responses from the two respondent groups were significantly different about borehole run-offs ($\chi^2 = 9.059$; df = 1; $p = 0.003$), dams ($\chi^2 = 7.096$; df = 1; $p = 0.008$) and wells ($\chi^2 = 6.551$; df = 1; $p = 0.010$) **(Table 5)**.

Asked about the preferred environment for mosquito breeding, 89% of participants from the general community and 80% of the HAS+LSM committee members considered standing water to be the preferred environment for mosquito breeding.

Table 5. Knowledge of anopheline breeding habitats. The chi-square tests indicate significant differences among participants from the general community and Health Animators+LSM committee members.

Characteristic	Frequency of responses (%)	
	Participants from the general community	Health Animators+LSM committee members
Total responses	767	466
Pit latrine ($\chi^2 = 0.193$; df = 1; $p = 0.660$)	79 (10.3)	45 (9.7)
Rice paddies ($\chi^2 = 2.139$; df = 1; $p = 0.144$)	4 (0.5)	0 (0.0)
Wells ($\chi^2 = 6.551$; df = 1; $p = 0.010$)	83 (10.8)	63 (13.5)
Drainage channels ($\chi^2 = 0.751$; df = 1; $p = 0.386$)	30 (3.9)	12 (2.6)
Borehole run-offs ($\chi^2 = 9.059$; df = 1; $p = 0.003$)	133 (17.3)	47 (10.1)
Dams ($\chi^2 = 7.096$; df = 1; $p = 0.008$)	97 (12.6)	72 (15.5)
Stream beds ($\chi^2 = 2.676$; df = 1; $p = 0.102$)	59 (7.7)	42 (9.0)
Freshwater marshes ($\chi^2 = 0.007$; df = 1; $p = 0.934$)	67 (8.7)	35 (7.5)
Tyre tracks ($\chi^2 = 0.005$; df = 1; $p = 0.945$)	4 (0.5)	2 (0.4)
Brick pits ($\chi^2 = 1.023$; df = 1; $p = 0.312$)	89 (11.6)	40 (8.6)
Construction ditches ($\chi^2 = 7.862$; df = 1; $p = 0.005$)	22 (2.9)	25 (5.4)
Hoof prints ($\chi^2 = 24.41$; df = 1; $p < 0.001$)	2 (0.3)	16 (3.4)
Ponds ($\chi^2 = 0.546$; df = 1; $p = 0.460$)	14 (1.8)	10 (2.1)
Rain pools ($\chi^2 = 0.070$; df = 1; $p = 0.792$)	48 (6.3)	27 (5.8)
Run-off from natural source ($\chi^2 = 1.349$; df = 1; $p = 0.246$)	9 (1.2)	2 (0.4)
Water storage containers ($\chi^2 = 22.457$; df = 1; $p < 0.001$)	10 (1.3)	25 (5.4)
Tree holes ($\chi^2 = 0.532$; df = 1; $p = 0.466$)	1 (0.1)	0 (0.0)
Bathroom run-offs ($\chi^2 = 1.848$; df = 1; $p = 0.174$)	13 (1.7)	3 (0.6)
Any place with water ($\chi^2 = 0.532$; df = 1; $p = 0.466$)	1 (0.1)	0 (0.0)
No idea ($\chi^2 = 1.065$; df = 1; $p = 0.302$)	2 (0.3)	0 (0.0)

Perception on habitat creation and importance

When asked about the preferred environment for mosquito breeding, 89% of participants from the general community and 80% of the HAS+LSM committee members considered standing water to be the preferred environment for mosquito breeding. The responses invited further questions prompting whether the standing water served any purposes in the communities. **Table 6** summarizes the responses of the respondents on habitat creation and importance. Forty-four percent and 55% of participants from the general community and HAs+LSM committee members, respectively, indicated that the sites are human-made. As regards importance, 37.5% and 43% of participants from the general community and HAs+LSM committee members, respectively, considered the habitats important. Of those participants in the former group who attached importance to the habitats, 67%, 29% and 4% related the habitats to domestic, agricultural and brick making purposes, respectively. As for the HAs+LSM committee members, slightly over half (51%) of those who felt that the habitats were important related the importance to domestic purposes. Three percent and 46% of the HAs+LSM committee members associated the habitats with brick making and agricultural purposes, respectively. Overall perception about habitat importance did not differ significantly in the two respondent groups ($\chi^2 = 1.495$; df = 1; $p = 0.222$).

Table 6. Perception on larval habitat importance and creation. The chi-square tests indicate significant differences among participants from the general community and Health Animators+LSM committee members.

Characteristic	Numbers (and percentage) of responses	
	participants from the general community	Health Animators+LSM committee members
Habitat importance ($\chi^2 = 1.495$; df = 1; $p = 0.222$)		
Total responses	146	104
Domestic purposes ($\chi^2 = 0.018$; df = 1; $p = 0.892$)	98 (67.1)	53 (51.0)
Agricultural purposes ($\chi^2 = 16.053$; df = 1; $p < 0.001$)	42 (28.8)	48 (46.1)
Brick-making purposes ($\chi^2 = 0.007$; df = 1; $p = 0.933$)	6 (4.1)	3 (2.9)
Habitat creation (X2 = 5.476; df = 1; $p = 0.019$)		
Total responses	155	113
Domestic purposes ($\chi^2 = 2.688$; df = 1; $p = 0.101$)	73 (47.1)	28 (24.8)
Agricultural purposes ($\chi^2 = 19.100$; df = 1; $p < 0.001$)	23 (14.8)	35 (31.0)
Brick-making purposes ($\chi^2 = 7.726$; df = 1; $p = 0.005$)	59 (38.1)	50 (44.2)

Factors influencing motivation and participation in LSM

Multivariate binary logistic regression analysis based on a set of variables (sex, level of education, source of Income, knowledge about malaria symptoms, knowledge of

people at risk, mode of spread, possibility to control mosquitoes, knowledge of mosquito control methods, knowledge of vector, ability to recognise larvae, ability to distinguish larvae, knowledge of anopheline breeding environment,knowledge of anopheline breeding site, pereceived importance of anopheline breeding sites, knowledge of origin of breeding sites, knowledge of larval habitat management method, awareness of *Bti*, knowledge of effects of *Bti*) showed that for the participants from the general community, motivation in LSM was driven by their knowledge of *Bti* as a mosquito control tool (Wald=0.253; df=1; $p < 0.001$) while the HAs+LSM committee members were motivated by the ability to recognise mosquito larvae (Wald=9.841; df=1; $p = 0.002$).

With regard to participation in the LSM activities, binary logistic regression with the same set of variables as above showed that participants from the general community were influenced by knowledge of mosquito aquatic habitats (Wald=5.057; df=1; $p = 0.025$) and knowledge of *Bti* as a mosquito control tool (Wald=20.286; df=1;$p < 0.001$). For the HAs+LSM committee members, participation in LSM activities was driven by their ability to recognise mosquito larvae (Wald=11.55; df=1; $p = 0.001$).

For the LSM committee-executed *Bti* application, logistic regression utilising same variables as in above cases showed that the ability to recognise larvae was the only predictor that influenced both motivation and participation, Wald=5.074; df=1; $p = 0.024$ and Wald =5.052; df=1; $p = 0.025$, respectively.

Discussion

Reporting of LSM activities, i.e. habitat draining and filling, by the LSM committees was irregular in most of the villages. The weekly *Bti* treatment of habitats, however, remained uninterrupted in most villages. Participation of the community-at-large in habitat draining and filling activities as part of LSM was low and did not reflect the impressive knowledge the community had about malaria as their major health risk and malaria vectors, their larval habitats and control. The LSM committee members and HAs exhibited, overall, higher motivation and participation in the LSM activities. The *Bti* applications were effective in controlling larval densities within water bodies, as evidenced by larval density reductions during post-spray surveys in relation to pre-spray surveys. The repeated weekly applications were associated with a decline in anopheline densities in villages. The ability to recognise mosquito larvae in water bodies, knowledge about mosquito aquatic habitats and about *Bti* as a mosquito control tool influenced both motivation and participation in the community-led LSM.

Although habitat draining and filling were part of the LSM activities under MMP these were, in practice, not applied as much by the community. It was observed that *Bti* application was the most preferred component of LSM, presumably because it was carried out by motivated LSM committees or because it was deemed to be relatively easy to execute in comparison with draining and filling activities. The extent to which the community participated in these activities is unclear as it was hampered by a lack

of reporting by the LSM committees. It is worth noting that the lack of reporting did not necessarily mean lack of action. It was observed by the MMP research team during the larval density surveys that the committees did not consistently document details about habitats treated with *Bti* despite taking such actions. For example, in one village, Esinele, no reports were written because there was no literate member in their LSM committee.

We observed that the weekly treatments of habitats with *Bti* were associated with reduced anopheline larval densities in LSM villages. Over the five rounds of larval sampling, significantly lower anopheline larval densities were recorded in the post than pre-spray surveys, which indicated reductions due to treatment. These results are likely due to continued suppression of larval populations in the habitats by the weekly *Bti* treatment, which also reduced adults emerging from the sites over time. *Bti* has shown to reduce larval and consequently adult populations (Fillinger et al. 2003, Shililu et al. 2003, Fillinger and Lindsay 2006, Majambere et al. 2010, Dambach et al. 2014, Djènontin et al. 2014). Non-larvicidal effects of *Bti* have also been observed and these have been shown to result in adults with a reduced fitness (Flores et al. 2004, Wang and Jaal 2005, Zahiri and Mulla 2005, Simsek et al. 2009).

No differences in anopheline larval densities were observed between non-LSM villages and LSM villages during pre-spray surveys. The comparison of pre-spray survey densities in LSM villages with the densities in non-LSM villages was based on the assumption that consistent and repeated weekly applications of *Bti* would induce longitudinal reductions in the anopheline larval densities and that the effects would be reflected even during subsequent pre-spray surveys. This absence of differences in larval densities could be attributed to (1) the high LLINs coverage following mass distribution of LLINs in 2016 in the study villages which might have suppressed vector populations overall, and (2) the unusually low precipitation experienced during the study period which might have reduced the number of habitats containing water and also larval populations. Another, third option may be the result of our experimental design: short distances between LSM and non-LSM villages might have allowed mosquitoes to fly between the villages. With this reasoning, reductions in larval and also adult populations in the LSM villages could thus potentially reduce mosquito populations in the nearby non-treatment villages. Anopheline mosquitoes fly further than the 500m set in this project with the aid of wind (Manoukis et al. 2011) though the majority will stay close between water bodies and houses where suitable hosts reside.

Our results from the KAP survey indicate that a lack of participation in the LSM activities did not result from lack of knowledge about the malaria topic. The majority of participants from the LSM villages had sufficient knowledge about people most at risk of malaria, its spread, vector larval habitats and control efforts. It was also very clear that, based on results of the current study, the community realised that vector control is possible. Despite all this knowledge, there was unimpressive participation

by members of the community in the habitat draining and filling activities. One of the reasons for not removing water bodies may have been the important functions the habitats served, as also revealed by our KAP survey. In this study the five most mentioned habitat types were human-made, and most of them served domestic and agricultural purposes. Similar conflicting interests were observed in Kenya (Imbahale et al. 2010, 2012) where communities were not willing to remove sites they deemed important for their livelihoods. Apart from the use of *Bti*, which is not very readily accessible by communities, other alternative larval control interventions which reduce larval densities without removing the water sources need to be explored (Takken and Knols 2009).

The HAs+LSM committee members were the most active groups in the LSM activities. Their motivation and participation were associated with their ability to recognise mosquito larvae which probably increased awareness of the risk of malaria and the need to manage the breeding sites. The role played by these specially trained members of the community might have also influenced their participation. The LSM curriculum developed by the MMP for LSM committees emphasised understanding of the malaria topic and also gaining leadership skills for proper execution and management of the intervention. It was clear that the tailored trainings given to the LSM committee members instilled both knowledge of the malaria risk and also sense of ownership of the intervention. This, to a greater extent, set the committee members apart from the other members of the community. It could, thus, be suggested that to successfully implement community-led disease control initiatives, investment should be directed towards making selected groups of people more aware of their problems as this would promote both sense of ownership of the initiative and also participation.

The current study was limited by the drought that was prevalent during the study period and which reduced larval habitats and also larval densities. This limits the extent to which we can attribute weekly applications of *Bti* in larval habitats to reductions in larval densities. Also, the distances between treatment and non-treatment villages in our study might not have been sufficient to prevent movement of mosquitoes between villages, hence also not sufficient to detect significant effects of the *Bti* treatment applied.

Conclusions

Community members who received tailored training about LSM from the project team were more motivated and active in LSM activities than members from the general community who received training from their project-trained counterparts. Knowledge about mosquito aquatic habitats and *Bti* as a mosquito control tool, as well as the ability to recognise mosquito larvae were the main factors that influenced both motivation and participation in the LSM activities. Community-led LSM, particularly *Bti* treatment, was effective in reducing larval densities of malaria vectors in villages where the intervention was applied. The effects of community-led LSM may have also contributed to vector reductions in nearby villages not participating in the

intervention, although other reasons may have contributed to the absence of differences between LSM and non-LSM villages. Concentrating efforts in making communities more aware of their risk of malaria and their role in malaria proliferation through habitat creation could potentially reduce risk factors, suppress creation of larval habitats, and promote ownership and participation in malaria vector control.

Acknowledgements

The study was generously supported by De Vos Fund, The Netherlands. We also thank the Dioraphte Foundation for financial support. The content is solely the responsibility of the authors and does not necessarily represent the official views of the funders. RSM is supported by an NIH-funded postdoctoral fellowship (T32AI007524). We thank Prosper Chaki for his role in field training on *Bti* application to both LSM committees and research team. We thank all the research assistants for their support in data collection. Davies Kazembe, Happy Chongwe, Asante Kadama, Richard Nkhata and Tinashe Tizifa are thanked for logistical support. We also thank the people in the study area for their willingness to participate in the study.

Supplementary material

Appendix A: Spin-the-bottle method for selecting larval habitats

Eligible habitats: any habitat the LSM committee is spraying with *Bti*, and which has a minimum depth along at least part of the edge that is less than 30cm.

Selection of Habitat 1

- Start at center of village.
- Spin a glass bottle on the ground.
- The first habitat (which the LSM committee is spraying with *Bti*) in the direction the bottle is pointing is Habitat 1 for larval density sampling.
- The habitat does not have to be a minimum distance from the center of the village.
- If there isn't a habitat (which the LSM committee is spraying with *Bti*) in that direction, spin the bottle again at the center of the village.

Selection of Habitat 2

- At location of Habitat 1, spin a glass bottle on the ground.
- The first habitat in the direction the bottle is pointing, *farther than 50m from Habitat 1*, is Habitat 2 for larval density sampling.
- If there isn't a habitat (which the LSM committee is spraying with *Bti*) in that direction farther than 50m from Habitat 1, then spin the bottle again at the location of Habitat 1.
- If there isn't a habitat (which the LSM committee is spraying with *Bti*) farther than 50m in any direction, then the first habitat in the direction the bottle is pointing is Habitat 2.

Selection of Habitat 3

- At location of Habitat 2, spin a glass bottle on the ground.
- The first habitat in the direction the bottle is pointing, *farther than 50m from the both Habitat 1 and Habitat 2*, is Habitat 3 for larval density sampling.
- If there isn't a habitat (which the LSM committee is spraying with *Bti*) in that direction farther than 50m from Habitat 1 and Habitat 2, then spin the bottle again at the location of Habitat 2.
- If there isn't a habitat (which the LSM committee is spraying with *Bti*) farther than 50m in any direction, then the first habitat in the direction the bottle is pointing is Habitat 3.

Note: If there are only 3 habitats, or fewer, sample at all of them. There would be no need for random selection in this case.

Appendix B: Semi-structured questionnaire for participants in the LSM arm of the study (English version)

Village:
Demographic Features
Age (years)
18-25
26 - 40
41 - 64
65+
Gender
Male
Female
Education
Illiterate
Primary
Secondary
Graduate & above
Main Source of Income
Employed
Agriculture
Business
Manual labour
None
Knowledge of Malaria, Spread and Symptoms
Do you have any knowledge of malaria?
Yes
No
Have you ever suffered from malaria? (diagnosed malaria)
Yes
No
Has anybody you know ever suffered from malaria? (diagnosed malaria)
Yes
No
What are the common symptoms of malaria? (multiple choice)
Fever
Nausea
Headache
Body aches
Vomiting
Shivering
Diarrhea

What are the common symptoms of malaria? (multiple choice)
Convulsion
No idea
Who do you perceive to be the most at risk for malaria? (open question)
In your opinion, how do people get malaria?
Mosquito bite
Fly bite
Witchcraft
Soaking in rain
Other (specify)
No idea
Knowledge about malaria control
Do you think it is possible to control mosquitoes?
Yes
No
If yes, how do you think mosquitoes can be controlled? (multiple choice)
Smoke
Mosquito coils
Mosquito spray
Fan
Covering of body with clothes
Mosquito net
Skin repellents
Cleaning house
Indoor Residual spraying
Removing standing water
Clearing of bushes
Other (specify)
No idea
If no, why do you think it is not possible to control mosquitoes? (open question)
What is the name of the malaria vector?
Female Anopheles
Male Anopheles
Culex
Other (specify)
No idea
Knowledge about mosquito larvae
Are you able to recognise mosquito larvae?
Yes
No

If yes, are you able to distinguish anopheline larvae from culicine?
Yes
No
Knowledge about mosquito breeding
In what type of environment do anopheline mosquitoes like to breed? (multiple choice)
Running dirty water
Garbage/Trash
Standing clean water
Standing dirty water
Running Clean water
Plants/ vegetation
Other (specify)
No idea
What are common aquatic habitats for anopheline mosquitoes? (multiple choice)
Pit-latrine
Rice paddies
Wells
Drainage Channels
Bore hole run-offs
Dams
Stream Beds
Freshwater marshes
Tire tracks
Brick pits
Hoof print aggregations
Ponds
Rain Pools
Run-offs from natural sources
Other (specify)
[Explanation of the correct breeding habitats of anophelines by the research assistant: E.g. clean standing water in sites like borehole run-offs, wells, etc]
Creation and importance of mosquito breeding sites
Do you think that these aquatic habitats are important for your livelihood?
Yes
No
Why do you think that these aquatic habitats are important for your livelihood? (open question)
Do you create these sites yourself?
Yes
No

For what purposes do you create these sites? (open question)
Knowledge about mosquito larval control methods
Eradication of breeding site of mosquito (multiple choice)
Draining
Filling up
Changing water in storage tanks
Others (specify)
No idea
Are you aware of *Bti* larviciding?
Yes
No
Negative effects of *Bti* on spray operators
Many
Few
None
No idea
Negative effects of *Bti* on livestock
Many
Few
None
No idea
Negative effects of *Bti* on crops
Many
Few
None
No idea
Negative effects of *Bti* on crop consumers and water users
Many
Few
None
No idea
Participation in LSM activities (Draining & filling)
Active
Less active
Not at All
Motivation in LSM activities (Draining & filling)
A lot
Little
None

for LSM Committee members only
Participation in *Bti* spraying
Active
Less Active
Not at All
Motivation in *Bti* spraying
A lot
Little
None

Chapter 7

General Discussion

Introduction

Because of stalling progress in malaria control, scientists are exploring new strategies that have synergistic effects on the current tools to further reduce the malaria burden (Koenraadt and Takken 2018). In this regard, larval source management (LSM) is increasingly gaining attention as a complimentary strategy for vector control (Fillinger et al. 2009, Mwangangi et al. 2011, Imbahale et al. 2012, Djènontin et al. 2014, Mazigo et al. 2019).

The growing interest in LSM can be explained by the strategy's potential to reduce populations of behaviourally versatile and insecticide-resistant malaria vectors. While LSM is a viable complimentary strategy for malaria control (Tusting 2014), its adoption in many African countries has been slow, possibly due to lack of information on how and where it can be implemented, lack of sufficient evidence of efficacy and concern about operational costs. To exploit the potential of LSM, knowledge about local vector ecology is needed and to make it sustainable, we need to know how best to engage local communities. In Malawi, knowledge about local anopheline larval ecology is lacking and hence no policy exists on larval control. Also, there remains a knowledge gap about community involvement in malaria control initiatives. This, too, hinders the development of policy around involving local communities in control efforts. Community involvement in LSM could reduce implementational costs and increase intervention coverage. Engagement of communities in malaria control is likely to increase commitment for activities that are often long lasting and require personal effort and time.

The main objective of this thesis was to assess the effects of habitat characteristics and community-based LSM on anopheline larval ecology and population dynamics in Malawi. In the following sections, I discuss the possible implications of the results presented in the different chapters of this thesis. As indicated in Chapter 1, successful implementation of community-led LSM is conditional upon: 1) knowledge about vector ecology, 2) effectiveness of the components of LSM on larval populations and 3) the willingness of the communities to participate in larval control activities.

Characteristics of productive anopheline larval habitats in southern Malawi

With the growing attention for LSM as a complementary tool for malaria control there is a need for a clear understanding of anopheline larval ecology. This knowledge is crucial for both development and targeted deployment of larval control programmes. Targeted LSM could be both cost-effective and feasible, especially in settings where available aquatic habitats are numerous. An understanding of biotic and abiotic factors near larval habitats has important implications for establishing larval control tools.

Gravid female mosquitoes actively select aquatic sites for oviposition based on cues emanating from the sites (Lindh et al. 2008, Ponnusamy et al. 2008, Nazni et al. 2009, Day 2016). This has implications for the population dynamics of the mosquitoes in

different sites, with sites considered less suitable by the gravid females becoming less productive in terms of larval densities. For example, presence of predators in larval habitats deters gravid females from ovipositing in such sites (Blaustein et al. 2004, Munga et al. 2006). Abiotic factors such as water temperature, turbidity and pH also influence oviposition site selection by the gravid females mosquitoes (Bayoh and Lindsay 2003, Minakawa et al. 2005, Murrell and Juliano 2008, Dida et al. 2018). However, plasticity in oviposition site selection has been documented in gravid female anophelines, especially when no favourable oviposition sites are available (Huang et al. 2006). Understanding the larval ecology of local malaria vectors provides information about general characteristics of productive habitats, thus creating opportunities for targeted larval control. Therefore, Chapter 2 of this thesis characterised anopheline larval habitats on the basis of habitat ecology and anopheline larval productivity in Majete, southern Malawi, to provide a basis for larval control in the country.

The results showed that larval habitats were widespread in the study area, with high seasonal variation in suitability for mosquito larvae. The anopheline densities were higher in aquatic habitats with bare soil making up part of the surrounding land cover, and in aquatic habitats in which culicine larvae were developing. However, the densities were significantly lower in highly turbid habitats than in clearer ones, and presence of predators in the aquatic habitats significantly reduced the probability of anopheline larvae being present. The preference of anophelines for habitats surrounded by bare grounds in this study has also been observed in *An. gambiae* s.l. which utilize shallow temporary puddles over bare soil as aquatic habitats (Gimnig et al. 2001, Minakawa et al. 2005, Huang et al. 2006, Fillinger et al. 2009, Ndenga et al. 2011). The co-occurrence of anophelines and culicines as observed in this study may have been caused by selection of culicine occupied habitats by gravid anophelines to reduce their own predation rates as the presence of the culicines provides alternative prey for local predators. Also, cues emanating from culicine larvae in the habitats may signal the presence of a relatively safe environment for oviposition by gravid anophelines (Mwingira et al. 2019). The negative effect of high turbidity on anopheline larval densities observed in this study has also been observed in *An. gambiae* s.l. (Ye-Ebiyo et al. 2003). High turbidity reduces light penetration into water which reduces photosynthetic processes (Chirebvu and Chimbari 2015), microbial growth (Muturi et al. 2008) and water temperature. Temperature affects the metabolic rate, which in turn affects resource uptake and energy allocation for growth, development, reproduction and excretion (Brown et al. 2004). The reduced anopheline occurrence in predator-infested habitats may have resulted from direct preying of the larvae by the predators as well as by avoidance by gravid mosquitoes to oviposit in these habitats. In their selection of suitable oviposition habitats, gravid mosquitoes are able to detect cues emanating from predators which leads to discrimination between habitats with and without predators (Blaustein et al. 2004, Munga et al. 2006). Information about which habitats support anopheline larval development is very crucial as it can be used to

determine locations that can sustain residual malaria transmission. Therefore, our findings are valuable for targeted larval control which could be both cheaper and more manageable.

Non-repellent effect of *Bti* on oviposition-site selection in *Anopheles coluzzii* mosquitoes

Field applications of an endotoxin-producing bacterial larvicide, *Bacillus thuringiensis* var. *israelensis (Bti)*, as component of LSM can reduce *Anopheles gambiae* s.l. larval densities by up to 95% (Fillinger et al. 2003, Shililu et al. 2003, Fillinger and Lindsay 2006, Majambere et al. 2010, Dambach et al. 2014, Djènontin et al. 2014). Continued efficacy of the bacterial larvicide is dependent upon the inability of gravid females to detect treated habitats and discriminately oviposit in untreated habitats. Little is known as to whether *Bti*-treatment of larval habitats changes the suitability of the habitats either by enhancing or inhibiting oviposition by gravid females. This would modulate the effectiveness of the bacterial larvicide. Discrimination of the treated sites by female mosquitoes may reduce overall effectiveness of the intervention. In Chapter 3, dual-choice experiments were carried out with gravid *An. coluzzii* females to assess the effect of habitat treatment with *Bti* on selection of oviposition sites.

Our results suggested that treatment of sites with *Bti* does not induce avoidance of these sites by gravid females. This suggests that the females would still oviposit in treated habitats, thereby exposing their offspring to the larvicide. However, our sample size was very small hence the results do not provide solid evidence that treatment of sites with the larvicide does not repel gravid females from ovipositing in such sites. Also, the cages in which the dual-choice tests were conducted were relatively small, 30cm x 30cm x 30cm, which reduced the distance between treated and untreated cups, and may have prevented gravid females from clearly distinguishing between cups with *Bti* and the controls. For more conclusive results further experiments need to be undertaken under field conditions, in which the treated and untreated sites are placed at a greater distance from each other. If indeed the larvicide does not induce discrimination of oviposition sites by gravid females, infusing the larvicide with attractants would render its use more efficient thereby constituting an effective lure and kill strategy (Menger et al. 2016).

Reduced adult fitness due to larval exposure to sublethal doses of *Bti* in *Anopheles coluzzii*

Numerous studies have shown that *Bti* is effective in controlling immature aquatic mosquito stages (Fillinger et al. 2003, Shililu et al. 2003, Fillinger and Lindsay 2006, Majambere et al. 2010, Dambach et al. 2014, Djènontin et al. 2014). These results often come from expert-led initiatives in which recommended *Bti* doses are applied in larval habitats. In such cases, 1) sufficient skills about target-habitat size measurements, 2) proper sprayer calibration and spraying techniques and 3) knowledge of the effects of

habitat biotic and abiotic factors are available. An important question is whether campaigns advocating for community involvement in *Bti* spraying could improve coverage of the intervention but at the same time compromise on quality due to community's lack of expertise in determining effective doses in relation to target habitat sizes, sprayer calibration and spraying, as well as habitat ecological factors. Effectively, community involvement in *Bti* spraying activities could potentially result in exposure of larvae to sublethal doses. Therefore, Chapter 4 of this thesis assessed the effect of larval exposure to sublethal doses of *Bti* on body size, survival rates and oviposition as fitness measures of adult *An. coluzzii*.

Most importantly, adult survival was reduced for both females and males that had been exposed to the LC_{70} concentrations of *Bti* in the larval stage. The *An. coluzzii* adults that did emerge after exposure to LC_{70} concentrations, were observed to have longer wings than the unexposed control groups. No effects of the sublethal doses, while controlling for wing length of gravid females, were observed in oviposition rate. It is not very clear how exposure to *Bti* as larvae reduces longevity of adults as that has not been fully investigated. However, based on existing evidence on effects of a *Bti* toxin, Cry1C, to brain cells of larvae of a lepidopteran, *Lymantria dispar,* in vitro (Cerstiaens et al. 2001), I suggest that the larvicide causes similar irreparable damage in mosquito larvae which results in reduced functioning and thus longevity in adult life. With regard to the observed increase in wing length due to larval exposure to the biocide, it remains unclear whether this resulted from activity of the toxins released by the larvicide or due to reduced resource-competition in treated trays where larval densities were reduced as a result of the killing effect of *Bti*. Similar effects can be expected to occur if *Bti* is applied under natural conditions, in which sub-lethal doses in the treated site can be the result of, for example, inadequate treatment by applicants or dilution with rain water.

These data, therefore, demonstrate that sublethal *Bti* doses can lead to an additional reduction in vectorial capacity (on top of larval mortality) for malaria vector populations by increasing mortality of adults that survived exposure to *Bti* in their larval stage. These results may take away concerns about community involvement in *Bti* larviciding as the effects of the sublethal doses, which are very likely to occur with community involvement in the larviciding activities, still negatively impact the vectors. However, the findings do not do away with the need to adhere to *Bti* doses that kill all larvae as those doses have instant impact in reducing vector populations and consequently the malaria burden.

Lessons learnt from involving communities in LSM in Chikwawa District, Southern Malawi

Although there is a growing realisation that LSM could be a complimentary strategy for malaria control, it should be noted that LSM seems most feasible where habitats are few, fixed and findable (WHO 2013b). Therefore, in settings where the conditions

for carrying out LSM cannot be met, operational success of the intervention may be conditional upon involvement of local communities, especially because they are most aware about where potential larval habitats are located. For effective community involvement, knowledge about the factors that may influence community acceptance and participation in LSM is very important as it could inform best practices for scale-up of community-led initiatives.

In Chapter 5 we explored factors that influence community acceptance and participation in community-led LSM for malaria control in the Majete area, southern Malawi. This research demonstrated that in the study areas, involvement of communities in the community-led LSM increased community awareness of malaria as their most important health risk, as well as acceptance of LSM as a tool that can reduce vector populations and hence, malaria risk. Similar results were observed in Madagascar where community-based distribution of long-lasting insecticide-treated nets (LLINs) improved household net ownership and population access beyond Roll-Back Malaria (RBM) targets after a 9-month community distribution pilot (de Beyl et al. 2017). These results highlight how community involvement can contribute to increased intervention coverage. It has been argued that implementing LSM using the community-approach is challenging due to requirement for consent and cooperation of the community (Dambach et al. 2018). Indeed, in our study, participation in LSM activities by members from the community-at-large was lower than of the selected groups, HAs and LSM committee members. For those who participated in the activities, participation was characterised by intense time and labour demands, and differential perceptions about incentives by the different groups in the community. Therefore, the results do not only indicate that community involvement is feasible, but also highlight the need to make the community-based activities less demanding. The impressive knowledge about the malaria topic by communities in this study showed that involving communities has potential to promote participation in malaria control efforts. For example, the high community knowledge about mosquito larval habitats can easily be directed towards action against habitat creation. With clear understanding of their risk factors for malaria, communities may be driven to take action without relying on monetary incentives. In the study area, numerous relief organisations give out money and other forms of relief which may contribute to the dependency syndrome (Harvey and Lind 2005). To counter this dependency, community-led projects need to foster dialogues with other projects within the study areas to promote community empowerment in ways that do not involve promoting dependency on aid.

In future intervention strategies, incorporating disease risk factors and how these can be reduced at local community level in health messages is relevant and will have a greater impact if all stakeholders including the government, non-governmental organizations and research organizations are involved in awareness campaigns. Most importantly, future intervention strategies must ensure that the communities are fully

informed about the impact of diseases on their livelihoods and the strategies that can be employed to restrict spread of the diseases.

Feasibility of community-led LSM

In Chapter 6, the potential of engaging communities in malaria control via LSM was assessed. Entomological surveys were used to assess the impact of community-executed LSM on anopheline larval control, while questionnaires were addressed to investigate factors influencing the community's actual participation in the community-led LSM. The findings of the study showed that community engagement in LSM increased awareness of malaria risk factors and its control and that *Bti* larviciding might have contributed in reducing anopheline larval densities in larval habitats. Further, the results showed that community action and reporting of the LSM activities undertaken was irregular.

The findings establish that community involvement in LSM is not only feasible but also has potential to control malaria vector populations. Similarly, the feasibility of community involvement in LSM was demonstrated in Rwanda (Ingabire et al. 2017). By involving communities, a number of key determinants of successful community-driven initiatives are exploited which promote (1) coverage of targeted areas through communities' comprehensive knowledge of larval habitats, (2) reduction in costs of implementation as human capital is locally available, (3) community support and ownership, and (4) sustainability of the initiatives.

The study showed that participation in habitat draining and filling activities by the community-at-large was very low while the LSM committee-executed larviciding was consistent. This suggests that for successful community-led interventions only selected and trained members from the community should be involved.

The findings also show that reporting of the LSM activities by LSM committees was irregular. This limits thorough understanding about the implementation of LSM by communities hence later decisions and policy about how to engage communities. By consistently reporting how the activities are executed at community level, vital lessons would be learnt which would inform future initiatives, particularly those involving communities. The findings also highlight the need for tailored trainings before community-led activities start.

The results, therefore, suggest that in settings where malaria remains a major health challenge and larval habitats are numerous, involving communities should be at the centre of control programmes. To exploit the full potential of community involvement in community-led initiatives, programmes should invest heavily in community awareness and instilling sense of responsibility and ownership of initiatives. For long term larval reductions, repeated applications of *Bti* by the communities or use of bio-larvicides with longer residual activity should be exploited. The use of innovative tools such as drone technology to map and treat mosquito larval habitats is currently

being exploited (Carrasco-Escobar et al. 2019) and this may further contribute to the future success of larval vector control programmes.

Future perspectives

The challenges faced by current standard vector control interventions directed against adult mosquitoes have inspired adoption of LSM as an additional intervention. Under settings where larval habitats are few, findable and fixed, LSM could further reduce vector populations (WHO 2013b), especially when applied along the standard tools (Fillinger et al. 2009). To effectively implement LSM, especially where larval habitats are undefined and numerous, communities need to be involved in both larval habitat mapping and execution of the components of LSM. While community involvement could improve intervention coverage, willingness by the communities to participate in the initiatives, concerns about potential application of inadequate doses of *Bti* by the community and lack of understanding of larval ecology would constitute major challenges. As shown in this thesis, application of *Bti* even at lower dose rates, reduces anopheline larval densities and further contributes to lowered vectorial capacity as a result of reduced longevity in adults. However, it remains unclear and requires research whether widespread treatment of potential mosquito aquatic habitats with the bacterial larvicide would result in the evolution of a behavioral trait that involves discrimination of the aquatic sites by gravid females.

The community-led LSM in the study areas was hampered by little interest by some members of the community to participate in LSM. Labour and time demands of the activities, and also differential incentivisation of the different groups of people within the community threaten community participation in the community-led LSM. Despite the challenges, involving communities in malaria control increased awareness of its risk factors in areas where the intervention was undertaken. The impressive community knowledge of malaria, its risk factors and control did not, however, lead to participation in the initiative by most of the community members. Increased participation would increase coverage of the intervention and possibly have greater impact. Future interventions should, therefore, explore means that could ensure that the community practices the knowledge attained such as: 1) approaches embracing innovations that make execution of LSM less demanding, both in terms of time and labour, 2) use of bio-larvicides with longer residual activity to reduce repeated applications, 3) use of high-accuracy drones capable of detecting mosquito larval habitats to lessen the demands attached to larval habitat mapping and 4) lobbying, at national level, for the inclusion of LSM in the regular health policies. Also, more research is needed to investigate how to keep the communities motivated without creating dependency on financial incentives. The forms and modes of delivery of incentives have potential to affect willingness to participate in interventions but remain less studied (Ikeoluwapo, Ajayi et al. 2012).

Conclusion

The results presented in this thesis reveal that (i) typical anopheline larval habitats in Malawi are characterized by low turbidity, by a surrounding of bare soil, and by the presence of culicine larvae. Habitats with these characteristics should thus be particularly targeted for malaria vector control efforts. However, for the most effective LSM, all available larval habitats need to be targeted owing to the plasticity of gravid anophelines in their selection of oviposition sites, (ii) application of *Bti* in larval habitats does not repel gravid females to oviposit, (iii) anopheline larval exposure to sublethal doses of *Bti* reduces the longevity of adult mossquitoes, (iv) community-based LSM increases community awareness of malaria, its risk factors and control methods and could be improved by making activities less time and labour demanding and, (v) *Bti* application by specially-trained members of the community reduces anopheline larval densities. Generally, the findings from this study show that community-led LSM is feasible and improves community awareness of health risks and methods of control

Summary

Current trends in the fight against malaria suggest that further progress will be difficult with the use of insecticide-based control measures alone. Without major reductions in the burden of malaria registered in the past few years, the use of additional interventions with synergistic effects on the current standard measures is required. Currently, interest in employing Larval Source Management (LSM) as a complementary tool is growing as it has shown to significantly reduce larval densities and consequently adult populations in settings where it has been applied along other interventions. LSM is commonly executed via 1) habitat modification, which includes physical transformation of a larval habitat through draining, filling and land levelling and 2) larviciding, commonly using an endotoxin-producing bacterial larvicide, *Bacillus thuringiensis* var. *israelensis (Bti)*. Knowledge on the ecology of anopheline larval habitats is therefore important as it informs where LSM should be targeted. Also, knowledge about community acceptance and participation in LSM is important as it affects the scalability and future sustainability of the intervention. The study described in this thesis focused on the potential of community-led LSM in Malawi. Chapter 2 describes the habitat ecology of malaria vectors in the Majete area, southern Malawi. In this area, anopheline larvae develop in habitats with little silt, surrounded by bare-grounds and occupied by culicine larvae. I conclude that larval control should be directed towards such anopheline-productive habitats which sustain malaria transmission. In Chapter 3, I investigated whether application of *Bti* induces discrimination of treated sites by gravid females seeking oviposition sites. I found that treatment of the sites with the bacterial larvicide does not repel ovipositing females from laying eggs in such sites. This finding implies that the female mosquitoes did not detect the presence of the larvicide in aquatic sites. In Chapter 4, we explored whether application of lower doses (sublethal) of *Bti* in larval habitats can negatively affect fitness parameters of malaria vectors and hence their ability to successfully transmit malaria. Sublethal *Bti* doses are likely to occur when applications are done under field conditions, especially by local communities who may lack the desired expertise in comparison with trained experts. Immature and adult life history parameters, including larval survival, adult longevity, wing size and oviposition of *An. coluzzii*, an important African malaria vector, were assessed in a laboratory setting. Our results show that larval densities were reduced when exposed to the sublethal doses. When exposed to *Bti* LC_{70} as larvae, the proportional hazard rate for mortality as adult females was about three times higher than in the control group. At the same LC_{70} dose rate, the mean wing length of the adult females increased by 12% compared to that of the control group. These findings are valuable as they demonstrate that larval exposure to *Bti*, even at lower doses, reduces the longevity of emerging adults which also reduces their vectorial capacity as they may not live long enough to effectively transmit the malaria parasite. In Chapter 5, we assessed whether communities would accept and are willing to participate in community-led LSM activities. Specifically, we explored factors that would motivate community acceptance and participation in

LSM. Our results show that community involvement in LSM as an additional tool for malaria control increases local awareness of malaria as a health problem, its risk factors and control strategies. The results also show that specially trained members of the community easily accepted the intervention and were more willing to participate in the associated activities than the rest of the community. Further, the findings highlight the need to make activities less demanding in terms of time and labour. It was also observed that the community needs incentives to participate in community-led interventions but though critical, forms and modes of delivery of incentives need to be further studied. In Chapter 6, we investigated whether community involvement in LSM is feasible and can result in reduced larval vector densities. Our results showed that groups from the community, which received tailored training from the research team, participated more actively in the LSM activities than the rest of the community. Also, larviciding using *Bti* was the more preferred component of LSM by the community than habitat modification. Interestingly, application of *Bti* reduced larval densities in intervention villages. The findings of this study suggest that community involvement in LSM is only feasible when the community understands their malaria risk factors and control methods. Also, the study demonstrates that community involvement in application of *Bti* has the potential to reduce larval densities but should be implemented after proper training of the spraying teams. In Chapter 7, the key findings of this research and the implications for community-led LSM in Malawi are addressed and recommendations for future investigations are provided. In conclusion, the results of the research described in this thesis show that participation of communities in LSM is feasible and can reduce the malaria burden via reduced larval densities.

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List of abbreviations

An *Anopheles*

Ae *Aedes*

Bs *Bacillus sphaericus*

Bti *Bacillus thuringiensis* var. *israelensis*

CPH Cox proportional hazard

IRS Indoor residual spraying

ITNs Insecticide treated nets

LC Lethal concentration

LLINs Long-lasting insecticide treated nets

LSM Larval Source Management

MMP Majete Malaria Project

PCR Polymerase chain reaction

RBM Roll-Back Malaria

s.l. sensu lato

spp. species

s.s. sensu stricto

WHO World Health Organization

Acknowledgements

My PhD journey has been both exciting and challenging. It was largely characterised by hard work, sleepless nights and funny moments, interactions and experiences. The work presented in this thesis highlights great support, contribution and selfless acts of many people, some in very subtle ways.

Firstly, I would like to express sincere gratitude to Prof. Willem Takken, Dr. Robert McCann, Dr. Henk van den Berg, Dr. Lucinda-Manda Taylor, Dr.Themba Mzilahowa, Dr. Jeroen Spitzen and Dr. Sander Koenraadt for the invaluable support and mentorship during my PhD. Your selfless contribution, guidance and encouragement was my driving force. Words fall short of expressing my sincere gratitude. The timely feedback bearing both encouraging and 'unsettling' comments pushed me out of my comfort zone and challenged me to succeed in my academic and research pursuits. The practical lessons learnt from your commitment and strife for excellence have made me motivated and driven scientist. I endeavour to pass on the attitude wherever I go.

I am specially grateful to Dr. James Chirombo for guidance in statistical analysis of my data. James, the long and odd hours invested will forever be appreciated.

I would like to extend my profound gratitude to Wageningen University & Research, the Netherlands for giving me the opportunity and academic support to pursue my PhD studies. I extend my gratitude to the Diorapthe Foundation, the Netherlands, who made this research and my PhD training possible. I gratefully acknowledge the Training and Research Unit of Excellence, Blantyre, Malawi for providing me with the platform and support to carry out my research. I am also grateful to Prof. Kamija Phiri, Prof. Michèle van Vugt, Dr Alinune Kabaghe, Dr Tinashe Tizifa, William Nkhono, Richard Nkhata, Tumaini Malenga, Dr Monicah Mburu, Asante Kadama and Zainab Longwe for giving me support to conduct my field data collection in Majete, Malawi. Special thanks also go to the Majete Malaria Project staff for the support rendered to me. I thank the staff of African Parks who facilitated many aspects of my field work.

To Paola Meijer, I thank you for support with field work and analysis of some sociological data. I am grateful to Pieter Rouweler, Joeri Groot, Seppe Salari, Frans van Aggelen and André Gidding for helping with rearing mosquito larvae for my laboratory studies.

To my colleagues and friends at the Laboratory of Entomology, Wageningen University & Research: Tessa, Marilyn, Helen, Margaret, Julian, Jeanine, Marieke, Sandeep, Jeroen, Daan, Alessia, Alexander, Antonio, Bram, Davy, Els, Filippo, Gabriela, Jeroen, Shaphan, Jessica, Julia, Julian, Julien, Karol, Katherine, Kay, Luuk, Maite, Max, Monicah, Mitchel, Peter, Pieter, Quint, Simone, Sivaprakaham, Stijn, Thibault and Yidong; thank you for your help in diverse ways. You made my time in the Netherlands memorable and educative.

ICEMR lab team: Dr. Karl Seydel, Joseph, Godfrey, Alex, Syze, Chifundo, Charles, Trancizeo, Andrew, Gertrude, Monica, Liz, Priscilla, Visopo, and Stanley, thank you for the support and discussions during the time I worked in the laboratory.

I am thankful to Angelique Bosch and Charlotte Schilt for their administrative assistance at Wageningen University & Research. Many thanks to Jacqueline de Graaff and Petra Cornelissen for support with my travel arrangements to the Netherlands. I am grateful to Ineke Jansen for helping out with the design of my thesis cover. Thank you Claudius and Lennart for organizing the PhD weekend meetings, they were very insightful.

To the African community and the united community of African students (UCAS) groups at Wageningen University & Research: Kim, Melvin, Bilhar, Michael-Babatunde, Dan, Domina, Lerato, Nathaniel, Nyati, Simon, Esther, Samuel, Deedi, Christella, Stephane, Severin, William, Taofik, Emily, Tele, Jared, Mohamad, Faith, Debbie, Brian, Joy, Isaac, Aron and many others, thank you for the moments we shared. The Friday night dinners were so refreshing and made me wish everyday was Friday. You made me feel at home. Special thanks to my friends Joshua (Kenya) and Bernard (Zambia) for organising the Friday meetings.

I extend my sincere gratitude to my wife and son, Grace and Steve, for the support and encouragement you gave me while I pursued my PhD. The many days I spent away from home were painful but very important. I am so much indebted to you! Many thanks to my siblings, cousins, nephews, nieces and friends. Your encouragement kept me going even when it seemed difficult at times.

Curriculum vitae

Steven Gowelo was born on 30 September 1985 in Lilongwe, Malawi. After completing his primary and secondary education, he enrolled with the University of Malawi where he obtained a Bachelor's degree in Biological Sciences (major) and Chemistry (minor) in 2009. From September 2009-March 2010 and April 2010-August 2011, Steven worked as a secondary school teacher at the Malawi Ministry of Education and BEDIR high school, respectively. Later in 2011 he joined Chancellor College (constituent college of the University of Malawi) as Research Assistant in a vector-borne disease control project conducted by the University of Malawi and Nagasaki University Institute of Tropical Medicine. In 2014, Steven joined Chancellor College as a Laboratory Technician. The next year, 2015, he joined the College of Medicine (another constituent college of the University of Malawi) as a Research Associate in a community-based malaria control project (Majete Malaria Project, MMP). While working with MMP, Steven pursued a Master of Biological Sciences (Entomology) degree by research at the University of Malawi (2016). During his MSc, he assessed the host range and *Plasmodium falciparum* sporozoite infectivity of *Anopheles funestus s.s.* and *Anopheles gambiae* s.l. in northern Malawi. In 2017, Steven enrolled for a PhD programme with Wageningen University & Research, the Netherlands. His research focused on participatory approach for malaria control in southern Malawi and the results of this work can be read in this thesis. Steve has recently started on a post doc position with the Partnership for Increasing the Impact of Vector Control (PIIVeC) at the Malaria Alert Centre, Blantyre, to study the dynamics of human trypanosomiasis in northern Malawi.

List of publications

Steven Gowelo, James Chirombo, Jeroen Spitzen, Constantianus J.M. Koenraadt, Themba Mzilahowa, Henk van den Berg, Willem Takken, Robert McCann. 2020. Effects of larval exposure to sublethal doses of *Bacillus thuringiensis* var. *israelensis* on body size, oviposition and survival of adult *Anopheles coluzzii* mosquitoes. Parasit. Vectors. 13: 259. **DOI: 10.1186/s13071-020-04132-z**.

Van Den Berg, H., M. Van Vugt, A. N. Kabaghe, M. Nkalapa, R. Kaotcha, Z. Truwah, T. Malenga, A. Kadama, S. Banda, T. Tizifa, **S. Gowelo**, M. M. Mburu, K. S. Phiri, W. Takken, and R. S. McCann. 2018. Community-based malaria control in southern Malawi: A description of experimental interventions of community workshops, house improvement and larval source management. Malar. J. 17: 1–12. **DOI : 10.1186/s12936-018-2415-1.**

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McCann, R. S., H. van den Berg, P. J. Diggle, M. van Vugt, D. J. Terlouw, K. S. Phiri, A. Di Pasquale, N. Maire, **S. Gowelo**, M. M. Mburu, A. N. Kabaghe, T. Mzilahowa, M. G. Chipeta, and W. Takken. 2017. Assessment of the effect of larval source management and house improvement on malaria transmission when added to standard malaria control strategies in southern Malawi: Study protocol for a cluster-randomised controlled trial. BMC Infect. Dis. 17. **DOI: 10.1186/s12879-017-2749-2**

Submitted

Steven Gowelo, James Chirombo, Constantianus J.M. Koenraadt, Themba Mzilahowa, Henk van den Berg, Willem Takken, Robert McCann. Characterisation of anopheline larval habitats in southern Malawi (Chapter 2 in this thesis) (submitted to ACTA TROPICA)

Steven Gowelo, Robert McCann, Constantianus J.M. Koenraadt, Henk van den Berg, Willem Takken, Lucinda Manda-Taylor. Community factors affecting participation in larval source management for malaria control in Chikwawa District, Southern Malawi (Chapter 5 in this thesis) (submited to Malaria Journal)

Themba Mzilahowa, **S. Gowelo.** *Anopheles funestus* sensu stricto Giles (Diptera:Culicidae) bites late in the morning at two rural villages in northern Malawi and its implications for malaria vector controls. (submitted to Malawi Medical Journal)

Amoah, B., R. S. Mccann, A. N. Kabaghe, M. Paula, **S. Gowelo**, M. Mburu, M. G. Chipeta, T. Tizifa, and E. Giorgi. 2020. On the relationship between *Plasmodium falciparum* prevalence and entomological inoculation rate. (submitted to PLoS Med.)

Yoshihide Maekawaa, Dylo Pemba, Justin Kumala, **Steve Gowelo**, Yukiko Higa, Kyoko Futamic, Kyoko Sawabe, Yoshio Tsuda. DNA barcoding of mosquitoes collected through a nationwide survey in 2011 and 2012 in Malawi, Southeast Africa (submitted to ACTA TROPICA)

In preparation

Steven Gowelo, Paola Meijer, Tinashe Tizifa, Themba Mzilahowa, Constantianus J.M. Koenraadt, Henk van den Berg, Lucinda Manda-Taylor, Alinune Kabaghe, Michèle van Vugt, Kamija Phiri, Robert McCann, Willem Takken. Community participation in habitat management and larviciding for the control of malaria vectors (Chapter 6 in this thesis)

Steven Gowelo, Noboru Minakawa, Dylo Pemba, Themba Mzilahowa. Bloodmeals, and *Plasmodium falciparum* sporozoite infectivity of two malaria vector species in karonga and nkhata bay districts in northern Malawi.

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)

Ecological and community factors for consideration in the implementation of community-based larval source management for Malaria control in Chikhwawa district, Malawi

Writing of Project proposal (4.5 ECTS)

A participatory approach for malaria control in southern Malawi: Effects of the environment and community on larval source management

Post-graduate courses (4.9 ECTS)

Good Clinical Practice (GCP) including ethics of health research; University of Malawi (2016)

Spatial modelling of vectors using VecMap; WUR (2017)

R and statistics; Malawi-Liverpool Wellcome Trust (2018)

Systematic review methods; Cochrane South Africa (2019)

R & Big Data; WUR (2019)

Mixed linear models; WUR (2019)

Good Clinical Practice (GCP); Global Health Network (2020)

Laboratory training and working visits (4.5 ECTS)

Molecular identification of malaria vectors; Blantyre Malaria Project (2018)

Deficiency, refresh, brush-up courses (15 ECTS)

Ecological methods; WUR (2017)

Ecological aspects of biological interactions; WUR (2017)

Analysis and prevention of health risks in the tropics; WUR (2017)

Competence strengthening / skills courses (2.2 ECTS)

Leadership and management; Malawi-Liverpool-Wellcome Trust (2017)

Scientific writing and presentation; ACEPHEM, Malawi (2018)

Scientific publishing; WUR (2019)

Reviewing a scientific manuscript; WUR (2019)

PE&RC Annual meetings, seminars and the PE&RC weekend (2.1 ECTS)

PE&RC First years weekend (2017)

PE&RC Day (2017, 2019)

PE&RC Last years weekend (2019)

Discussion groups / local seminars / other scientific meetings (4.5 ECTS)

PhD lunch group discussion; Laboratory of Entomology (2017, 2019)

One health meetings; Laboratory of Entomology (2017, 2019)

Journal club; Malawi-Liverpool Wellcome Trust, Malawi (2017, 2018, 2019)

International symposia, workshops and conferences (4.8 ECTS)

Research Dissemination Conference; oral presentation; University of Malawi (2018)

Keystone symposia; oral and a poster presentation; Hilton Addis Ababa (2019)

Lecturing / supervision of practicals / tutorials (8.7 ECTS)

Research methods; College of Medicine, University of Malawi (2018)

Medical parasitology; Chancellor College, University of Malawi (2019)

Introduction to Invertebrate Zoology; Chancellor College, University of Malawi (2019)

Supervision of MSc students (3 ECTS)

Knowledge, perceptions and practices on the risk of malaria and larval habitats in Chikwawa district, rural Malawi

The research described in this thesis was conducted under the Majete Malaria Project that was financially supported by the Dioraphte foundation, The Netherlands.

Cover design by Ineke Jansen and Steven Gowelo

Thesis layout by Steven Gowelo

Printed by GVO Printers & Designers, Ede, The Netherlands