Rice and Malaria in Africa: Seeking Vector Control in Rice Cultivation Practices

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I, Kallista Hay Ching Chan, confirm that work presented in this thesis is my own.

Where information has been derived from other sources, I confirm this has been indicated in the thesis.

[redacted]

Kallista Hay Ching Chan

08/08/2022

To Côte d'Ivoire,

specifically, Baoulé country.

She has captured my heart

with her constant source of

tchieh!

eh-heh,

yako &

voilà.

Abstract

Background

Demand for rice is rapidly growly in sub-Saharan Africa. There is ongoing and substantial investment in the extensification of irrigated lowland rice production. Unfortunately, irrigated rice fields, which are continuously flooded for the majority of the growing season, are ideal breeding sites for African malaria vectors. A better understanding of the relationship between rice cultivation and malaria risk, with an intent of identifying improved methods of rice production that minimises malaria vector production, is urgently needed.

Methods

To examine the association between rice growing, malaria vectors and malaria risk in sub-Saharan Africa, two systematic reviews and meta-analyses were conducted. To gain a more thorough understanding of the ecology and epidemiological significance of malaria vectors produced in rice fields, the population dynamics of mosquitoes were monitored for four cropping seasons in Côte d'Ivoire. Experimental field trials were also established to investigate the effect of various rice cultivation practices on vector productivity. To explore rice farmers' views and perspectives on their contribution to mosquito production, in-depth interviews and focus group discussions were conducted in two rice communities in Côte d'Ivoire.

Main findings

Rice-farming communities in sub-Saharan Africa are exposed to a greater number of malaria vectors, where one hectare of irrigated rice is capable of producing 700,000 malaria vectors during a cropping season. Rice communities are also exposed to greater malaria risk. As malaria transmission intensity reduces, the effects of irrigated lowland rice cultivation on malaria are expected to become more conspicuous. Potential interventions that grow rice

with fewer mosquitoes exist, namely rice-fish co-culture, various forms of intermittent irrigation, and land preparation techniques. Whilst farmers were not aware of the link between rice and mosquitoes, they were willing to adopt vector control interventions.

Conclusions

This thesis highlights that the associations between rice and malaria should not be interpreted as a trade-off between food security and human health. Instead, the development of modified rice-growing methods that improve rice yield (and are hence more attractive towards farmers) whilst minimising malaria vector production should be prioritised. The effect of irrigated systems (other than rice) on malaria should be explored.

Chapter findings

Chapter 1 describes the background on malaria epidemiology and rice cultivation in sub-Saharan Africa. It also outlines the trends in both malaria control and rice intensification, highlighting the key knowledge gaps.

Chapter 2 outlines the aims and specific objectives of this thesis. It provides context on the study sites where this work was undertaken.

Chapter 3 consists of a systematic review and meta-analysis which reveals that since 2003, communities living near irrigated rice areas in Africa are not only exposed to more intense malaria transmission but also have a higher prevalence of malaria infection than non-rice communities.

Chapter 4 also consists of a systematic review and meta-analysis, which found that chemical larvicides can reduce malaria vectors in rice fields by 77% whilst biological larvicides can do so by 60% and rice-fish co-culture by 82%. Intermittent irrigation is effective at reducing the abundance of late-stage anopheline larvae, but not overall immature abundance.

Chapter 5 describes the population dynamics of malaria vectors in experimental field trials in Côte d'Ivoire. It reveals that peak *Anopheles coluzzii* productivity occurred in the first four weeks after transplanting and can be attributed to the aquatic conditions during these early stages of the rice season. It also estimates that a total of 700,000 malaria vectors is produced in one hectare of rice during a cropping season.

Chapter 6 outlines experimental field trials conducted in Côte d'Ivoire and Tanzania, which tested the effect of various rice cultivation practices on malaria vector density, rice yield, water consumption, and greenhouse gas emissions. The trials revealed that puddling of more than seven days at land preparation, directly seeded fields, and fields applied with fertilisers produced more malaria vectors. Whilst Tanzanian trials demonstrated that alternate wetting and drying irrigation produced 71% fewer late-stage anophelines, Ivoirian trials demonstrated that it produced 41% less methane.

Chapter 7 consists of a qualitative study investigating rice farmers' views and perspectives on rice farming and its effect on mosquitoes. The study revealed that farmers did not perceive a link to exist between rice cultivation and mosquitoes but were receptive towards potential riceland mosquito control.

Chapter 8 discusses the main findings of Chapters 3 to 7, study limitations, implications of the findings and recommendations for further research.

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Abbreviations

A4NH	Agriculture for Nutrition and Health
ACT	Artemisinin-based combination therapy
AWD	Alternate wetting and drying
Bs	*Bacillus sphaericus*
Bti	*Bacillus thuringiensis israelensis*
CDC	Centers for Disease Control and Prevention
CF	Continuous flooding
CGIAR	(Formerly) Consultative Group for International Agricultural Research
CI	Confidence interval
CITS	Controlled interrupted time series
CTS	Controlled time series
DALY	Disability-adjusted life year
DAP	Diammonium phosphate
DAS	Days after seeding
DAT	Days after transplanting
DDT	Dichlorodiphenyltrichloroethane
DS	Direct seeding
EC	Emulsifiable concentrate
EIR	Entomological inoculation rate
EPOC	Effective practice and organisation care
FAO	Food and Agriculture Organisation
FD	Forced drainage
FGD	Focus group discussions
GRADE	Grading of recommendations, assessment, development, and evaluation
HBR	Human biting rate
HLC	Human landing catch
IDI	In-depth interviews
IHI	Ifakara Health Institute
II	Intermittent irrigation

IPR	Institut Pierre Richet
IRR	Incidence rate ratio
IRS	Indoor residual spraying
IRRI	International Rice Research Institute
ITN	Insecticide-treated net
IVM	Integrated vector management
LLIN	Long-lasting insecticide-treated net
LSM	Larval source management
MSF	Monomolecular surface film
NB	Negative binomial
NPK	Nitrogen phosphorus potassium
OPOH	Our Planet Our Health
OR	Odds ratio
PAR	Participatory action research
PCR	Polymerase chain reaction
PEEM	Panel of Experts on Environmental Management for Vector Control
PRISMA	Preferred reporting items for systematic reviews and meta-analysis
PSC	Pyrethrum spray catches
RAFT	Resilience Against Future Threats
RBM	Roll Back Malaria
RDT	Rapid diagnostic test
RE	Random effects
ROB	Risk of bias
ROM	Ratio of means
RR	Risk ratio
SD	Standard deviation
SE	Standard error
SSA	Sub-Saharan Africa
TP	Transplanting
UNEP	United Nations Environment Programme
WHO	World Health Organisation

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All fieldwork activities were conducted in Bouaké, Côte d'Ivoire through the London School of Hygiene and Tropical with partners at Africa Rice Center, Institut Pierre Richet and Université Alassane Ouattara. Additional fieldwork was conducted in Bagamoyo, Tanzania in partnership with International Rice Research Institute and Ifakara Health Institute.

My role and the contributions of my collaborators to the studies included in this thesis are summarised below.

Chapter 1 describes the background on malaria epidemiology and control as well as rice cultivation in sub-Saharan Africa and outlines the key knowledge gaps. I wrote all the content for this chapter.

Chapter 2 outlines the study design and objectives as well as the study sites where this work was undertaken.

Chapter 3 consists of a published paper that assesses the association between rice and malaria through a systematic review and meta-analysis. I designed the study, conducted the data search and extraction, analysed the data and wrote the manuscript. Jo Lines verified the data whilst Christian Bottomley advised on statistical analyses.

> Chan, K., Tusting, L.S., Bottomley, C., Saito, K., Djouaka, R. and Lines, J., 2022. Malaria transmission and prevalence in rice-growing versus non-rice-growing villages in Africa: a systematic review and meta-analysis. The Lancet Planetary Health, 6(3), pp.e257-e269.

Chapter 4 was prepared as a pending publication that evaluates the efficacy of riceland mosquito control and rice cultivation practices on reducing malaria risk. I designed the study, conducted the data search and extraction, analysed the data, and wrote the manuscript. Christian Bottomley advised on statistical analyses. Christian Bottomley, Kazuki Saito, Jo Lines and Lucy Tusting advised on analysis methods and reviewed the manuscript.

Chapter 5 was prepared as a pending publication that describes the population dynamics of mosquitoes in rice fields. I designed the study, supervised the field work, analysed the data and wrote the manuscript. Data was collected by field teams in Institut Pierre Richet coordinated by Raphael N'Guessan and Alphonsine Koffi. Christian Bottomley, Ellie Sherrard-Smith and Kimberly Fornace advised on analysis methods.

Chapter 6 was prepared as a pending publication that evaluates the effect of rice cultivation practices on malaria vectors, rice yield, water consumption and greenhouse gas emissions. I designed the study, supervised the field work in Côte d'Ivoire, analysed the data on malaria outcomes and wrote the manuscript. Edith Madumla and George Iranga supervised the field work in Tanzania. Elliott Dossou-Yovo and Paul Iboko advised on study design and analysed the data on agronomic and climate outcomes. Christian Bottomley advised on analysis methods.

Chapter 7 was prepared as a pending publication that describes rice farmers' views and perspectives on rice farming and its effect on mosquitoes and malaria. I designed the study and with Dimi Théodore Doudou, I supervised the fieldwork conducted by a PhD candidate (from Université Alassane Ouattara) Aimé-Charles Kouadio Konan. I analysed the data and wrote the manuscript. Dimi Théodore Doudou, Robert Aunger, and Lucy Tusting advised on analysis methods and reviewed the manuscript.

Chapter 8 discusses the main findings, study limitations, implications of the findings and recommendations for further research.

1 Introduction

1.1 Malaria control in Africa: looking towards elimination

Consistently ranked as one of the top 10 causes of early death, malaria remains the most important vector-borne disease worldwide; in 2020, there were an estimated 241 million malaria cases and 627,000 malaria deaths globally [1,2]. Considerable effort has been made to reduce its burden. The “global” malaria eradication programme, which launched in 1955, was a success in many European, American, and Asian countries, even allowing some to declare themselves “malaria-free” [3]. However, the campaign almost completely excluded sub-Saharan Africa, because of the ‘physical, economic, and developmental difficulties’, combined with the continent’s high endemicity and prolonged transmission [4,5]. Nonetheless, due to the rapid and widespread expansion of DDT resistance and operational, economic, and political issues, the programme came to a halt in 1969.

Malaria control then remained fairly untouched for the following two decades, until the 1990s, when major initiatives, including World Health Organisation (WHO) and The Global Fund, instigated the Roll Back Malaria Partnership (RBM). It was predicated on the sense that there were “new” tools, namely insecticide-treated nets and artemisinin-based combination treatments, that could be mobilised [6]. The initiative not only had an aim to halve malaria morbidity and mortality by 2010, but also included Africa as part of the campaign. Bill and Melinda Gates’ proclamation for malaria eradication and calls for “universal coverage” of key interventions in 2007 also led to a massive scaling-up of indoor residual spraying (IRS) and insecticide-treated nets (ITNs). Since, there has been unprecedented success and the dynamics of malaria transmission in Africa have changed dramatically. According to estimates by Bhatt et al., the fraction of African populations under hyper- or holo-endemic transmission intensity has decreased from 33% to 9% [7].

Progress in this decline appears to have stalled in the last five years – the main challenges in Africa being the lack of funding and insecticide resistance.

Nevertheless, 75% of cases are found only in 13 African countries and many countries in the rest of sub-Saharan Africa are planning for elimination [2]. The right approaches to prepare and tackle this new chapter remain poorly defined, but it is apparent that the current and proposed strong reliance on insecticides and drugs is inadequate. Under this scenario, the absence of malaria will not be rendered stable unless chemical-based interventions are maintained [8]. Thus, a more multi-sectoral response (a whole-of-government response involving other sectors such as agriculture, education, the environment, housing, social development, and transport) and intensive surveillance and vigilance are required to prevent the re-establishment of local transmission. It is vital that any avoidable, man-made ecosystems that are highly receptive to malaria transmission (which is when competent vectors, a suitable climate and susceptible human populations are present) are not being actively created [4,9] (see Box 1.1).

Rice agro-ecosystems in sub-Saharan Africa are extremely receptive to malaria transmission. Mostly grown in tropical climates, rice does not only attract a large workforce, but also malaria vectors. Rice fields are excellent breeding sites for many species of mosquitoes, but particularly *Anopheles gambiae* sensu lato, which is the most important malaria vector in Africa. The high longevity and anthropophily of this species make it exceptionally efficient in transmitting malaria and this efficiency is the main reason why Africa suffers 96% of the world's malaria mortality [2,10]. Unfortunately, these mosquitoes are very well adapted to, and can breed abundantly in, the aquatic conditions of rice paddies[1]. Thus, the optimal conditions that rice fields provide for the proliferation of *An. gambiae* s.l. pose a major threat to malaria elimination in Africa.

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[1] Larvae of various *Anopheles* species are also common in major rice-producing areas in Asia. The difference is that, as adults, these are short-lived and animal-biting and are therefore unimportant in malaria transmission.

Box 1.1. Environmental management for mosquito and malaria control: brief history and current applications in Africa.

To avoid creating or eliminate ecosystems that are highly receptive to malaria transmission, environmental management should be considered. Historically, environment-based interventions have been largely successful in controlling malaria. In ancient Rome, even before the discovery of the role of mosquitoes as vectors of malaria (in 1897), drainage of stagnant waters and swamps was often used to improve public health and agricultural production [11,12].

In the Americas and Asia, environmental modification and manipulation programmes could successfully reduce malaria incidence and malaria-attributable mortality [13]. Notable examples include drainage activities combined with vegetation clearing in the Suez Canal in Panama and in the Malaysian Peninsula (in rubber plantations) in the early 1900s [14], the use of intermittent irrigation in Indian and Chinese rice fields from as early as 1940s [15] and water level management and reservoir clearance in the Tennessee Valley Authorities in the 1930-50s [16].

In sub-Saharan Africa, very few cases of environmental management have been attempted (or documented), most likely because the vectorial capacity and ensuing force of infection were very high in most settings, so high that malaria was deemed saturated (see Appendix 1.1), with effects of environmental management unlikely to yield a noticeable reduction in malaria disease burden) [12,17]. There were two instances of programmes that successfully reduced or eliminated mosquito breeding habitats and clinical malaria outcomes, but both, conducted in the 1930/40s, were targeted at smaller populations: copper mining communities in Zambia [18] and military personnel in Nigeria [19].

Interest of environment management for mosquito and malaria control had waned with the rise of DDT and other chemical insecticides during the Global Malaria Eradication programme in the 1950-60s. However, with reduced malaria transmission nowadays and concerns over reliance on chemical control, sustainability and insecticide resistance, environmental control should be reconsidered in African settings.

1.2 Rice cultivation in Africa: expanding its irrigation potential

Rice is the most widely consumed staple food in the world. Although not as much rice is grown in Africa as compared to Asia, Africa has had significant expansions in rice cultivation; since 1990, harvest has increased by over 185% by 2020 [20]. This growth can be attributable to Africa's substantial population growth, urbanisation, and changes in family dynamics (more women entering the workforce), which lead to increasing popularity of convenience foods like rice [21,22]. However, Africa is dominated by rain-fed rice agro-ecosystems, which, susceptible to droughts and variation in rainfall levels, do not provide adequate food security. It has therefore been difficult for Africa to keep up with its increasing demand and close the yield gap, and its rising dependence on imports is highly unsustainable; sub-Saharan Africa has a self-sufficiency rate of only 48% [23].

Accordingly, to increase food security, boost economic growth and achieve self-sufficiency, many African Ministries of Agriculture have launched national strategic plans and policies to increase rice production [24,25]. Numerous international institutions have also invested in resources to boost irrigation development, which can help Africa realise its rice production potential; an estimated 42.5 million hectares of land in Africa can be irrigated, of which only 6% is currently irrigated [26,27]. Because of these plans, rice-harvested areas are expected to increase [28,29]. Côte d'Ivoire, for example, has already had exceptional growth, from less than 400,000 hectares of rice-harvested areas in 2007 to 1 million hectares in 2016 [30]. There is still large untapped potential for irrigation in Africa, extending to about 24 million hectares or 1.8 times greater than the existing irrigation area [26].

Within the context of malaria, this anticipated growth in rice paddies is worrying as it conflicts with the agenda of many African Ministries of Health, who are planning for malaria elimination.

1.3 Rice fields as ecosystems

To understand the relationship between mosquitoes and rice fields in Africa, it is essential to recognise that rice fields are dynamic ecosystems. They are in no way homogenous, spatially nor temporally. In terms of spatial variation, rice is grown in places with a range of climate, soil quality, and hydrology but can generally be categorised into 5 agro-ecosystems, based on their hydrological characteristics: upland, rain-fed lowland, irrigated lowland (paddies), deep water and mangrove swamps (Figure 1.1A). Among which, rain-fed upland rice is the most widespread in West Africa (47%), followed by rain-fed lowland rice (34%), mangrove swamps (4%) and irrigated rice (3%) [31].

Temporally, as a rice season progresses, rice fields change in microhabitat. Depending on rice variety, soil and aquatic conditions, plants take around 4 to 6 months to grow from seed to maturity (Figure 1.1B) [31]. Rice fields are also exposed to natural phenomena such as rainfall and flooding as well as man-made farming practices [32]. Farming practices are diverse and variable, and include many methods of land preparation (ploughing, hoeing, levelling), crop establishment (transplanting, direct seeding), weeding as well as many differences in the application of fertilisers, herbicides, and pesticides (in frequency, timing, and amount) (see Box 1.2).

In irrigated agro-ecosystems, the most common water management method is continuous flooding, because it provides a constant flow of water can dramatically increase rice productivity. Rice is a water-loving plant and water affects its physical characteristics, the nutrient and physical status of soils as well as the nature and extent of weed growth [31]. This stable body of water, continuously present for at least 4 months, in turn permits many cycles of vector breeding. It is also subject to various rice operations, which can impact mosquito abundance too, positively or negatively.

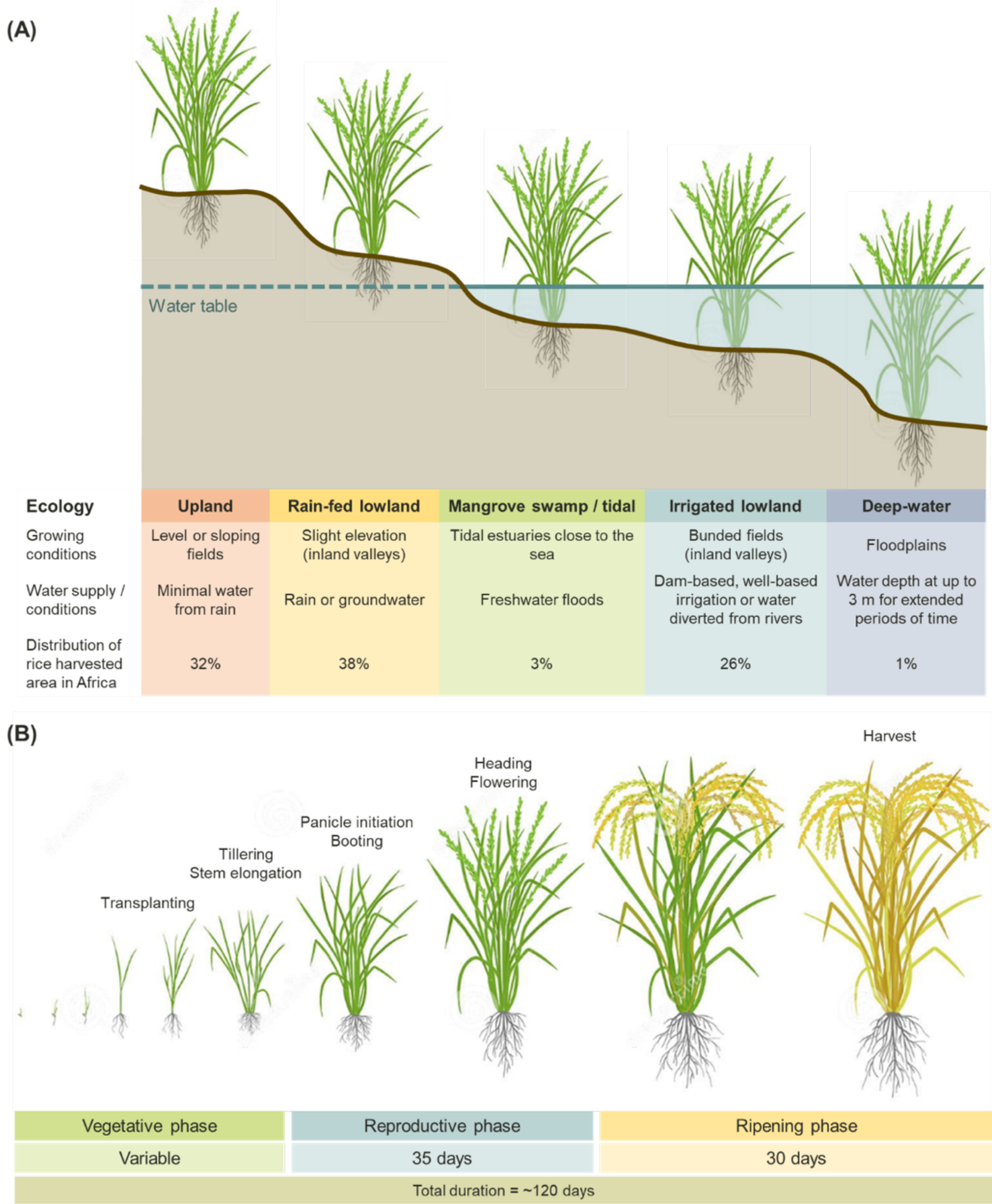

Figure 1.1 (A) The major rice agro-ecosystems, their growing and water conditions and their distribution across Africa, adapted [22,24], and **(B)** the growth stages and phases of rice.

Box 1.2. Guide to rice cultivation practices.

Rice cultivation practices can be generally categorised to land preparation, crop establishment, crop management (including water, weed, nutrient and pest/disease management) and harvest. There are many variations of each part of the growing process and only the (most popular) practices that are recommended by agronomists are described here.

Pre-planting: land preparation

Rice is best grown in clay or loamy soils that are rich in organic matter. The soil needs to be at its best physical condition for crop growth in order to control weeds, recycle nutrients through the decomposition of rice stubble and weeds, and minimise water and fertiliser loss. Land preparation, a process of 3-4 weeks, starts with bund construction (dikes of land surrounding fields to slow down water runoff) and pre-irrigation for 3-5 days before primary tillage (i.e., ploughing and harrowing to mix and overturn the soil whilst breaking it up into smaller portions) occurs. Fields are then irrigated for 2-3 weeks (i.e., puddling) before being drained for basal fertiliser application and secondary tillage and levelling. These two steps allow uniform distribution of fertilisers into the soil and water to help seeds or seedlings to become established more easily. Based on the basic biology of mosquito larvae, it can be expected that puddling encourages (perhaps two or three generations of) mosquito breeding.

Planting: crop establishment

Amongst numerous techniques to establish rice plants, transplanting and direct seeding (which includes dibbling and drum seeding) are the two main techniques. Transplanting is the more traditional and popular method where seeds are raised in nursery beds to pre-germinate seedlings (up until around 21 days) before they are transferred to the main field. It is often conducted in areas with good soil fertility and water availability. Done either manually by hand or by machine, transplanting requires more labour, but it is often preferred due to greater weed control and greater yield relative to the number of seeds planted (~40 kg/ha required). For direct seeding, seeds (either dry seeds in rainfed rice or pre-germinated seedlings [i.e., seeds submerged in water for 12-

48 hours until shoots sprout] in irrigated rice systems) are sown directly in the main field. Although it utilises more seeds (~60-80 kg/ha) and water, less labour and inputs are required.

Rice varieties are chosen depending on the region of cultivation, farmer, or consumer preferences and whether there is a need for improved varieties against certain diseases or climatic conditions. For transplanted rice, seedlings are usually planted at distances of 20 x 20 cm, but they can be planted with closer spacing depending on the availability of plants and cost of labour.

Crop management: water management

In general, fields must be constructed to control water flow. This is done through land preparation: (a) channel and bund construction, (b) puddling to create a hard pan and (c) levelling (where an unlevelled field requires extra water to ensure even coverage of water).

The most common method of water management is continuous flooding, where water levels are maintained at around 3 cm above the soil surface after transplanting or seeding and increased to 5-10 cm a few weeks after transplanting until one week before harvest. This method ensures that rice has sufficient water throughout growth, but essentially controls weeds and pests, both of which can severely affect yield. Unfortunately, it also provides the perfect conditions for mosquito breeding (details covered in main text) and methane production. Under continuous flooding, water blocks oxygen from penetrating the soil, creating ideal conditions for anaerobic micro-organisms to degrade the organic matter into methane [33]. Thus, any farming method that interrupts or shortens flooding periods can reduce methane emissions, including alternate wetting and drying (AWD) irrigation.

AWD starts 1-2 weeks after transplanting, where water depth is allowed to drop 15 cm below the soil surface (i.e., passive drainage through percolation and evaporation) before the field is re-flooded to 5 cm. This cycle is repeated until one week before harvest, except for one week during flowering. AWD has been shown in Asia to be capable of reducing greenhouse gas emissions by 48%, and water consumption by 38%, whilst maintaining rice yield [34].

Crop management: weed management

Most weeds, through competition for nutrients, moisture, and sunlight, are harmful to the rice plant. They can significantly reduce yield and grain quality whilst increasing production costs. Thus, fields should be weed-free for at least up to 45 days after sowing. Weed management is normally built into land preparation (ploughing and allowing them to emerge before tillage), crop establishment (close crop spacing) and water management (continuous flooding, maintaining wet fields following herbicide application) but additional methods can be categorised as mechanical and chemical methods. Mechanical weeding can be conducted via manual weeding or equipment such as hoeing or rotary weeders whereas chemical herbicide application uses either contact or systemic herbicides. Depending on the type of weeds present, pre- or post-emergence herbicides can be applied. In small-scale rice farms across Africa, hand weeding is the most widely practiced management method whilst herbicides are often used alongside other control options [35]. Based on mosquito biology, it is anticipated that weeding affects their oviposition and development too: fields that are heavily infested with weeds, where water surfaces are not exposed to sunlight, may not be preferred by *An. gambiae* [36].

Crop management: nutrient management

Typically, field soil does not provide all the nutrients required by the rice plant at each different growth phase. Thus, the right amount of fertilisers needs to be applied at the right time to obtain optimal yields; premature or delayed application can lead to nutrient losses. First, basal fertilisers are incorporated into pre-planted soil, followed by top dressings at tillering and panicle initiation. Fields must be flooded with 2-3 cm of water for at least one week during top dressing applications to maximise nutrient retention. Instead of synthetic chemicals such as diammonium phosphate (DAP) or nitrogen-phosphorous-potassium (NPK), organic materials (e.g., manure and biochar) can be used if easily available. However, animal manure is inconvenient: it tends to be expensive to purchase and transport, limited in its nutrient composition (that is required for the rice plant) and tends to release its nutrients slowly. It has been speculated that fertilisers applied in rice fields encourage mosquito development [37,38].

Crop management: pest and disease management

Other than weeds, rice yield is constantly subject to loss through the actions of pests (rats, snails, nematodes, and insects), diseases and other phenomena (such as soil acidity, water availability and temperatures). To manage these problems, prevention by good field practice through using clean seeds and equipment, accurate fertiliser application and planting at similar times to neighbouring farmers (to reduce disease and pest pressure on fields). In general, integrated pest management is recommended, where biological and cultural (e.g., mixed cropping, crop rotation, synchronous planting) control are encouraged, and chemical control is only adopted if the former two practices were not adequate.

Harvest

Depending on the variety of rice, harvest occurs between 105-150 days after crop establishment. It is the process of collecting mature rice crop from fields and entails cutting, stacking, handling, threshing, cleaning and hauling.

1.4 The biology of malaria vectors in rice fields

Because of the flooded nature of most rice paddies, they can be inhabited by many different mosquito species throughout all stages of plant development (from transplanting to harvest). Due to the variability of a rice agro-ecosystem, however, vector species composition and densities tend to change with time. In the early stages of rice development, the recommended growing conditions for irrigated rice are very similar to the optimal conditions for *An. gambiae* s.l. larvae development: sunlit, shallow (2-10 cm depth), fresh and clean water (not de-oxygenated) and damp mud surfaces suitable for oviposition [10,31]. This is because *An. gambiae* is a 'pioneer' species: it is one of the first insects to colonise a newly created body of suitable water, and while the water is still new, a large proportion of mosquito larvae may survive to adulthood [39]. But if water remains stable for a few weeks (i.e., "older" water), it will gradually be colonised by a variety of predators, so an increasing proportion of young larvae will be eaten before they mature [40]. In rice agro-ecosystems, this means that the first few weeks after transplantation is often a period of peak productivity [41–44].

As the season progresses, the development of rice plants can change the aquatic conditions of paddies. Depending on rice variety (and distance between plants), plant growth can leave the water surface still largely exposed and sunlit, but for certain varieties, maturation establishes a closed canopy which shades the water surface [44]. Thus, in some parts of Africa, a succession of vector species is observed: for the first few weeks after transplanting, when rice plants are still relatively short, *An. gambiae* s.l. is most abundant, but when rice is nearly ready for harvest, *An. funestus* takes over in the now shaded water [45,46].

Other than the natural progression in plant development, most rice management practices can affect vector densities, positive or negatively. In Kenya, peaks of *An. arabiensis* were seen shortly after fertiliser application [37]. In other instances, herbicides and pesticides exhibited some larvicidal properties, but also killed many predators and subsequently, resulted in an overall increase in larval populations [41,47]. The presence and density of aquatic vegetation in paddies also have profound effects on larvae: sparse amounts enable protection from predators, whereas dense vegetation (e.g., *Azolla*, weeds) can reduce oviposition [46,48,49]. Water management obviously also plays a key role in influencing vector density (see Box 1.3).

Box 1.3. Intermittent irrigation of paddy fields

Reducing or eliminating standing water in rice fields can minimise mosquito breeding, so “intermittent irrigation”, which involves the periodic flooding and drying of fields for several days to prohibit complete larval development, is strongly advocated by medical entomologists and malariologists. A series of experimental trials have demonstrated that it is a viable and successful method of vector control, capable of reducing vector densities up to 95% [27]. However, its efficacy was highly site-specific, dependent on factors such as climate, soil composition and farmer compliance [48]. Intermittent irrigation, created with the primary purpose of reducing mosquito production, also did not necessarily lead to optimal rice productivity, and thus, limited farmer incentive. Although intermittent irrigation is similar to alternate wetting and drying irrigation, it is different in terms of methods of drainage (Figure 1.2): whilst field water is actively drained during intermittent irrigation based on timing, field water is passively drained through evapotranspiration and percolation during AWD and relies on surrounding environmental conditions.

1.4.1 Natural wetlands vs. irrigated rice

Irrigated rice schemes are often installed in settings that were previously natural freshwater wetlands, with their own natural mosquito fauna. It is therefore reasonable to ask whether the conversion to rice makes any differences. Data collected by Chandler, Highton & Hill in the 1970s, re-drawn in Figure 1.3, shows that the natural wetlands did indeed produce a large number of mosquitoes, but these were mostly nuisance mosquitoes, comprising a diverse mixture of animal-biting non-vector species [42,46,50]. In the rice fields, overall mosquito abundance was similar, but there was a major change in species composition: species diversity was greatly reduced, and the two main malaria vectors (*An. gambiae* s.l. and *An. funestus*) made up 90% of the catch.

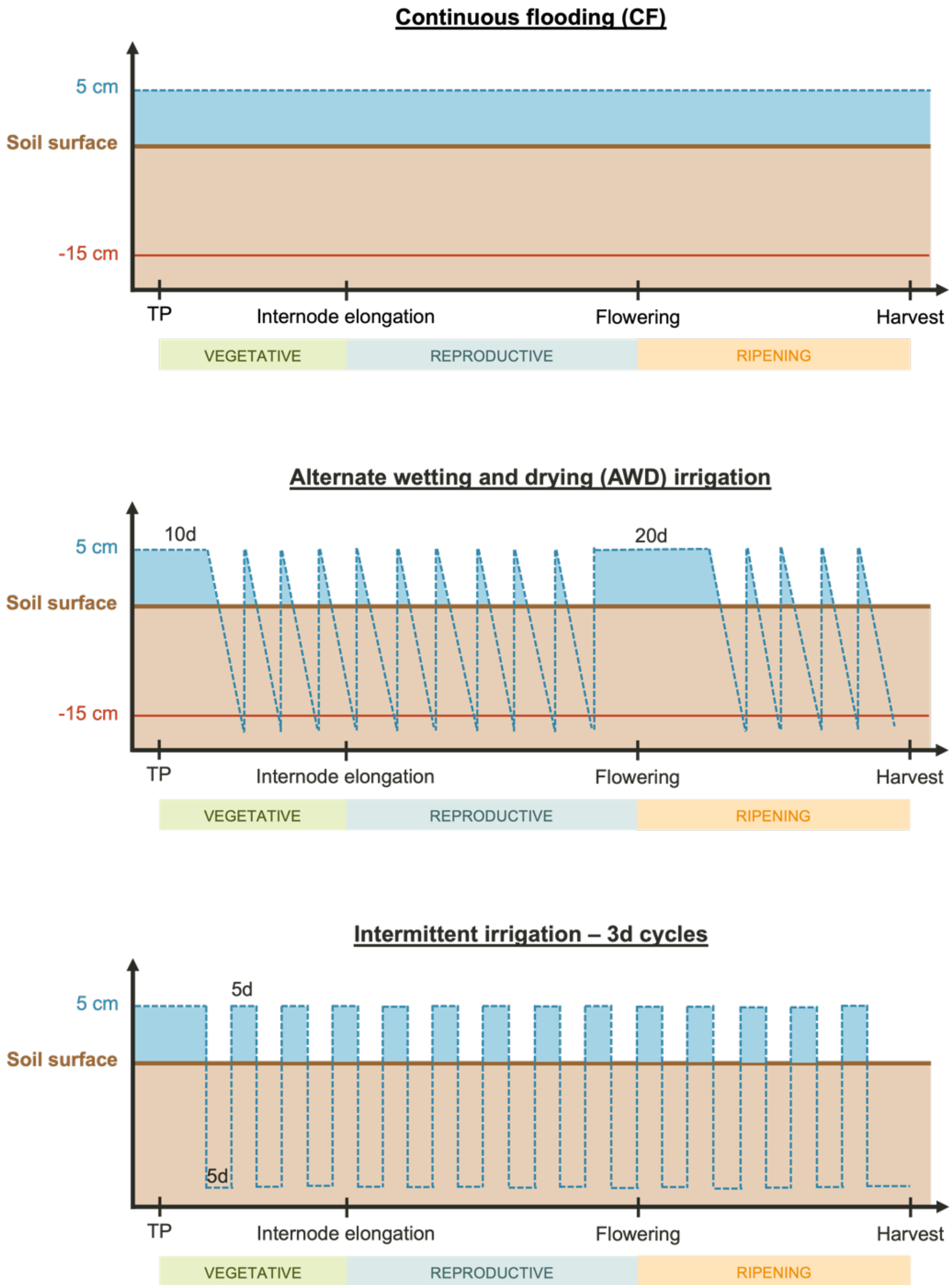

Figure 1.2. Diagrammatic representation of water management techniques in irrigated rice cultivation: continuous flooding, alternate wetting and drying irrigation and intermittent irrigation.

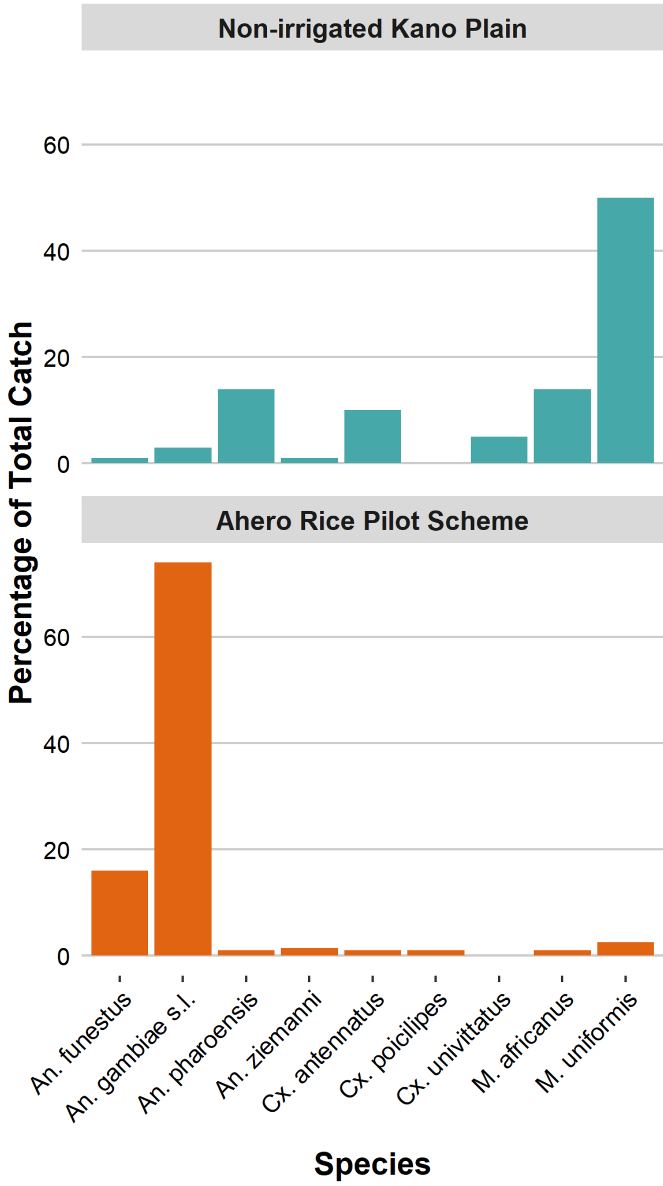

Figure 1.3. Mosquito species found in wetlands vs. irrigated rice. A comparison of indoor CDC light trap catches in two neighbouring locations in Kenya (1971-72): natural, non-irrigated wetlands (turquoise) vs. irrigated rice fields (orange). The total numbers caught are similar, but species composition has completely changed: in the natural wetlands, there is a diverse mixture of mainly non-vector species; in the irrigated rice area, malaria vectors make up 95% of the catch. Histogram is re-drawn from Chandler, Highton, and Hill [42].

1.4.2 Rice-specialist mosquitoes

Since the early 20th century, it has been known (and never contradicted) that rice fields are ideal breeding sites for African malaria vectors [51]. In both East and West Africa, there are sibling species within the *An. gambiae* species complex which specialise in riceland breeding. In East Africa, this is *An. arabiensis,* which is associated with more arid environments [10]. Thus, even in relatively humid settings that would normally be more suitable for *An. gambiae* s.s., *An. arabiensis* is the dominant species in rice fields. For example, in Mwera Zanzibar, a 1980s study comparing catching methods observed that the *gambiae*:*arabiensis* ratio was approximately 50:50 in human bait catches, 75:25 in indoor resting catches and 5:95 in larvae sampled from nearby rice fields [Lines et al., unpublished observations].

In West Africa, *An. coluzzii* (previously known as *An. gambiae* s.s. M form) is the rice-specialist member of the complex [52]. The process of speciation, by which *An. coluzzii* is splitting away from *An. gambiae* s.s., is thought to be recent and ongoing in some places. It seems this process has been driven, at least in part, by the novel breeding opportunities offered by the recent appearance of large, irrigated rice schemes in West Africa [53]. *An. coluzzii* thus is becoming specialised in irrigated rice fields, whilst *An. gambiae* s.s. retains its original adaptations to smaller, temporary habitats like puddles and footprints [54].

1.4.3 Greater vector abundance in rice-growing areas

By the 1940s, it was well known that *An. gambiae* vectors could breed prolifically in rice fields. Adults of these species are especially abundant in villages near rice schemes. For example, in Tanzania, a 12-month study caught a total of 11,000 *An. arabiensis* mosquitoes in a village near a rice irrigation scheme, compared to 5000 and 3000 in irrigated sugarcane and savannah areas, respectively [55]. Similarly, in Burkina Faso, human biting rates (HBR) of *An. gambiae* in rice-growing areas were 10-fold higher than that of a savannah area (36.9 vs. 3.5 females/man/night) [56].

1.5 The effect of rice cultivation on malaria

If there is a greater abundance of malaria vectors in rice areas, it raises the question of whether rice communities are at a greater risk of acquiring malaria. There were a few reports of a clear and strong association between rice and malaria in Africa. In Madagascar, peaks of vector abundance and malaria cases coincided with rice cropping seasons [57–59]. In the Burundian highlands, vectorial capacity of *An. arabiensis* was 150 times greater in rice- than cotton-growing areas, and correspondingly, parasite prevalence amongst children under 5 years old were 50% and 17% respectively [60,61]. However, this clear epidemiological pattern has not been uniform across mainland Africa: in 2001, Ijumba and Lindsay reviewed a set of studies where malaria outcomes had been measured in irrigated rice and nearby non-rice communities [62]. They found that although rice villages had comparatively more vectors, they did not necessarily have higher malaria risk, and called this the "paddies paradox". Over the next few years, a further series of such studies were conducted, which expanded the body of evidence. When it was reviewed by Keiser *et al*. in 2005, confirmed and amplified the "paddies paradox" [63].

It was speculated that this phenomenon occurred due to greater "protection" amongst rice villages and inequities between the rice and non-rice villages. First, wealth creation amongst rice communities led to better socio-economic conditions, housing, education, and access to healthcare services (commercial mosquito nets, anti-malarial drugs, etc.), all of which were hypothesised to compensate for the amplified mosquito numbers [56,64–68]. Second, the numerous mosquitoes caused nuisance, which forced the need for bed-net usage, which in turn decreased human-vector contact [56,69]. For both of these aspects, the community effect of greater bed-net usage in rice communities could have also reduced the force of infection in these communities, even if the total number of mosquito bites was not reduced. Third, it was suggested that higher densities of larvae developing in rice fields generated smaller adults with reduced longevity, and hence lowered efficiency in malaria transmission [66,70,71]. In effect, all these factors allowed rice communities to benefit from more protection against vectors and malaria, which, consequently, reduced transmission. This “paddies paradox” concept was highly influential and ever since, agencies promoting irrigated rice production in Africa have used it to provide reassurance that, despite the exceptionally high production of mosquitoes, rice development in Africa brings more development benefits and is not harmful with respect to malaria [72–74].

The studies that brought about the paradox conclusion were carried out before 2005, when most African settings had very intense background levels of transmission. These high intensities occurred in non-rice villages too, making it harder to detect effects of any further increases in intensity, in other words, “saturation” [75,76]. “Saturation”, explained in further detail in Appendix 1.1, occurs when a large proportion of infectious mosquito bites fall upon people who are already infected. It means that a significant difference in epidemiological indicators like prevalence may not be seen between rice and non-rice areas, even if there was indeed more intense transmission in rice areas according to entomological measures like entomological inoculation rate (EIR). This raises the possibility that in some of those studies, rice communities were subjected to more frequent inoculations of malaria parasites, but there was no observed impact on prevalence because non-rice communities had already reached “saturation” of malaria infection.

In recent years, the malaria situation across Africa has completely transformed: massive scale-up of effective anti-malaria interventions has led to remarkable declines in transmission intensities and intervention coverage has become not only much higher, but also much less inequitable [7,77,78]. Both of these changes (lower transmission intensities and in more recent years) could have implications for the “paddies paradox”. This calls for a re-examination of the relationship between rice cultivation and malaria.

1.6 Rationale

Preventing rice cultivation is not an option since rice is necessary to feed a growing African population. However, the current methods of rice cultivation can have negative side effects on malaria. Therefore, research is urgently needed to understand how the health and agricultural sectors can come together to solve this problem. The health sector, even with its remarkably powerful interventions and working at full stretch, can deliver only a partial and not a complete solution. So perhaps the role of agriculture is to simply stop being part of the problem, and start being part of the solution. To achieve this, a better understanding of how rice can be grown without benefitting malaria vectors is necessary [79]. Whilst the rice-malaria relationship has been the topic of many studies between 1984 and 2002, in the form of case studies, reviews and workshops, many elements of this relationship remain unexplored [27,48,80–82]. They range from miniscule (e.g., preferences in mosquito oviposition) to large-scale characteristics (e.g., malaria risk across different landscapes of rice agrosystems).

Therefore, the aim of this PhD is to examine the effect of rice cultivation practices on vectors, specifically to find improved (combinations of) methods of growing rice that minimise growing malaria vectors. This strategy has arisen primarily due to the opportunity to collaborate with agronomists of Africa Rice Centre (AfricaRice), a pan-African rice research organisation, who have recognised the need to improve farming systems to be more sustainable and environmentally friendly, as well as improving the livelihoods and health of rice farmers. For example, since rice cultivation contributes to 11% of the anthropogenic greenhouse gas emissions, AfricaRice has also been working closely with climate change scientists to find methods of rice cultivation that minimise carbon emissions [83].

The effect of the vastly varied rice cultivation practices on vectors must be properly understood. Some of these effects have been previously summarised by Lacey and Lacey and International Rice Research Institute (IRRI)[2], but these two reviews, conducted worldwide and 30 years ago, did not have a strong focus on Africa [48]. Thus, an update is required, with particular attention

[2] In collaboration with the WHO/FAO/UNEP Panel of Experts on Environmental Management for Vector Control (PEEM)

towards African malaria vectors and identifying any control methods that may be effective in reducing them.

Rice experts are constantly striving to improve practices to reduce the large yield gap in African rice production. They anticipate future obstacles and search for ways to mitigate their impact on rice production, such as how labour shortages are expected to bring about a shift in crop establishment methods (from transplanting to direct-seeding) and towards more effective nutrient management [84]. Agronomists are also promoting sustainable rice production, which entails harnessing practices to minimise environmental impact and improve farmer livelihoods [85]. One such practice of interest is alternate wetting and drying irrigation (refer to Box 1.1 for more details), a climate-smart water-saving technology that significantly produces less methane [86,87]. In this sphere, important comparisons can be made between mosquitoes and methane: like mosquitoes, methane is produced as a side-effect of irrigation. However, unlike their response towards mosquitoes, the rice sector has acknowledged and started to address the problem of greenhouse gas emissions. Thus, they now need to address the problem of malaria vectors in Africa: when techniques in rice cultivation are being renewed at rice research institutes like AfricaRice, their effect on vector abundance should also be determined so that agriculture and health co-benefits can be realised. Vector abundance could be added onto the list of measures that agronomists take into account of whilst they are experimenting with rice-growing technologies. Such integration would also be considered more sustainable than other control methods that rely heavily on the health sector, such as large-scale larvicide application or providing more ITNs to rice farmers.

If the research and development task to develop rice-growing methods that maintain (or improve) yield and minimise mosquito production is to be adopted by rice experts, methods of sampling mosquitoes in rice fields must be improved. However, mosquito monitoring poses a few challenges related to precision, bias and logistics. First, "dipping" is often used as the standard technique to sample mosquito immatures. Although, in comparison to area samplers, it is efficient in providing rapid estimates of relative density, it is not as precise in estimating absolute population density [88–90]. Second, most larval *Anopheles* sampling methods are designed for small, temporary breeding sites [89]. Rice paddies have neither characteristic: they span across large areas and are relatively permanent, lasting 4-5 months per season. This creates inherent biases in the estimates obtained by dipping. Third, mosquito breeding is very patchy and unstable, so it is difficult to clearly distinguish the appropriate conditions which allow mosquitoes to proliferate [89]. Riceland mosquito monitoring therefore requires regular visits with short intervals. Fourth, for most purposes, vector abundance is quantified by the number of adult females per person, trap, or house, but to measure productivity, estimates of the number of emerging adults per hectare of rice field would be required. However, on the one hand, regularly enumerating adult populations emerging from rice paddies is too labour-intensive [91]. On the other hand, larval density may not be the most representative measure, as it can overestimate vector quantities by not factoring in mortality. As a compromise, dipping for pupae may be more appropriate. All things considered, it is vital to understand the population distribution of mosquitoes (especially pupae) within rice fields, in order to conduct representative (and efficient) riceland mosquito sampling and help evaluate the effect of rice cultivation practices on vector productivity.

If rice cultivation practices that minimise mosquito production are to be adopted by farmers, methods that clearly benefit farmers are required. Rice farmers, who often live close to their fields, are very familiar with mosquitoes and, their children, with malaria [92–95]. In some African countries, malaria was even perceived to disrupt rice operations [96]. Nonetheless, with the exception of a few cases, farmers' motives to adopt new technologies fundamentally involve economic benefits, rather than issues concerning mosquito nuisance or health (or even the environment) [27,94,97,98]. These observations solicit questions on how rice farmers feel towards mosquitoes, how they view themselves in mosquito production, what would motivate them to change their rice-growing practices and how they could cooperate to implement collective vector control.

By exploring all these components, the relationship between rice and malaria can be better understood. Importantly, recommendations for irrigated lowland rice development in Africa that can maximise rice productivity whilst simultaneously minimising vector productivity can be developed.

2 Aims, objectives, and study sites

2.1 Overall aim

To identify potential routes of advancing malaria control in rice fields.

2.2 Objectives

Specific Objective 1

To re-assess the literature on the relationship between rice cultivation, malaria vectors and malaria in Africa, through:

a) A re-examination of the association between rice growing and malaria **(Chapter 3)**, and
b) A systematic review on the effect of rice cultivation practices and mosquito control interventions on riceland malaria vectors **(Chapter 4)**

Specific Objective 2

To investigate the mosquito population dynamics in rice fields **(Chapter 5)**, specifically the:

a) Mosquito species composition during a rice season,
b) Temporal distribution of mosquitoes throughout a rice season,
c) Spatial distribution of mosquitoes within a rice field, and
d) Vector productivity of rice fields during a rice season

Specific Objective 3

To investigate the effect of selected, modified rice cultivation practices on mosquito productivity and species composition through field experiments **(Chapter 6)**

Specific Objective 4

To explore rice farmers' views and perspectives on the effect of rice farming practices on mosquitoes **(Chapter 7)**

2.3 Study area description

These studies were conducted in a West African and an East African country: Côte d'Ivoire and Tanzania. Table 2.1 describes the malaria and rice situation in both countries [99–102].

Table 2.1. Malaria epidemiological and rice profiles of study countries.

	Côte d'Ivoire	**Tanzania**
Population at risk of malaria	Entire population (26 million)	Entire population (59 million)
Malaria parasites (%)	*Plasmodium falciparum* (95%), *P. malariae* and *P. ovale* (<5%)	*Plasmodium falciparum* (95%), *P. malariae* (<5%)
Transmission season(s)	Transmission is year-round, with peaks during the rainy seasons	Transmission is year-round with peaks occurring at the end of each rainy season
Main vector(s)	*An. gambiae* s.s., *An. coluzzii* and *An. funestus* s.s	*An. gambiae* s.s., *An. arabiensis* and *An. funestus* s.s.
Malaria development goal	To reduce malaria incidence and mortality by 75% by 2025 (compared to the 2015 baseline of 147 cases per 1000 population at risk)	To reduce malaria prevalence in children under 5 years of age from 7% in 2017 to <3.5% in 2025
Importance of rice	The third most important food staple (following yams and plantains) in the country	Second most important food and commercial crop after maize. Second largest producer of rice in Eastern and Southern Africa after Madagascar.
Distribution of rice agro-ecosystems	64% upland 19% rainfed lowland 14% irrigated	71% rainfed lowland 20% upland 9% irrigated
National rice production and harvested area	Presently, annual national rice production is unable to meet even half of an estimated 1.5 million tonnes of national consumption.	Rice is cultivated on around 700 million hectares

National rice development strategy	Côte d'Ivoire still has much unexploited potential, with abundant rainfall and significant land area suitable for rice cultivation. 1. Development of a seed sector 2. Rehabilitation of all sites previously developed for irrigated rice growing and carry out development on floodplains	The rice sub-sector has long been identified as a strategic priority for agricultural development due to its potential for improving food security and income. 1. Double the area under rice cultivation from 1.1. million ha (2018) to 2.2. million ha by 2030 2. Double on-farm rice productivity from 2 t/ha to 4 t/ha by 2030

In central Côte d'Ivoire, studies were conducted in the Gbêkê region of the Vallée du Bandama district (Figure 2.1A). According to the 2016 Malaria Indicator Survey, this region has a malaria prevalence of 38% amongst children under 5 [103]. Seasons are distinguished by rainfall (annual average of 1373 mm), where rainy season occurs from April to October whilst dry season is between November to March [104]. There is an annual minimum and maximum temperatures between 22°C and 33°C and mean relative humidity of 71% in this region [105]. In this area, subsistence agriculture is a major livelihood, the dominant crop being rice [106].

In east coast Tanzania, studies were conducted in the Bagamoyo district of the Pwani region (Figure 2.1B). Malaria transmission in this district is considered moderate, with 5% prevalence amongst children under 5 in 2017 [107]. Malaria peaks tend to occur during the rainy seasons from May to July and November to December [101]. There is an average annual rainfall of 1818 mm, relative humidity of 77%, minimum temperature of 22°C and maximum temperature of 31°C [108,109]. Most of the land area is devoted to crops, particularly maize and rice paddy [110].

2.4 Study design and site selection

These studies were conducted in different locations and at two spatial scales, plot- and village-level (Table 2.2).

Table 2.2. Study sites and descriptions.

Location	Description	Objective(s)
Experimental rice plots at AfricaRice's research station (M'bé), Côte d'Ivoire	- Located within low-lying plains, which compose the vast majority of land in West Africa - Characterised by the presence of water control, a slope of 0 to 1% and poorly drained and deep clayey soils[1] [78] - Experimental rice plots were selected based on land availability within the station	2 and 3
Experimental rice plots at IRRI's research station (Bagamoyo), Tanzania	- Located within the Ruvu river basin - Soils were moderately deep to deep, imperfectly to poorly drained, grey-brown massive heavy clays [111] - In this area, flooded rice is commonly produced on foot slopes and flood plains on hydromorphic soils[1] [112]	3
Rice-farming villages near Bouaké, Côte d'Ivoire	- Rice villages where communities work in the two neighbouring two irrigation schemes (M'bé and Lokapli) - Villages were selected based on their distance from rice fields (<5 km), if their dominant crop was rice and if farmers conducted two cropping seasons of rice	4

[1] Clay soils are heavy soils that benefits from high nutrients and become stiff when they are dry

[2] Soils characterised by the reduction or localised segregation of iron, owing to the temporary or permanent waterlogging of the soil pores, which causes a lack of oxygen over a long period

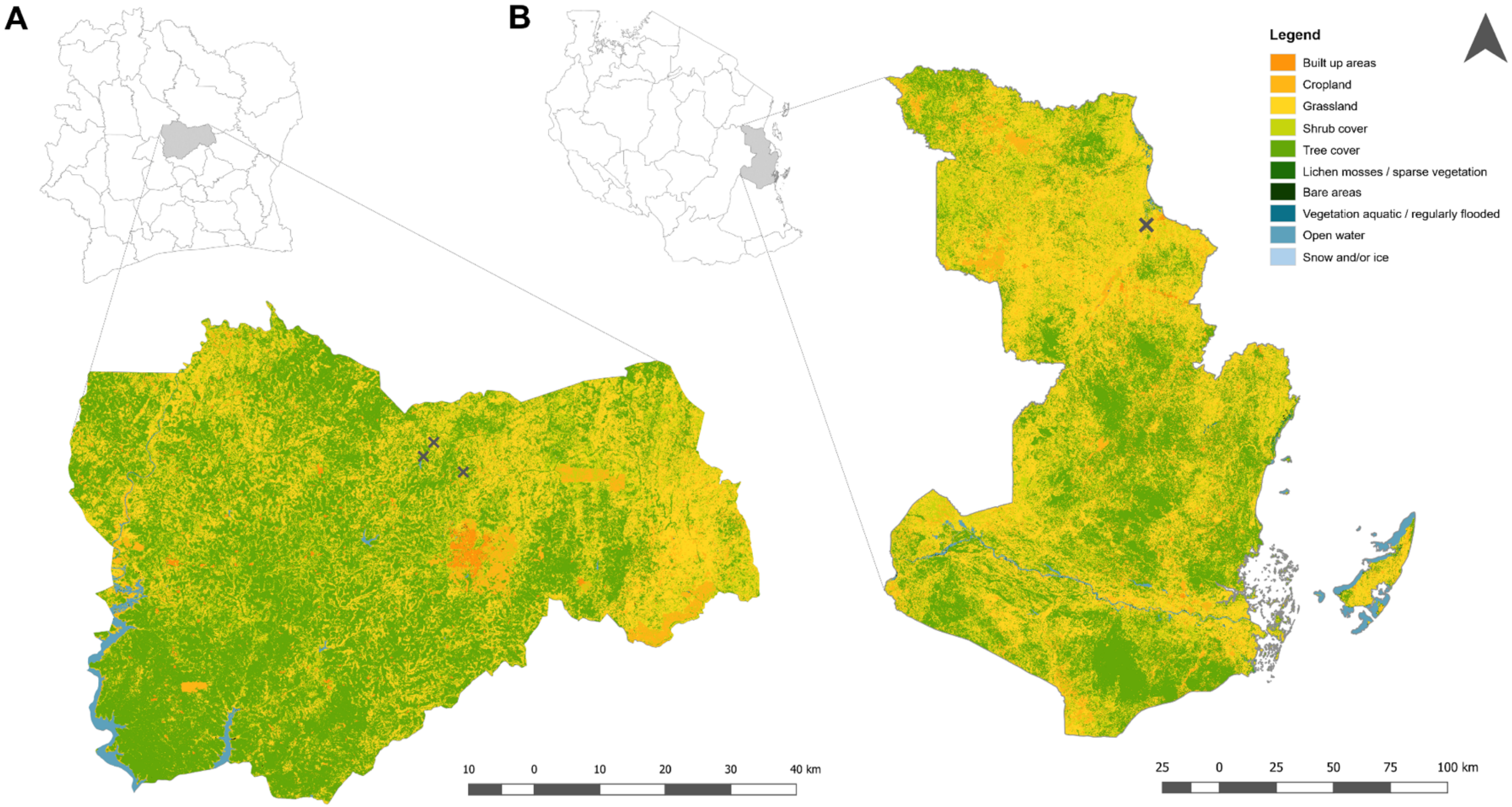

Figure 2.1. Geographical location of the **(A)** Gbêkê region relative to Côte d'Ivoire and **(B)** Pwani region relative to Tanzania, and the location of the study areas, in relation to land cover (obtained from European Space Agency Climate Change Initiative, 2016).

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Surname	Chan		
Thesis Title	Rice and malaria in Africa: Seeking vector control in rice cultivation practices		
Primary Supervisor	Jo Lines		

If the Research Paper has previously been published, please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	The Lancet Planetary Health		
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Date	12/08/2022	**Date**	12/08/2022

3 Malaria transmission and prevalence in rice-growing versus non-rice-growing villages in Africa: a systematic review and meta-analysis

Authors

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3.1 Abstract

Background

Rice fields in Africa are major breeding sites for malaria vectors. However, when reviewed in the 1990s, in settings where transmission was relatively intense, there was no tendency for malaria indices to be higher in villages with irrigated rice fields than in those without. Subsequently, intervention coverage in sub-Saharan Africa has been massively scaled up and malaria infection prevalence has halved. We re-examined this rice–malaria relationship to assess whether, with lower malaria transmission, malaria risk is greater in rice-growing than in non-rice-growing areas.

Methods

For this systematic review and meta-analysis, we searched EMBASE, Global Health, PubMed, Scopus, and Web of Science to identify observational studies published between Jan 1, 1900, and Sept 18, 2020. Studies were considered eligible if they were observational studies (cross-sectional, case-control, or cohort) comparing epidemiological or entomological outcomes of interest between people living in rice-growing and non-rice-growing rural communities in sub-Saharan Africa. Studies with pregnant women, displaced people, and military personnel as participants were excluded because they were considered not representative of a typical community. Data were extracted with use of a standardised data extraction form. The primary outcomes were parasite prevalence (*P. falciparum* parasite rate age-standardised to 2–10-year-olds, calculated from total numbers of participants and number of infections [confirmed by microscopy or rapid diagnostic test] in each group) and clinical malaria incidence (number of diagnoses [fever with *Plasmodium* parasitaemia confirmed by microscopy or rapid diagnostic test] per 1000 person-days in each group). We did random-effects meta-analyses to estimate the pooled risk ratio (RR) for malaria parasite prevalence and incidence rate ratio (IRR) for clinical malaria in rice-growing versus non-rice-growing villages. RRs were compared in studies conducted before and after 2003 (chosen to mark the start of the mass scale-up of antimalaria interventions). This study is registered with PROSPERO (CRD42020204936).

Findings

Of the 2913 unique studies identified and screened, 53 studies (including 113 160 participants across 14 African countries) were eligible for inclusion. In studies done before 2003, malaria parasite prevalence was not significantly different in rice-growing versus non-rice-growing villages (pooled RR 0·82 [95% CI 0·63–1·06]; 16 studies, 99 574 participants); however, in post-2003 studies, prevalence was significantly higher in rice-growing versus non-rice growing villages (1·73 [1·01–2·96]; seven studies, 14 002 participants). Clinical malaria incidence was not associated with residence in rice-growing versus non-rice-growing areas (IRR 0·75 [95% CI 0·47–1·18], four studies, 77 890). Potential limitations of this study include its basis on observational studies (with evidence quality rated as very low according to the GRADE approach), as well as its omission for the effects of seasonality and type of rice being cultivated. Risk of bias and inconsistencies was relatively serious, with I^2 greater than 90% indicating considerable heterogeneity.

Interpretation

Irrigated rice-growing communities in sub-Saharan Africa are exposed to greater malaria risk, as well as more mosquitoes. As increasing rice production and eliminating malaria are two major development goals in Africa, there is an urgent need to improve methods for growing rice without producing mosquitoes.

Funding

Wellcome Trust Our Planet Our Health programme, CGIAR Agriculture for Nutrition and Health

3.2 Introduction

Rice cultivation and malaria are linked in sub-Saharan Africa because of two biological characteristics of the most important African mosquito vector, *Anopheles gambiae* sensu lato (s.l.; the species complex referring to *Anopheles gambiae* sensu stricto [s.s.], *Anopheles coluzzii,* and *Anopheles arabiensis*). The first of these characteristics is that adults of this species complex are long-lived and prefer to bite humans, making them exceptionally efficient in transmitting malaria [10]; this fact is why Africa accounts for 96% of the world's malaria mortality burden, with approximately 602 000 of the 627 000 global malaria deaths occurring in the region in 2020 [2]. The second characteristic is that the larvae of *An. gambiae* s.l. are very well adapted to, and can breed abundantly in, the aquatic conditions in rice fields [48]. Against this biological background, in many African countries, ministries of agriculture and their partners are planning for a massive expansion in irrigated rice cultivation, in response to rapidly increasing consumer demand [113]. Rice is the fastest growing food in Africa; harvested areas increased by over 600% from 1961 to 2019 [20]. Meanwhile, ministries of health and their partners are working towards the eventual elimination of malaria. Therefore, it is important to consider the potential interactions between these two development processes, and whether they might interfere with one another.

The links between rice and malaria were studied in a series of case studies in west and east Africa during the 1990s and early 2000s [62,63]. An overall review of the findings revealed that, although mosquito vectors (especially *An. gambiae* s.l.) were substantially more abundant in villages beside irrigated rice fields than in nearby non-rice-growing areas, the prevalence of malaria in rice-growing villages was unexpectedly either the same as or slightly lower than that in non-rice-growing control communities. Ijumba and Lindsay coined the term “paddies paradox” to describe this phenomenon [62]. Investigations into the possible causes of this paradox suggested that, in many cases, rice cultivation also brought substantial economic benefits, particularly improvements to family income (and hence better access to commercial mosquito nets and antimalarial drugs) and community infrastructure (housing, transport, and health services). Thus, families could protect themselves and respond to malaria episodes more promptly and effectively [56]. Density-dependent effects could also contribute: some studies [71,114–117] found a reduction in vectorial capacity at high mosquito densities through reduced adult longevity (probably due to greater larval competition) and reduced blood feeding success (probably due to greater use of bed nets). For 20 years, this paradoxical conclusion has helped to reassure rice experts in Africa that they are contributing to development and not making the malaria problem worse [73].

We were prompted to re-examine this conclusion because the malaria situation across sub-Saharan Africa has changed radically in the past two decades. The massive upscaling in coverage of modern malaria control interventions (such as insecticide-treated nets and antimalarial drugs) has greatly reduced the intensity of transmission for most of the at-risk population in Africa, where the population exposed to hyperendemic or holoendemic transmission has fallen from 33% to 9% [7]. Moreover, there is clear evidence that intervention scale-up has reduced previous inequities in bed-net coverage, suggesting less severe inequality between rice-growing and non-rice-growing villages [78]. Furthermore, the paddies paradox was often interpreted as an implication that the extra mosquitoes from rice fields were generally harmless, which was misleading. Therefore, we re-examined whether these recent changes in malaria epidemiology have altered the relationship between malaria risk and irrigated-rice cultivation in Africa.

3.3 Methods

3.3.1 Search strategy and selection criteria

We did a systematic review and meta-analysis following the PRISMA reporting guidelines [118]. EMBASE, Global Health, PubMed, Scopus, and Web of Science were searched without language restrictions to identify studies published between Jan 1, 1900, and Sept 18, 2020 (the end date of our search). Combinations of the following keywords and Medical Subject Headings were used: malaria, *Plasmodium falciparum*, prevalence, incidence, risk, Africa, rice, padd*, and irrigation. The full search strategy is summarised in Appendix 3.1. Additional references were identified using citation searches of obtained articles, conference proceedings (such as the Multilateral Initiative on Malaria's Pan-African Malaria Conferences and the American Society of Tropical Medicine and Hygiene) and contact with authors.

The inclusion criteria for epidemiological studies were as follows: studies with participants of any age residing in sub-Saharan Africa; studies with a cross-sectional, case-control, or cohort design; studies conducted in rice-growing and non-rice-growing areas; and studies reporting on any epidemiological outcomes of interest (parasite prevalence or malaria incidence). Studies with pregnant women, displaced people, and military personnel as participants were excluded because they were considered not representative of a typical community. The inclusion criteria for entomological studies were as follows: studies with a cross-sectional, case-control, or cohort design; studies conducted in rice-growing and non-rice-growing areas; and studies reporting on any entomological outcomes of interest (human biting rate, sporozoite rate, and entomological inoculation rate), reported as summary estimates. The titles and abstracts of studies identified by the searches were screened by KC and JL, and, for those that were potentially relevant, full texts were assessed. Any conflicts were resolved by LT.

The protocol for this study is available online.

3.3.2 Data analysis

Data on the following study variables were extracted using a predefined and standardised form: participants (age and recruitment methods), sampling method (i.e., type of mosquito trap and ascertainment of malaria positivity [microscopy or rapid diagnostic test]), exposures (i.e., residence in rice-growing or non-rice-growing area), comparisons (type of rice growing [number of cropping seasons] vs type of non-rice-growing area [control area]), epidemiological and entomological outcomes (parasite prevalence, malaria incidence, human biting rate, sporozoite rate, entomological inoculation rate), summary measures (odds ratio [OR], risk ratio [RR], and incidence rate ratio [IRR], including adjusted values), study design, setting (physical environment [i.e., semi-arid, forest, highlands, coastal]), sample size, vector species, long-lasting insecticidal net and indoor residual spraying coverage, and malaria transmission intensity. Data were extracted by KC and a 10% sub-sample was randomly selected for validation by JL. Any duplicate data (i.e., multiple reports from the same study) were excluded.

The primary outcomes were epidemiological outcomes in human participants: parasite prevalence (confirmed by microscopy or rapid diagnostic test, in any age group) and malaria incidence (fever with *Plasmodium* parasitaemia confirmed by microscopy or rapid diagnostic test, in any age group). Secondary outcomes were entomological indices of interest: human biting rate (the number of mosquitoes in contact with a person per night), sporozoite rate (the percentage of female *Anopheles* mosquitoes with sporozoites in the salivary glands), and entomological inoculation rate (the estimated number of infective bites per person per year, which is a product of human biting rate and sporozoite rate). Indoor and outdoor human landing catches were considered the gold standard for measuring entomological outcomes, followed by Centers for Disease Control and Prevention (CDC) light traps or pyrethrum spray catches.

For continuous outcomes (human biting rate and entomological inoculation rate), the arithmetic or geometric means, corresponding SDs or SEs, and number of participants in exposed and control groups were extracted. For dichotomous outcomes (sporozoite rate and parasite prevalence), the total numbers of participants and events in each group were extracted. For count data (clinical malaria episodes), the number of events and the total person-time at risk in each group were extracted. Adjusted effect sizes of entomological and epidemiological outcomes, where reported, were also extracted. Study authors were contacted for missing data.

Analyses were structured first by outcome, second by vector species (if applicable), and third by study design. All eligible studies were included in a qualitative analysis. Studies were also analysed semi-quantitatively if sufficient data to calculate crude effects were reported (but 95% CIs were not reported) and quantitatively if crude or adjusted effects with 95% CIs were reported. Because age is an important source of heterogeneity in parasite prevalence data, *P. falciparum* parasite rates were age-standardised to 2–10-year-olds ($PfPR_{2-10}$, i.e. up to 119 months old) to enable study comparability using a modified Pull and Grab algorithm, via an R package called ageStand [119].

Entomological and epidemiological data were combined in meta-analyses via the R metafor package [120]. Regardless of heterogeneity (I^2), random-effects models were used to calculate pooled (crude or adjusted) effect measures from quantitative studies only (ratio of means [ROM] for quantitative outcomes, RR for dichotomous outcomes, and IRR for clinical malaria), as well as corresponding 95% CIs, to illustrate the effect of rice cultivation on each outcome of each study. Separate meta-analyses were done for crude and adjusted results.

To evaluate the effect of the recent changes in malaria on the rice–malaria relationship, effect sizes were analysed in two ways. First, we did a subgroup analysis in which studies were separated by whether they were done before 2003 or from 2003 onwards; this cut-off year was chosen partly because it was the time at which previous reviews reached the paddies paradox conclusion, but mainly because it was when intervention scale-up started [121]. Antimalarial interventions started scaling up in sub-Saharan Africa between 2001 and 2005, varying between countries, and so 2003 was chosen as the midpoint to represent this change. A sensitivity analysis between these years (2001 and 2005) was done to evaluate the robustness of the year 2003 as a cut-off point. Second, a Pearson's correlation test was done between study effect sizes (log-transformed) and their underlying malaria intensity (parasite prevalence in the control group), weighted for the precision of the effect sizes. Results from the meta-analyses and subgroup analyses were depicted using bar graphs.

Risk of bias for cross-sectional and cohort studies was assessed using the Newcastle-Ottawa Scale [122]. Publication bias was assessed by the visual inspection of funnel plots and the Egger's test for funnel plot asymmetry [123]. Quality and strength of the evidence were evaluated using the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) approach [124].

This study is registered with the International Prospective Register of Systematic Reviews (CRD42020204936).

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

3.4 Results

Our search yielded 2913 studies after removal of duplicates (Figure 3.1). 53 studies [42,45,55,56,59–61,64–70,125–163] (with a total of 113 160 participants) met the inclusion criteria, various subsets of which were included in the quantitative, semi-quantitative, and qualitative analyses depending on the outcome of interest (Appendix 3.2) [124]. (43%) studies reported data on parasite prevalence, five (9%) on malaria incidence, 36 (68%) on human biting rate, 22 (42%) on sporozoite rate, and 19 (36%) on entomological inoculation rate. A description of the included studies can be found in the Appendix 3.2. All studies were conducted between 1971 and 2016 in rural settings across 14 sub-Saharan African countries. 27 studies were done in west Africa (eight countries), six studies in central Africa (Cameroon), and 20 studies in east Africa (five countries). Descriptions of study areas reported that the type of rice grown varied, and included swamps, rain-fed rice, (small-scale) traditional flooded irrigated rice, and (large-scale) rice irrigation schemes. Control villages were usually 5–20 km away from rice-growing villages and engaged in traditional crop farming, market gardening, sugar plantations, pastoralism, or were savannah areas and inland valleys without rice cultivation.

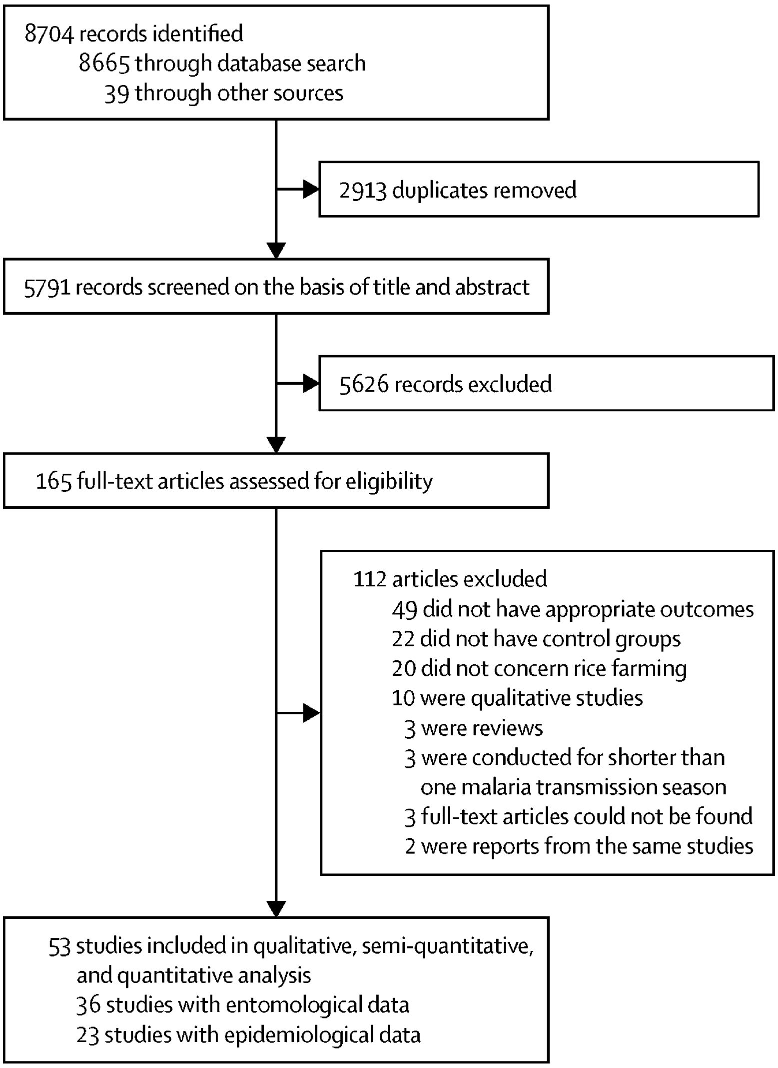

Figure 3.1. Study selection

22 studies reported malaria prevalence in rice-growing and non-rice-growing villages and were included in the meta-analysis, with 16 studies [61,64,65,67–70,126,127,129,132–134,136,141,144] conducted before 2003 and seven studies [67,152,154,158,161–163] since 2003 (one study included analyses both before and since 2003; Figure 3.2A). Before 2003, rice-growing was not associated with increased malaria prevalence (crude RR 0·82 [95% CI 0·63–1·06], 16 studies, 99 574 participants; adjusted OR [aOR] 0·73 [95% CI 0·57–0·89], two studies, 11 955 participants; Appendix 3.3).49, 52 From 2003 onwards, however, there was a 73% greater risk of malaria infection in rice-

growing than in control villages (1·73 [1·01–2·96], seven studies, 14 002 participants; 7·69 [2·72–12·66], one study, 1019 participants) [161]. A Wald-type test indicated that the pooled RR estimated from studies conducted since 2003 was significantly different from that of studies before 2003 (p=0·014). The sensitivity analysis found that 2003 was a robust year to mark the start of the scale-up of interventions; the pooled RRs from post-scale-up studies were unaffected by the choice of the cut-off year, but the pre-scale-up RR moved towards 1 as cut-off year increased (Appendix 3.3). When we assessed whether the effect of rice growing on malaria was influenced by the underlying malaria intensity (*Pf*PR_{2-10}), we found an increase in effect size with decreasing malaria prevalence in the control (non-rice-growing) villages (coefficient –0·429 [95% CI –0·698 to –0·160], p=0·019). Where malaria prevalence was very high (>75%) in control villages, there was almost no difference in prevalence in rice-growing villages; areas where prevalence was medium to high (26–75%) in control villages mostly had a lower prevalence in rice-growing villages; and, conversely, in areas with low prevalence (≤25%) in control villages, malaria risk was usually higher in rice-growing villages (Figure 3.2B).

There was no association between rice cultivation and clinical malaria (IRR 0·75 [95% CI 0·47–1·18], four studies, 77 890 participants; Figure 3.2C) [68,136,141,158]. Although a trend with time is visible, this trend was not significantly different from zero (Pearson's product moment correlation test weighted for precision of effect sizes: coefficient 0.827, p=0.173).

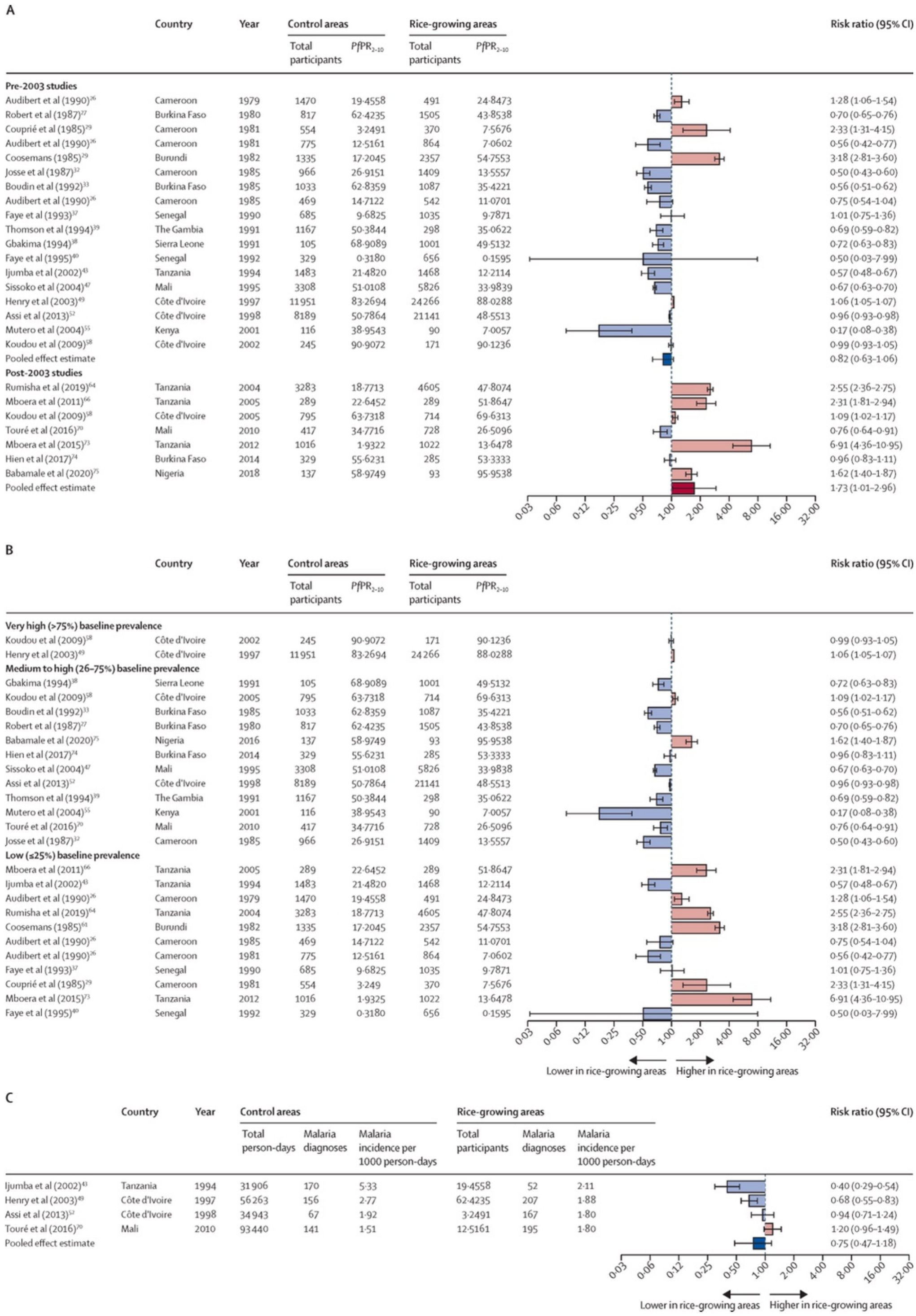

Figure 3.2. Meta-analyses of the association between residence in rice-growing areas and malaria epidemiological outcomes. Crude risk ratios for malaria infection prevalence ($PfPR_{2–10}$) plotted ordered **(A)** by year of study and

subgroup (before and after 2003), and **(B)** by underlying malaria intensity (*Pf*$PR_{2–10}$ in control group). An increase in effect size was found with decreasing malaria prevalence in the control (non-rice-growing) villages (coefficient –0·417 [95% CI –0·688 to –0·034], p=0·038). **(C)** Crude incidence rate ratios for clinical malaria incidence (per 1000 person-days) ordered by year of study. Pooled effect estimates based on quantitative studies, calculated using random-effects models, are presented at the bottom of the graphs (and separately for each subgroup in panel A). Error bars are 95% CIs. *Pf*$PR_{2–10}$=*Plasmodium falciparum* parasite rate age-standardised to 2–10-years age group.

36 studies collected entomological outcomes, all of which reported comparative figures on Anopheles human biting rates in rice-growing and non-rice-growing villages. Human biting rates were mostly measured directly using human landing catches (27 studies), and, in some circumstances, indirectly using CDC light traps (seven studies) or pyrethrum spray catches (two studies). In most studies (n=35), *An. gambiae* s.l. was the dominant vector, followed by *Anopheles funestus* and *Anopheles pharoensis* (Figure 3.3). It was not determined which sibling species of the *An. gambiae* s.l. species complex was predominant because only eight studies conducted identification to that level. Where sibling species identification was done, the dominant species were *An. arabiensis* in Cameroon and east Africa (seven studies), and *An. gambiae* s.s. (molecular form unknown) in Nigeria (one study). Meta-analysis of the four quantitative studies [59,67,148,150] that measured the human biting rate of *An. gambiae* s.l. (from 1971 to 2016) showed a pooled effect (ROM) of 6·54 times (95% CI 1·99–21·46) higher human biting rate in rice-growing villages than in non-rice growing villages. Vector densities were consistently higher in rice-growing than in non-rice-growing communities (Figure 3.3 and Figure 3.4). After taking into account 31 semiquantitative studies [[42,45,55,56,60,61,66,69,125,126,128,130,131,134,135,137–139,142–146,149,151,153,155–157,159,160] (those reporting crude effects without CIs), the median vector density in rice-growing villages was 34·0 bites per person per night (IQR 13·4–63·0), which is more than eight times greater than

in non-rice villages (4·2 bites per person per night [1·0–12·8]). In the three most extreme cases, human biting rates were more than 30 times greater in rice-growing than in non-rice-growing villages. *An. gambiae* s.l. collected from rice-growing villages had 71% lower sporozoite rates than those found in non-rice-growing villages (RR 0·29 [95% CI 0·19–0·46], 17 studies) [55,56,59,66,67,69,126,130,143,146,150,151,153,155–157,159]. A Pearson's product moment correlation test (weighted for precision of effect sizes) did not reveal a significant trend with time (coefficient 0.437, p=0.06).

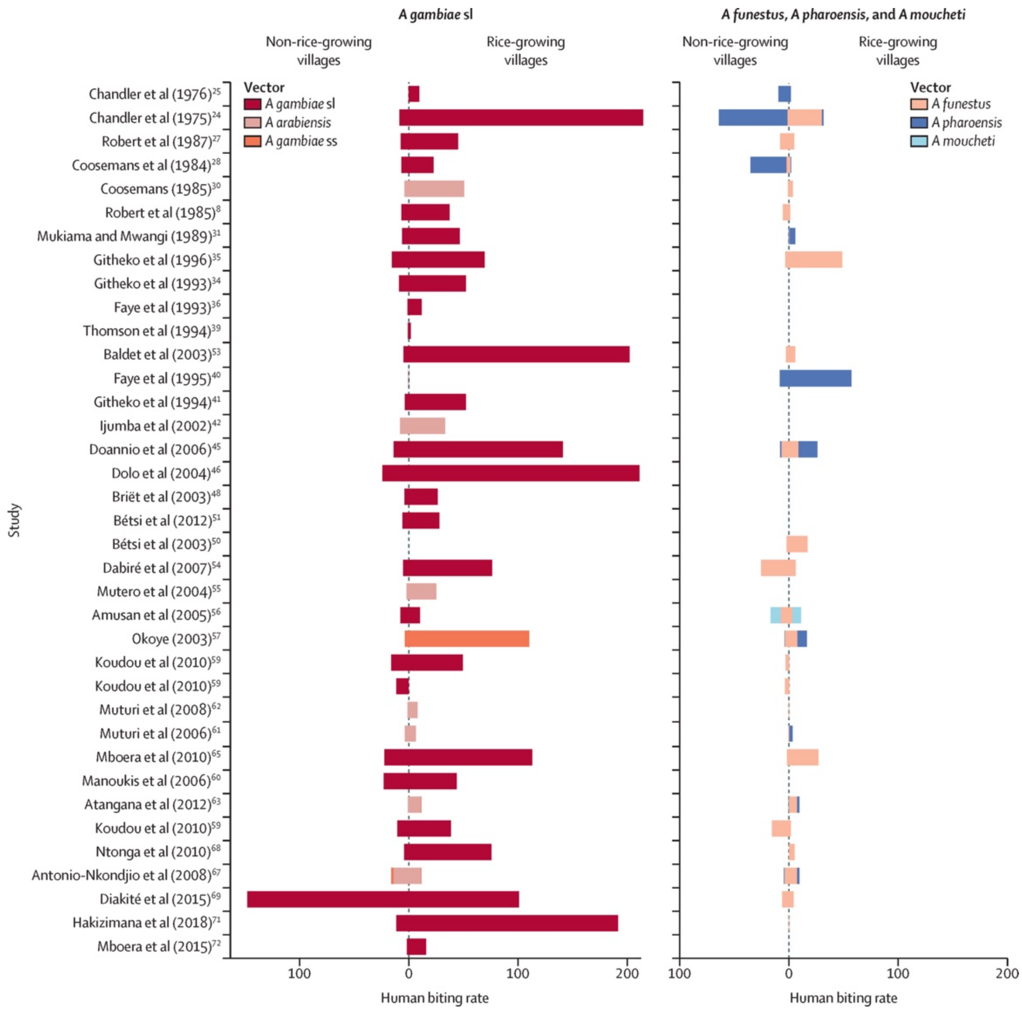

Figure 3.3. Human biting rate in non-rice-growing and rice-growing villages. Comparison of the human biting rate (mosquitoes per person per night) of major malaria vectors in non-rice-growing and rice-growing villages in Africa,

by vector species (*An. gambiae* s.l. [including *An. arabiensis* and *An. gambiae* s.s.] and other species [*An. funestus*, *An. pharoensis*, and *An. moucheti*]). Studies are ordered by year of study (some studies had data for more than one year). In most instances, *An. gambiae* s.l. was the dominant species in rice-growing areas and *An. funestus* and *An. pharoensis* were found in lower densities.

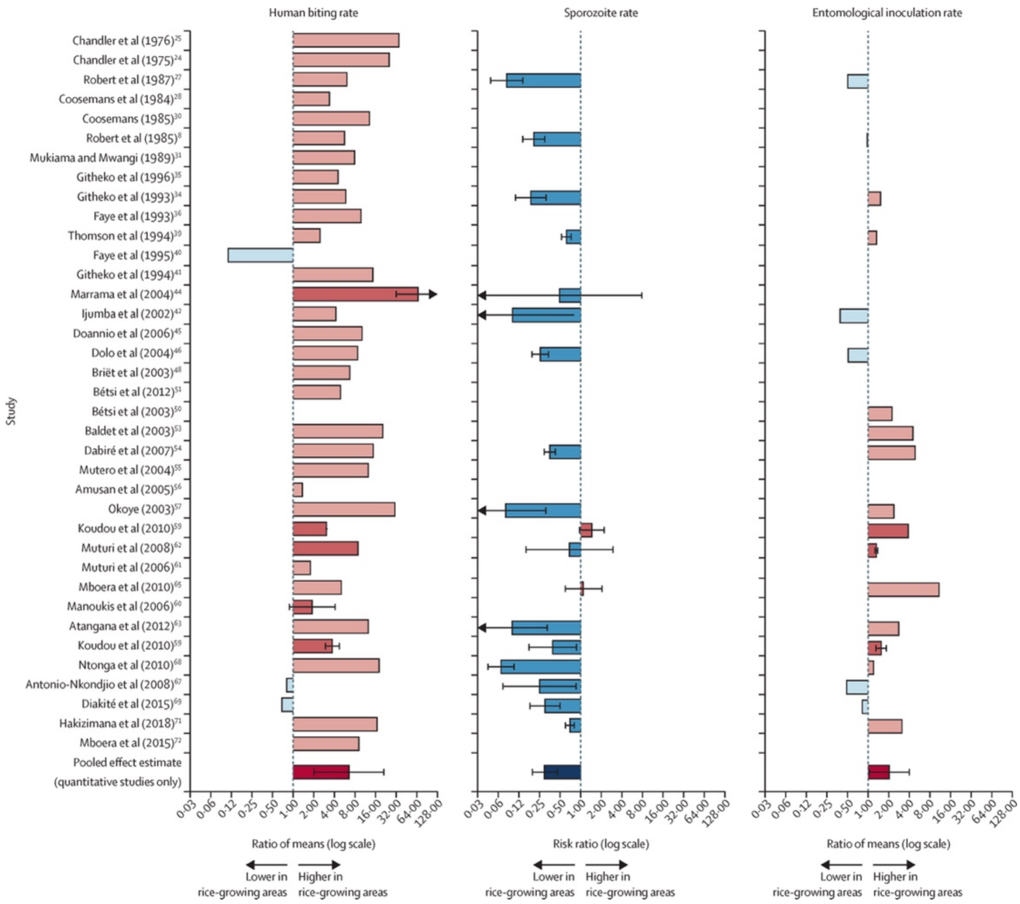

Figure 3.4. Meta-analyses of the association between residence in rice-growing areas and entomological outcomes. Association of *Anopheles gambiae* s.l. and/or *An. funestus* s.l. human biting rate, sporozoite rate, and entomological inoculation rate with rice-growing areas (as compared with non-rice-growing areas). Error bars are 95% CIs (for quantitative studies only). Studies are ordered by year of study. Semi-quantitative studies are represented by lighter-coloured bars and quantitative studies are represented by darker-coloured bars. Pooled effect estimates based on quantitative studies

and calculated using random-effects models are presented at the bottom of each graph.

In quantitative studies that reported the entomological inoculation rate of *An. gambiae* s.l., this rate was doubled in rice-growing compared with non-rice-growing villages (ROM 2·03 [95% CI 1·02–4·06], two studies [three analyses]) [147,150]. In semiquantitative studies, estimates of entomological inoculation rate were higher in rice-growing villages than in non-rice-growing villages in ten studies [69,130,139,142,143,146,151,153,156,159] and lower in six studies (Figure 3.4) [55,56,66,126,155,157]. Including the results from quantitative studies [147,150], the proportion of studies in which the entomological inoculation rate was higher in rice-growing than in non-rice-growing villages was 68% (13 of 19 analyses, sign test p=0·1671).

Of the studies that reported the human biting rates of *An. funestus* in rice-growing and non-rice-growing areas, only one was eligible as a quantitative study [147]. In this study, an 89% lower abundance of *An. funestus* was observed in rice villages (ROM 0·11 [95% CI 0·08–0·14]). A visualisation of the semiquantitative studies suggests a mixed effect: 13 studies [42,45,59,61,125,128,137,139,142,146,151,153,155] found more *An. funestus* in rice-growing areas than in non-rice areas whilst ten studies [56,60,126,143,145,147,149,150,157,159] found fewer *An. funestus* (Figure 3.3 and Appendix 3.4).

Concerning shifts in the ratio of sibling species between rice-growing and non-rice-growing villages, only two studies (both conducted in Cameroon) [151,155] did not report a complete dominance of one species, and the constituent species did not change radically. We also looked for species shifts among *Anopheles* vectors and observed that, in west Africa [56,66,69,126,131,134,137–140,142,143,145–148], the majority of vector populations in rice villages were *An. gambiae* s.l., while higher proportions of *An. funestus* were found in control villages (Figure 3.3). In east Africa, no conspicuous patterns were seen [42,45,55,60,61,125,128,130,135,153,159,160].

Table 3.1. GRADE quality of evidence for the association between rice cultivation and epidemiological and entomological malaria outcomes.

Outcomes	Summary of findings		Quality of the evidence					Overall quality of the evidence
	Relative effect (95% CI)	No. of participants (studies)	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	
HBR: *An. gambiae* s.l.	ROM[0] 4·69 (1·30 – 16·90)	823 (5)	Serious [1]	Serious [2]	Serious [3]	Serious [5]	Undetected [8]	VERY LOW [1,2,3,5,8] due to risk of bias, inconsistency, indirectness, imprecision
Sporozoite rate: *An. gambiae* s.l.	RR[0] 0·29 (0·19 – 0·46)	212 705 (18)	Serious [1]	Serious [2]	Not serious [4]	Serious [5]	Undetected [8]	VERY LOW [1,2,4,5,8] due to risk of bias, inconsistency, imprecision
EIR: *An. gambiae* s.l.	ROM 2·03 (1·02 – 4·06)	2 334 (3)	Serious [1]	Serious [2]	Serious [3]	Serious [6]	Undetected [9]	VERY LOW [1,2,3,6,9] due to risk of bias, inconsistency, indirectness, imprecision
Malaria infection: before 2003	RR 0.82 (0·63 – 1·06)	99 158 (16)	Serious [1]	Serious [2]	Not serious [4]	Serious [6]	Undetected [8]	VERY LOW [1,2,4,6,8] due to risk of bias, inconsistency, imprecision
Malaria infection: after 2003	RR 1·73 (1·01 – 2·96)	14 002 (7)	Serious [1]	Serious [2]	Serious [3]	Serious [7]	Strongly suspected [10]	VERY LOW [1,2,3,7,10] due to risk of bias, inconsistency, indirectness, imprecision, publication bias
Clinical malaria	IRR[0] 0·71 (0·48 – 1·06)	77 890 (4)	Serious [1]	Serious [2]	Serious [3]	Serious [7]	Undetected [9]	VERY LOW [1,2,3,7,9] due to risk of bias, inconsistency, indirectness, imprecision

Patient or population: People of all ages living in rural areas of malaria-endemic sub-Saharan Africa
Settings: Burkina Faso, Burundi, Cameroon, Côte d'Ivoire, Ghana, Kenya, Madagascar, Mali, Nigeria, Rwanda, Sierra Leone, Tanzania, The Gambia
Exposure: Rice cultivation
GRADE working group grades of evidence:
High quality: further research is very unlikely to change confidence in the estimate of effect
Moderate quality: further research is likely to have an important impact on confidence in the estimate of effect and may change the estimate
Low quality: further research is very likely to have an important impact on confidence in the estimate of effect and is likely to change the estimate
Very low quality: the estimate is very uncertain
[0] ROM stands for ratio of means, RR for risk ratio and IRR for incidence risk ratio
[1] Serious risk of bias: All studies were non-randomised and observational (downgrade quality of evidence by 1 level)
[2] Serious inconsistencies: Minimal overlap of confidence intervals and considerable heterogeneity (I2 > 90%, p < 0·0001) (downgrade by 1)
[3] Serious indirectness: Studies were conducted only in West and East Africa. These results may not be generalizable to Central Africa.
[4] No serious indirectness: Studies were conducted in a variety of sites in rural settings across SSA. These findings are generalizable elsewhere.
[5] Serious imprecisions: At least one study showed a small number of events with wide 95% confidence intervals (downgrade by 1)
[6] Serious imprecisions: At least one study showed a small number of events with wide 95% confidence intervals and there is uncertainty about the magnitude of effect of the intervention, as it fails to exclude benefit or harm (downgrade by 1)
[7] Serious imprecisions: There is uncertainty about the magnitude of effect of the intervention, as it fails to exclude benefit or harm (downgrade by 1)
[8] Publication bias not detected: Egger's test for bias found no evidence for funnel plot asymmetry (bias coefficient < 1·00, p > 0·05)
[9] Publication bias not detected: Insufficient studies to construct funnel plots
[10] Publication bias strongly suspected: Egger's test for bias found some evidence for funnel plot asymmetry (bias coefficient = 2·82, p = 0·005) (downgrade by 1)

Risk of bias within individual cohort and cross-sectional studies was generally at an intermediate level (Appendix 3.5). There was no evidence of publication bias in the meta-analysis of all outcomes except in malaria infection in the subgroup of studies done since 2003, where there was evidence of funnel plot asymmetry suggesting bias towards publication of positive findings (bias coefficient 2·82, p=0·0014; Appendix 3.6). There were insufficient studies to test for asymmetry in the meta-analysis of entomological inoculation rate and clinical malaria. The GRADE approach indicated that the quality of evidence for the comparisons between rice-growing and non-rice-growing villages was very low (Table 3.1).

3.5 Discussion

To assess whether declining malaria rates in sub-Saharan Africa have changed the relationship between rice cultivation and malaria, we compared entomological and epidemiological malaria indicators between rice-growing and non-rice-growing villages using data from 53 observational studies. The results confirmed that before 2003, infection prevalence was not higher in rice-growing than in non-rice-growing communities. Conversely and most importantly, since 2003, prevalence was almost two times higher in rice-growing than in non-rice-growing communities. Additionally, the intensity of malaria transmission, measured as the entomological inoculation rate, tended to be higher in rice-growing areas: the lower sporozoite rates found in rice-dwelling *An. gambiae* s.l. did not generally compensate for their greater numbers.

Previous reviews based on studies done before 2003 showed that malaria prevalence was not higher in rice-growing than in non-rice-growing communities [62,63]. Our re-examination of pre-2003 studies produced findings consistent with those reviews, and also showed that many sub-Saharan African countries had high malaria transmission intensities during this period. However, in more recent studies (applicable to the current malaria situation), we found higher infection prevalence in rice-growing than in non-rice-growing areas.

The differences between time periods could be explained by the introduction of the Roll Back Malaria initiative and the background developmental processes (general economic development, including housing) in Africa, both of which have changed the malaria picture in Africa drastically [7]. In the past, rice-growing communities, compared with their non-rice-growing counterparts, tended to be wealthier and therefore had better socioeconomic conditions and access to drugs and mosquito nets, which might have constituted a protective factor against malaria [74]. However, Roll Back Malaria brought about a massive upscaling of coverage of modern antimalaria interventions, including vector control, diagnostics, and treatment. Coverage has since become much more equitable within and between communities [78]. Similarly, general development across the continent brought about better infrastructure, transport, and housing, as well as better health systems [164]. Consequently, it can no longer be assumed that rice-growing villages have much better defences against malaria, or that non-rice-growing villages have no defences against the disease. It is presumed that the magnitude of change depends on which village characteristics were previously giving the differential protection between rice-growing and non-rice-growing villages; whether increased equity in antimalarial interventions or general development provided greater protection is a question that arises. As a consequence of the Roll Back Malaria initiative, there has also been a concomitant and equally widespread decline in the general intensity of transmission [15]. Thus, the fraction of the population at risk who are exposed to high intensity transmission has substantially decreased. Many of those who were previously intensely exposed are now exposed only to low levels of transmission. Hence, no longer under “saturation” (Appendix 1.1), the differences in exposure between rice-growing and non-rice-growing villages are now observable in human clinical outcomes.

Overall, malaria vector densities were six times higher in rice-growing than non-rice-growing areas. This finding was expected, because the ecological conditions of the early stages of rice fields are exactly those preferred by larvae of *An. gambiae* s.l. (fresh sunlit water of 2–10 cm depth, still or very slow-flowing, with silt or clay, without suspended organic matter, and non-deoxygenated) [48,165]. However, the magnitude of difference is perhaps surprisingly high. The tendency for sporozoite rates to be lower in rice-growing areas is presumably due to density-dependent reductions in the vectorial capacity of the vector population [71], which could happen through a reduction in adult lifespan (e.g., speculated by some to be because of competition for food in the larval stage despite the often vast area of inundated rice fields) or a reduction in adult feeding success (e.g., because extreme biting nuisance drives most people to use bed nets) [114–117]. There are also cases in which specific mechanisms dependent on unusual local conditions were operating. For instance, in Tanzania, there was evidence that the introduction of rice had removed the marshy breeding sites of *An. funestus* (a very efficient vector) and replaced them with rice fields, which *An. arabiensis* (a less efficient, although still important, vector) is better suited for breeding in [55]. In one study in The Gambia, there were two annual crops of rice and two corresponding peaks of mosquito abundance, but only one annual peak of malaria transmission, which was during the rainy season. Apparently, during the hot dry season, vectors were abundant but not transmitting the parasite, either because they were too short-lived or because it was so hot that the parasites were killed inside the vectors [166].

Previous reviews of whether rice-growing communities have a greater malaria burden have suggested that in rice-growing villages: (1) vector abundance tends to be higher; (2) sporozoite rates tend to be lower; and (3) the lower sporozoite rates compensate for the increased vector abundance, and there is no systematic tendency for malaria transmission to be more intense in rice-growing villages [62,63]. Our findings are consistent with (1) and (2), but not (3). Specifically, in 14 of the 19 studies, the reduction in sporozoite rate was not enough to compensate for the increase in vector abundance, and the pooled estimate suggests that malaria transmission in rice-growing villages tends to be about twice as intense as that in non-rice-growing villages. In other words, being a rice cultivating village is, and apparently always was, associated with exposure to more intense transmission for unprotected people[3]. It was never correct to assume the notion that the mosquitoes generated from rice fields did not increase malaria risk [72].

This study has several limitations. First, it was based on observational studies, which can be subject to selection and information bias as well as confounders. Exposure and control groups might have low comparability: rice-growing and non-rice-growing communities could have been intrinsically different in their characteristics, even before the introduction of rice cultivation schemes (i.e., there could be prerequisites that affect both malaria risk and the suitability of a village for irrigated rice fields – such as being situated in wetland areas[4]). Second, observational studies can be prone to confounding because factors such as socioeconomic status, housing conditions, and access to health care (e.g., antimalarial drugs and bed nets[5]) are not always accounted for. Although we attempted to reduce confounding of this nature by presenting adjusted effect measures, very few studies reported them. The rating of very low quality of evidence according to the GRADE system indicates low confidence in the effect estimate [124]. Nonetheless, we were not expecting, nor looking for, a true effect of rice cultivation on malaria risk; rather, we were more concerned about the direction of effect, which, although different in magnitude, was

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[3] This may seem less relevant nowadays when bed-net coverage and usage in rice communities are moderately high [92,314]. However, in recent years, sleeping-under-net-coverage has peaked at around 55% [2]. Moreover, nets are holding measures; they give only partial and temporary protection (especially in the context of insecticide resistance). Thus, one still needs to consider the degree of additional risk to which individuals living near rice fields are exposed, other factors being equal.

[4] Through observational studies comparing rice and non-rice villages, it is difficult to be sure that the higher malaria risk is attributable to rice cultivation itself: rice-cultivating areas could perhaps be inherently wetter than non-rice areas. On the other hand, the studies of Chandler and Highton in Kenya provide persuasive evidence that non-rice wetlands produce a wide range of mainly non-vector mosquitoes, whereas in rice fields the great majority of the emerging mosquitoes are malaria vector species [42].

[5] Note that when net coverage is very high, it is possible that human bait catches might tend to catch a larger proportion of the blood-seeking females in the vicinity, leading to an over-estimate of population size. The reported biting rates do not make allowance for this.

relatively consistent across studies given our a priori subgrouping. Third, a number of factors were not, and could not be, considered. Because of limited reporting, seasonality (wet vs dry, and seasonal vs perennial), intrinsic differences in landscapes of study sites, and characteristics of rice cultivation (type of rice grown, size of irrigation schemes, and distance of rice-growing communities from their fields) could not be accounted for. Control groups were also variable, each associated with different degrees of vector density. Additionally, of the seven post-2003 studies from which the pooled RR of malaria prevalence was calculated, three were done in central Tanzania by the same research group and could therefore be subject to bias [152,154,161].

Considering that this review was based only on observational studies, it has highlighted the need for replicated studies comparing before and after the introduction of rice crops, and if possible, intervention studies to measure the effect of rice cultivation on malaria risk. Given the complex relationship between vector abundance, vectorial capacity, and malaria prevalence, future studies should include all entomological and epidemiological indicators to provide a clearer picture of the rice and malaria story. Such studies should also address questions of equity by including information on bed-net coverage, use of antimalarial drugs, socioeconomic factors, and housing.

Despite low-quality evidence, subgroup analyses comparing studies before and after the scale-up of malaria interventions suggested that this turning point has changed the rice–malaria relationship in Africa. Rice fields tend to produce large quantities of mosquitoes, and, in most cases, any reductions in other vectorial capacity parameters (e.g. feeding success and/or longevity) is inadequate to compensate for this increase in abundance, such that, on balance, there is greater exposure to infective mosquitoes in rice-farming communities. Thus, if we want to greatly expand rice cultivation in Africa and at the same time work towards malaria elimination, then we will need to develop ways to reconcile these two goals. In short, we need to find ways of growing rice without producing mosquitoes. Although various methods of controlling mosquitoes in rice fields have been studied, in most cases, these methods are only partially effective or are effective for only part of the season or in specific circumstances. What we need to know is how to combine these methods to provide effective control for the entire season and in a wide variety of rice-growing settings.

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Research in context

Evidence before this study

Rice fields in Africa are major breeding sites for malaria vectors, bringing greater abundance of *Anopheles* species in rice-growing villages. When reviewed two decades ago, it was observed that these extra mosquito vectors did not increase the incidence of malaria in humans, and some reductions in malaria infection prevalence were observed. Since then, antimalaria intervention coverage across sub-Saharan Africa has greatly increased and become more equitable, and malaria infection prevalence has halved, calling for a re-examination on this rice–malaria relationship. Between May 23, 2018, and Sept 18, 2020, we searched EMBASE, Global Health, PubMed, Scopus, and Web of Science, without restriction on language or date of publication, to identify community-based studies that compared malaria risk between rice-growing and non-rice-growing areas in sub-Saharan Africa. Combinations of the following keywords were used: malaria, *Plasmodium falciparum, Anopheles,* mosquito, prevalence, incidence, risk, Africa, rice, paddies, paddy, irrigation, human biting rate, sporozoite rate and entomological inoculation rate. Risk of bias of eligible studies was generally at an intermediate level.

Added value of this study

In this systematic review and meta-analysis, we assessed whether the decline in malaria transmission has changed the associations between rice cultivation and malaria risk, by comparing older studies included in previous reviews with more recently published studies. It was confirmed that, before 2003, infection prevalence was not higher in rice-growing communities. However, after 2003, malaria prevalence was almost two times higher in rice-growing communities. It was also confirmed that, as underlying malaria intensity decreased, there was an increase in the strength of the association between rice cultivation and malaria risk. Malaria transmission (measured as the rate of infective biting on exposed residents) was also greater in rice-growing areas, indicating that although rice-field malaria vectors might have somewhat lower sporozoite rates, this reduction does not compensate for their substantially greater numbers.

Implications of all the available evidence

African ministries of health are considering how to eliminate malaria, while ministries of agriculture are actively planning the expansion and intensification of irrigated rice production. These objectives are both desirable, but our updated review indicates that the latter process might interfere with the former, as rice cultivation brings increased malaria risk. To reconcile these two goals, African countries urgently need to develop and promote methods of growing rice without growing malaria vectors.

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SECTION A – Student Details

Student ID Number	1602712	**Title**	Miss
First Name(s)	Kallista Hay Ching		
Surname	Chan		
Thesis Title	Rice and malaria in Africa: Seeking vector control in rice cultivation practices		
Primary Supervisor	Jo Lines		

If the Research Paper has previously been published, please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?			
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SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	Nature Scientific Reports
Please list the paper's authors in the intended authorship order:	Kallista Chan, Christian Bottomley, Kazuki Saito, Jo Lines, Lucy S. Tusting
Stage of publication	Submitted

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper.	I conceived the study, developed the protocol, conducted all analyses and wrote the paper. Other authors assisted in verifying data and suggesting analysis methods.

SECTION E

Student Signature	[redacted]	**Supervisor Signature**	[redacted]
Date	12/08/2022	**Date**	12/08/2022

4 The control of malaria vectors in rice fields: a systematic review and meta-analysis

Authors

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4.1 Abstract

The relatively stable aquatic conditions of irrigated lowland and rainfed rice, which is grown across 145 million hectares in more than 100 countries, are capable of generating large numbers of mosquito vectors of malaria, which causes more than 600,000 deaths per year worldwide. Many methods can control these vectors, but a systematic review has not previously been conducted. This study assesses whether larviciding, fish or intermittent irrigation can significantly reduce malaria vectors in rice fields whilst increasing rice yield. After a literature search for studies reporting the effect of larval control and rice cultivation practices on malaria vector densities in rice fields, 33 studies were eligible for meta-analysis.

Larviciding was effective at reducing rice-field malaria vectors. Pooled analysis of five controlled time-series (CTS) studies with chemical insecticides showed an overall combined reduction of larval densities of 77% compared to no larviciding. Eight CTSs with biological larvicides showed a pooled reduction of 60% compared to no larviciding. Cultivating rice and fish together provided good control too: a pooled analysis of three CTSs showed an overall 82% reduction in anopheline larvae compared to no fish. Pooled analysis of four studies suggested that intermittent irrigation (using various timings and frequencies of drainage) is effective at reducing the abundance of late-stage anopheline larvae (pooled reduction = -35%), but not overall immature abundance, compared to continuous flooding.

We conclude that many interventions such as larvicides, fish and intermittent irrigation can provide riceland malaria vector control, but the critical obstacle to wider use is farmer acceptability. Future research should be led by the agricultural sector, with inputs from entomologists, to investigate malaria control co-benefits within high-yielding rice cultivation practices.

4.2 Introduction

Rice is one of the major food grains of the world, acting as a staple food crop for about half of the world's population. Demand for rice is ever-increasing, especially in Africa, with continental production having increased 117% in the last 20 years [20]. In order to keep up with such demand and achieve self-sufficiency, there has been enormous investment of resources towards boosting rice production, including the expansion of rice-harvested areas [25,26].

Unfortunately, in addition to providing food security and improved farmer livelihoods, irrigated and rainfed lowland rice production systems also generate a large number of mosquitoes. Depending on the region where rice is grown, different sets of mosquito species can be found inhabiting the water, and in some parts of the world, rice fields are a major source of the most important malaria vector species of that region [48]. Examples include central China, sub-Saharan Africa (SSA), and parts of central Asia, Indonesia and Peru, where rice-cultivating areas can produce very high densities of competent malaria vectors, with adult female mosquitoes being up to 10-fold more abundant than in neighbouring areas without rice cultivation [48,160,167,168]. Thus, rice-growing areas can have high inherent malaria transmission capacity, posing a major public health problem. In many previously malarious countries such as Portugal, Spain, Turkmenistan and China, rice areas were identified as the last hotspots of transmission, and targeted control of mosquito breeding in the rice fields was often required to achieve malaria elimination and to prevent resurgence [169–172]. This rice-malaria relationship is especially important in SSA because African vectors are extraordinarily efficient at transmitting malaria. More than 90% of the world's 627,000 deaths due to malaria occur in African children under five years of age [2]. There is recent evidence that in Africa, there is a significant association between rice and intensified malaria transmission, and this association has grown stronger over time [Chapter 3] [173].

For these reasons, interventions to suppress vector breeding in rice fields have been studied since the 1930s. Malariologists have investigated many methods of larval source management (LSM) in rice fields (e.g., the use of chemical and biological larvicides) and, sometimes in collaboration with agronomists, different agricultural techniques (e.g., irrigation method, plant height and pesticide use). Reviews written over 30 years ago concluded that these interventions have mixed effects on malaria vector densities and that despite numerous studies, there are still major gaps in our understanding of what works, when and where [27,48,80]. In most cases, these reviews presented experimental trials in rice fields as individual case studies without any pooled effect measures. They also rarely included the effect of these interventions on rice production and water consumption and the technology readiness of the intervention (i.e., the farmers' propensity to adopt and incorporate a technology within their rice cultivation practices), all of which are priorities to agronomists when considering methods of rice cultivation.

We are, of course, interested in rice field-based interventions that would be effective in reducing malaria. However, large and expensive trials are needed to demonstrate epidemiological effectiveness, and only a small subset of candidate interventions could ever be evaluated in this way. Typically, therefore, studies begin by comparing the various candidate interventions in terms of their efficacy. In most studies, efficacy is measured as a reduction in the abundance of mosquitoes growing in, and/or, emerging from individual rice plots. The assumption here is that if an intervention fails to reduce mosquito breeding in individual rice plots, then it is not worth testing further. Conversely, if the intervention performs well in this test, it may indeed be worth testing on the larger scale. Three basic approaches have been used to quantify vector breeding in rice plots: (a) the abundance / density of larvae (all instars combined) and pupae, (b) the abundance / density of late-stage larvae (third and fourth instars) and pupae, and (c) the abundance of recently emerged adults. For the purposes of this review, we assumed that these are both adequate as indicators of efficacy[6].

As an update and supplement to the previous narrative reviews, we conducted a systematic review and meta-analysis to assess whether, by and large, riceland LSM and rice cultivation practices can reduce malaria vector abundance, whilst increasing rice yield and reducing water use.

4.3 Results

4.3.1 Search results and study characteristics

The literature search yielded 11,153 studies after removing duplicates (Figure 4.1). From these, 47 publications were eligible for inclusion. All 47 were included for qualitative analysis, while 33 were included for quantitative analysis, of which 26 were controlled time series (CTS) and 7 were controlled interrupted time series (CITS) studies. Data in CTS studies are collected at the same multiple time points in control and intervention groups only after treatment application whereas data in CITS studies are collected both before and after treatment application(s) (Appendix 4.1) [174]. In total, since studies often tested multiple interventions, there were 84 comparisons. Table 4.1 summarises all eligible studies (some repeated as they had multiple comparisons) by interventions, publication period and geographical region. Most studies were conducted between 1981 and 2000 (66%) and in America (n=21, all in USA), followed by Africa (n=13) and South Asia (n=12, all in India).

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[6] However, it is important to note that these indicators are not exactly equivalent. In particular, some interventions may not have an effect on the abundance of first instars but a strong effect on the survival of larvae from first to fourth instar. For these interventions, it is better to use approach (b) instead of (a) as an indicator.

4.3.2 Risk of bias

High risk of bias was found across numerous domains of the EPOC risk of bias for CTS studies, particularly for allocation concealment (where technicians and investigators could foresee intervention assignment) and blinding (Appendix 4.2). Amongst the seven CITS studies, there was a high risk of bias for both allocation sequence generation (where non-random methods were used) and allocation concealment. Another common design weakness is a general lack of information on baseline features in both CTS and CITS studies.

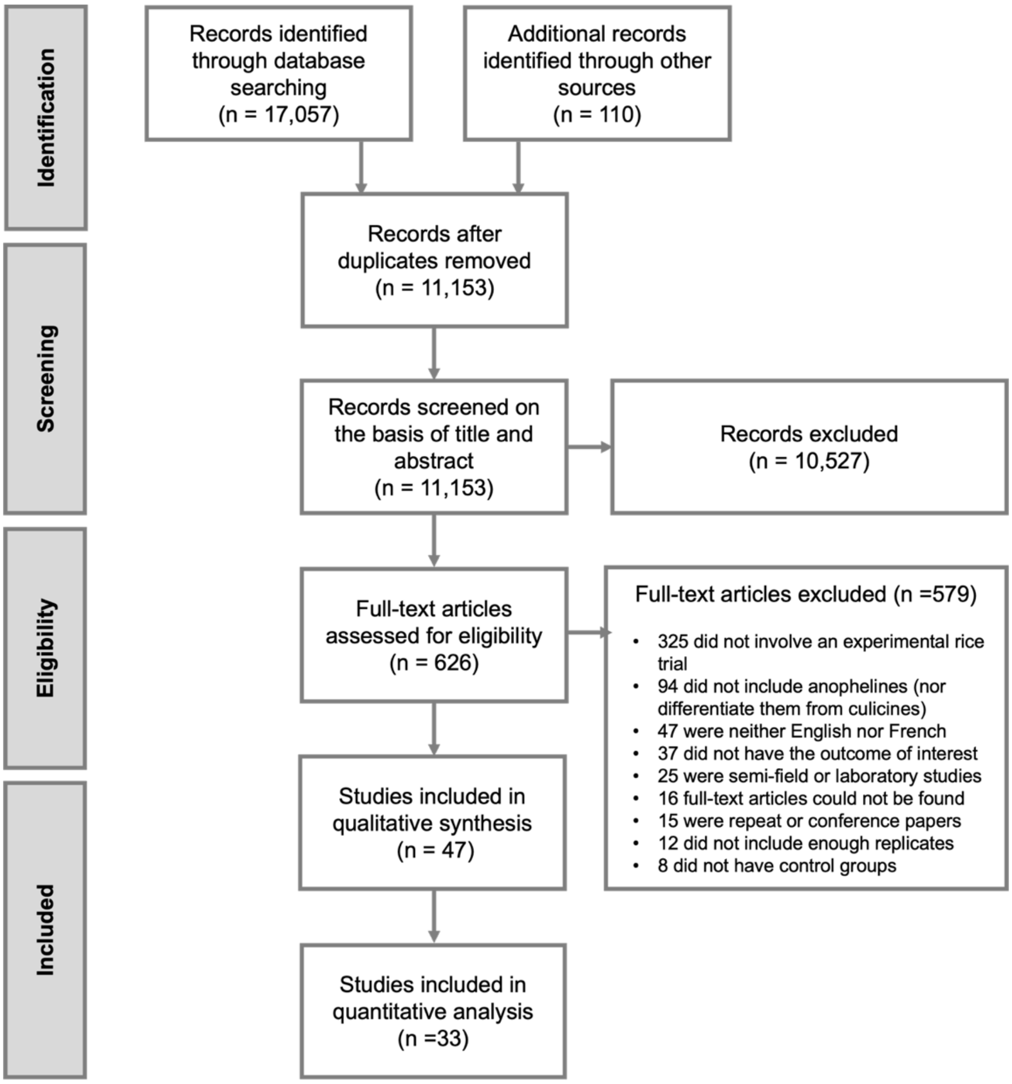


Figure 4.1. Study selection process.

Table 4.1. Interventions tested by studies included in the qualitative and quantitative analysis (n=47* studies), stratified by publication period and geographical region.

	Larviciding				Biological control		Environmental management / rice cultivation practices		
	Oils and surface agents	**Synthetic organic chemicals**	**Biological larvicides**	**Insect growth regulator**	**Fish**	**Copepod, *Azolla*, neem**	**Irrigation**	**Other: land preparation, water height, plant height**	**Total**
Publication period									
1941-1950		1					2		**3**
1951-1960		1							**1**
1961-1970									**0**
1971-1980	1	3			1				**5**
1981-1990		3	9*	1	4*		2*	2	**21**
1991-2000	1	1*	4*		2	3*	3*	2	**16**
2001-2010		1*	3*		1			2	**7**
2011-2021	1						1*	1*	**3**
Geographical region									
Africa	3	2*	3*		1*		1*	3*	**13**
South Asia		2	2*		1*	2*	4*	1	**12**
America		4*	9*	1	3	1	1*	2	**21**
East and SE Asia		2*	2*		3		1	1	**9**
Europe							1		**1**
Total	**3**	**10**	**16**	**1**	**8**	**3**	**8**	**7**	

*Studies with multiple comparisons that are treated separately here: Allen et al. 2008, Bolay and Trpis 1989, Djegbe et al. 2020, Kramer et al. 1988, Palchick et al. 1986, Rajendran et al. 1991, Rao et al. 1995, Teng et al. 2005, Yu et al. 1989.

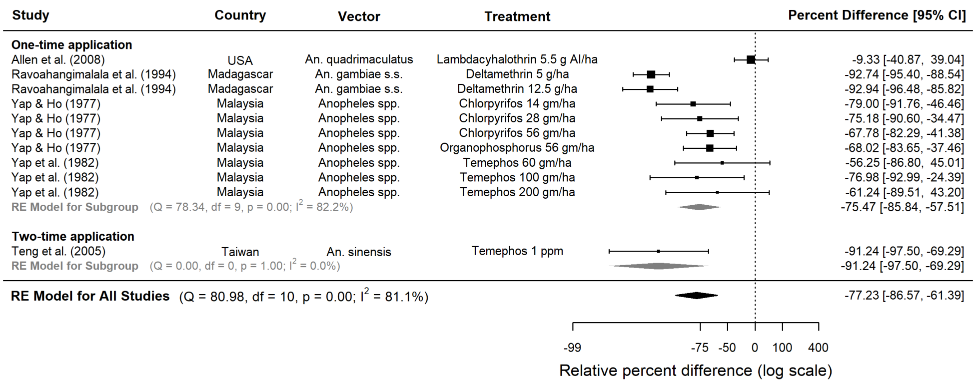
There were insufficient studies (n<10) to construct funnel plots and test for asymmetry for most meta-analyses except for studies that looked at larvicides or water management techniques. Regression tests for funnel plot asymmetry found no evidence for publication bias for the meta-analyses on chemical insecticides (Appendix 4.3A) or water management techniques (Appendix 4.3B). However, there was evidence of publication bias for the meta-analyses of CTS studies on bacterial larvicides (p=0.02, Appendix 4.3C).

4.3.3 Larviciding

Compared to no monomolecular surface films (MSF), MSFs for riceland vector control were not associated with reduced anopheline immature densities in one CITS study but were associated with a 57% reduction in anopheline immatures in two CTS studies (95% confidence interval [CI] 69.4, 40.3, p<0.0001, Table 4.2). Taking larval stages into consideration, MSFs were associated with a 50% reduction in early instar anophelines and a 55% reduction in late instars (Appendix 4.4).

Across six eligible studies, synthetic organic chemicals were effective in reducing anopheline larval numbers regardless of their application frequency: the pooled reduction was 77% in five CTS studies (95% CI 86.6, 61.4, p<0.0001) and 72% in one CITS study (95% CI 89.5, 26.9, p=0.01) (Figure 4.2A, Table 4.2). Pyrethroids (e.g., deltamethrin) and organophosphates (e.g., temephos and iodenphos) provided a high level of control, reducing up to 90% larvae in Asian and African rice fields. Across the CTS studies, vector density evaluation usually occurred at least 6 times, from 24 hours to 2 months after insecticide application. One quantitative study included adult malaria vectors as an outcome but found no association between iodenphos and human biting rate [175] (Appendix 4.5). However, qualitatively, two studies in the US observed significant reductions in adult density upon using organophosphates (Appendix 4.6) [176,177].

(A) Synthetic organic chemicals



(B) Biological larvicides

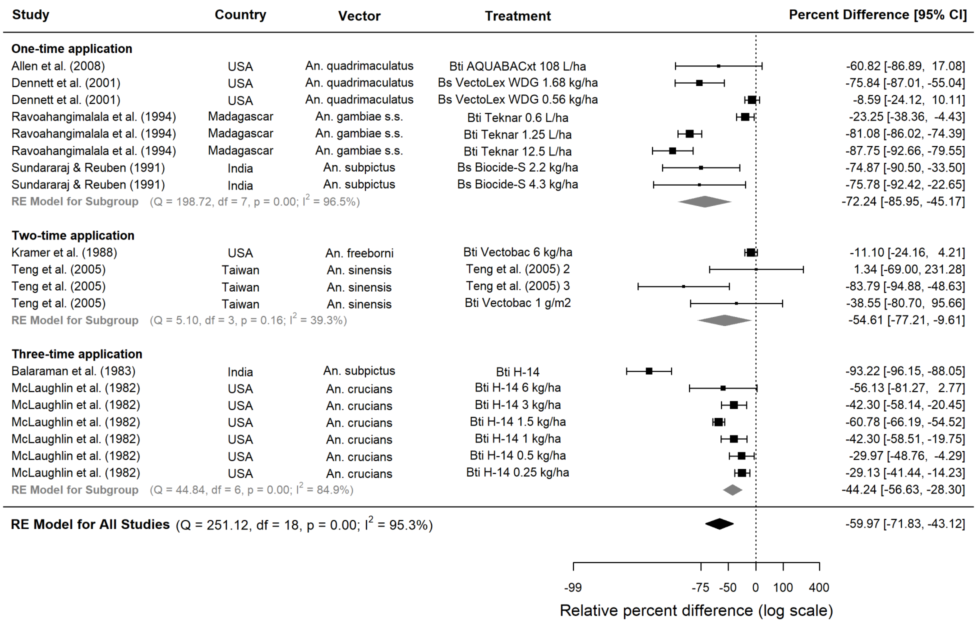


Figure 4.2. Pooled estimate of the effect of **(A)** synthetic organic chemicals and **(B)** biological larvicides on *Anopheles* larval densities in rice fields. Five controlled time series studies on **(A)** synthetic organic chemicals and eight controlled time series on **(B)** biological larvicides were included, conducted between years 1975 and 2004. Squares represent the relative effectiveness of individual studies, where square size represents the weight given to the study in the meta-analysis, with error bars representing 95% CIs; diamonds represent the pooled effects from random effects (RE) sub-group and meta-analyses.

Across all eligible studies, biological larvicides were mostly applied once or twice throughout an experiment and vector density evaluation usually occurred at least three times, from 24 hours to 6 weeks after insecticide application. Pooling across all frequencies and timings of applications, bacterial larvicides were associated with 60% fewer riceland anopheline larvae in eight CTSs (95% CI 71.8, 43.1, p<0.0001, Figure 4.2B) but not in two CITSs (Table 4.2). The most effective larvicides were *Bti*-based, against *An. gambiae* s.s. in Madagascar and *An. sinensis* in Taiwan. Three studies showed that bacterial larvicides produced greater reductions in the density of older immature stages, reducing pupae by up to 91%, followed by 67% in late and 47% in early-stage larvae (Appendix 4.4). In studies evaluating the combination of bacterial larvicides and rice-fish systems compared to no intervention, the results were mixed: two CITSs showed an 88% reduction in anopheline immatures (95% CI 95.0, 71.3, p=0.003), whilst two CTSs showed no association (Table 4.2). According to six studies that were only analysed qualitatively, both bacterial larvicide cum insect growth regulators and insect growth regulators alone could reduce riceland *An. quadrimaculatus* (Appendix 4.6).

4.3.4 Biological control

The simultaneous cultivation of rice and fish was effective in reducing the abundance of anopheline immatures, where a pooled reduction of 82% was found in three CTSs (95% CI 91.4, 60.2, p<0.0001) and 87% in three CITSs (95% CI 93.9, 72.7, p=0.001). In South Korea, *Aphycypris chinensis* (belonging to the carp or minnow family) was highly effective in reducing *An. sinensis* immatures whilst *Tilapia mossambicus* was not [178,179]. In Liberia, rice fields stocked with *T. nilotica* were associated with 88% lower *An. gambiae* s.l. numbers. *Gambusia affinis* (mosquitofish) were more effective against *An. freeborni* in the US when higher rates were stocked (Table 4.2). Other forms of biological control, including copepods, *Azolla* (mosquito fern) and neem, were not associated with lower numbers of anopheline larvae in rice fields (Table 4.2).

Table 4.2. Summary of findings of meta-analyses of the effect of riceland mosquito control on *Anopheles* larval density (the number of larvae and pupae per dip or area sampler), arranged by the type of control, study design and geographical region.

Study	Country	Predominant vector	Details of intervention (application method, rate, dose, frequency, timing, fish species)	Study design	Plot size (no. of replications*)	Relative percent difference (95% CI)
Larviciding						
Surface agents						
Reiter 1980	Kenya	*An. gambiae* s.l.	Monomolecular surface film (lecithin solution) at rate of 2.47 L/ha	CTS[1]	600 m^2 (9)	-60.0 (-74.0, -38.5)
Reiter 1980	Kenya	*An. gambiae* s.l.	Monomolecular surface film (lecithin solution) at rate of 4.94 L/ha	CTS	600 m^2 (15)	-57.1 (-76.3, -22.3)
Bukhari et al. 2011	Kenya	*An. gambiae* s.l.	Monomolecular surface film (Aquatain, silicone-based) at 1 ml/m^2 (1st application) and at 2 ml/m^2 (2nd application)	CTS	2000 m^2 (6)	-29.1 (-79.0, +138.7)
					RE model for all studies	**-57.2 (-69.4, -40.3)**
Karanja et al. 1994	Kenya	*An. arabiensis*	Monomolecular surface film (Arosurf MSF) at 4 L/ha every 14 days	CITS[2]	100 m^2 (4)	-91.6 (-99.9, +486.3)
Synthetic organic chemicals						
Allen et al. 2008	USA	*An. quadrimaculatus*	Lambda-cyhalothrin, aerial application at 5.5 g AI/ha, once (1x) prior permanent flooding	CTS	13-15 ha (2)	-9.3 (-40.9, +39.0)
Ravoahangimalala et al. 1994	Madagascar	*An. gambiae* s.s.	Deltamethrin emulsionable concentrate 25 5 g/ha, 1x	CTS	58-110 m^2 (2)	-92.7 (-95.4, -88.5)
Ravoahangimalala et al. 1994	Madagascar	*An. gambiae* s.s.	Deltamethrin emulsionable concentrate 25 12.5 g/ha, 1x	CTS	43-58 m^2 (2)	-92.9 (-96.5, -85.8)
Yap & Ho 1977	Malaysia	*Anopheles* spp.	Chlorpyrifos (Dursban) at 14 gm/ha, 1x	CTS	(3)	-79.0 (-91.8, -46.5)
Yap & Ho 1977	Malaysia	*Anopheles* spp.	Chlorpyrifos (Dursban) at 28 gm/ha, 1x	CTS	(3)	-75.2 (-90.6, -34.5)
Yap & Ho 1977	Malaysia	*Anopheles* spp.	Chlorpyrifos (Dursban) at 56 gm/ha, 1x	CTS	(3)	-67.8 (-82.3, -41.4)
Yap & Ho 1977	Malaysia	*Anopheles* spp.	Organophosphorus (Dowco-214) at 56 gm/ha, 1x	CTS	(3)	-68.0 (-83.6, -37.5)
Yap et al. 1982	Malaysia	*Anopheles* spp.	Temephos (Abate 500E) 60 gm/ha, 1x	CTS	69-365 m^2 (2)	-56.3 (-86.8, +45.0)
Yap et al. 1982	Malaysia	*Anopheles* spp.	Temephos (Abate 500E) 100 gm/ha, 1x	CTS	69-365 m^2 (2)	-77.0 (-93.0, -24.4)
Yap et al. 1982	Malaysia	*Anopheles* spp.	Temephos (Abate 500E) 200 gm/ha, 1x	CTS	69-365 m^2 (2)	-61.2 (-89.5, +43.2)
Teng et al. 2005	Taiwan	*An. sinensis*	Temephos (Abate 1-SG) at 1 ppm, 2x (20d interval)	CTS	119-194 m^2 (4)	-91.2 (-97.5, -69.3)
					RE model for all studies	**-73.1 (-83.8, -55.4)**
Kamel et al. 1972	Egypt	*An. pharoensis*	Iodofenphos (NUVANOL N20U), aerial application at 1.5 L/ha, 1x	CITS	50-120 ha (2)	-93.2 (-98.1, -76.2)
Kamel et al. 1972	Egypt	*An. pharoensis*	Iodofenphos (NUVANOL N20U), aerial application at 3 L/ha, 1x	CITS	50-120 ha (2)	-50.2 (-83.3, +49.0)
					RE model for all studies	**-72.3 (-89.5, -26.9)**
Biological larvicides						
Allen et al. 2008	USA	*An. quadrimaculatus*	*Bacillus thuringiensis* var. *israelensis* (*Bti*), AQUABACxt, aerial application at 108 L/ha on a 61-m swath, 1x	CTS	13-15 ha (3)	-60.8 (-86.9, +17.1)
Dennett et al. 2001	USA	*An. quadrimaculatus*	*Bacillus sphaericus* (*Bs*), VectoLex WDG, aerial application at 1.68 kg/ha, 1x	CTS	2000 m^2 (2)	-8.6 (-24.1, +10.1)
Dennett et al. 2001	USA	*An. quadrimaculatus*	*Bs,* VectoLex WDG, aerial application at 0.56 kg/ha, 1x	CTS	2000 m^2 (2)	-11.1 (-24.2, +4.2)
Ravoahangimalala et al. 1994	Madagascar	*An. gambiae* s.s.	*Bti,* Teknar HP-D liquid concentrate, at 0.6 l/ha, 1x	CTS	58-68 m^2 (2)	-81.1 (-86.1, -74.4)
Ravoahangimalala et al. 1994	Madagascar	*An. gambiae* s.s.	*Bti,* Teknar HP-D liquid concentrate, at 1.25 l/ha, 1x	CTS	58-78 m^2 (2)	-87.7 (-92.7, -79.5)
Ravoahangimalala et al. 1994	Madagascar	*An. gambiae* s.s.	*Bti,* Teknar HP-D liquid concentrate, at 12.5 l/ha, 1x	CTS	58-87 m^2 (2)	-93.2 (-96.1, -88.0)
Sundaraj & Reuben 1991	India	*An. subpictus*	*Bs,* Biocide-S 1593M, at 2.2 kg/ha, 1x after transplantation	CTS	440 m^2 (3)	-74.9 (-90.5, -33.5)
Sundaraj & Reuben 1991	India	*An. subpictus*	*Bs,* Biocide-S 1593M, at 4.3 kg/ha, 1x after transplantation	CTS	440 m^2 (3)	-75.8 (-92.4, -22.7)

Study	Country	Predominant vector	Details of intervention (application method, rate, dose, frequency, timing, fish species)	Study design	Plot size (no. of replications*)	Relative percent difference (95% CI)
Kramer et al. 1988	USA	*An. freeborni*	*Bti,* Vectobac (200 ITU/mg), at 6 kg/ha, 2x (when mosquito densities were high)	CTS	1000 m^2 (3)	-56.1 (-81.3, +2.8)
Teng et al. 2005	Taiwan	*An. sinensis*	*Bti,* Vectobac G, at 1 g/m^2, 2x (20d interval)	CTS	119-194 m^2 (4)	-83.8 (-94.9, -48.6)
Teng et al. 2005	Taiwan	*An. sinensis*	*Lagenidium giganteum* T, 1.5 ppm and 30 oz/acre, 2x (20d interval)	CTS	119-194 m^2 (4)	-38.5 (-80.7, +95.7)
Teng et al. 2005	Taiwan	*An. sinensis*	*Lagenidium giganteum* A, 1.5 ppm and 30 oz/acre, 2x (20d interval)	CTS	119-194 m^2 (4)	+1.3 (-69.0, +231.3)
Balaraman et al. 1983	India	*An. subpictus*	*Bti* serotype H-14 (VCRC B-17), with dose 27 x 10^5 spores/ml, 3x	CTS	1000 m^2 (5)	-75.8 (-87.0, -55.0)
McLaughlin et al. 1982	USA	*An. crucians*	*Bti,* H-14 (Abbott 6108b 300 T.U./mg), at 6.0 kg/ha, 3x	CTS	30 m^2 (3)	-42.3 (-58.1, -20.4)
McLaughlin et al. 1982	USA	*An. crucians*	*Bti,* H-14 (Abbott 6108b 300 T.U./mg), at 3.0 kg/ha, 3x	CTS	30 m^2 (3)	-60.8 (-66.2, -54.5)
McLaughlin et al. 1982	USA	*An. crucians*	*Bti,* H-14 (Abbott 6108b 300 T.U./mg), at 1.5 kg/ha, 3x	CTS	30 m^2 (3)	-42.3 (-58.5, -19.7)
McLaughlin et al. 1982	USA	*An. crucians*	*Bti,* H-14 (Biochem-Bactimos 666 1800 T.U./mg), at 1.0 kg/ha, 3x	CTS	30 m^2 (3)	-30.0 (-48.8, -4.3)
McLaughlin et al. 1982	USA	*An. crucians*	*Bti,* H-14 (Biochem-Bactimos 666 1800 T.U./mg), at 0.5 kg/ha, 3x	CTS	30 m^2 (3)	-29.1 (-41.4, -14.2)
McLaughlin et al. 1982	USA	*An. crucians*	*Bti,* H-14 (Biochem-Bactimos 666 1800 T.U./mg), at 0.25 kg/ha, 3x	CTS	30 m^2 (3)	-23.2 (-38.4, -4.4)
				RE model for all studies		**-60.0 (-71.8, -43.1)**
Bolay & Trpis 1989	Liberia	*An. gambiae* s.l.	*Bti,* Teknar HP-D, at 0.1 g/m^2	CITS	150 m^2 (3)	-75.8 (-96.0, +46.3)
Yu et al. 1993	S. Korea	*An. sinensis*	*Bti,* H-14 (Bactis-P), at 0.1 kg/ha	CITS	1000 m^2 (6)	-67.6 (-97.0, +251.1)
				RE model for all studies		**-76.3 (-95.4, +21.9)**
Larviciding and biological control						
<u>Bacterial larvicide and fish</u>						
Kramer et al. 1988	USA	*An. freeborni*	*Bti,* Vectobac (200 ITU/mg), at 6 kg/ha + *Gambusia affinis* at 1.1 kg/ha	CTS	1000 m^2 (3)	-31.0 (-68.3, +50.3)
Kramer et al. 1988	USA	*An. freeborni*	*Bti,* Vectobac (200 ITU/mg), at 6 kg/ha + *G. affinis* at 3.4 kg/ha	CTS	1000 m^2 (3)	-82.8 (-91.9, -63.4)
				RE model for all studies		**-65.7 (-91.2, +34.2)**
Bolay & Trpis 1989	Liberia	*An. gambiae* s.l.	*Bti,* Teknar HP-D, at 0.1 g/m^2 + *Tilpania nilotica* (300)	CITS	150 m^2 (3)	-88.1 (-96.1, -63.9)
Yu & Lee 1989	S. Korea	*An. sinensis*	*Bti,* H-14, at 1 kg/ha + *Aplocheilus latipes* at 2 /m^2	CITS	150 m^2 (2)	-67.0 (-79.8, -46.2)
				RE model for all studies		**-88.0 (-95.0, -71.3)**
Biological control						
<u>Fish</u>						
Kramer et al. 1988	USA	*An. freeborni*	*G. affinis* at 1.1 kg/ha	CTS	1000 m^2 (3)	-77.7 (-88.2, -56.1)
Kramer et al. 1988	USA	*An. freeborni*	*G. affinis* at 3.4 kg/ha	CTS	1000 m^2 (3)	-88.6 (-94.2, -77.9)
Victor et al. 1994	India	*An. subpictus*	3 indigenous carps (*Catla catla, labeo rohita, cirrhinus mrigala)* + 3 exotic carps *(Cyprinus carpio, Hypopthalmithys molitri, Ctenopharyngodon idella)* stocked at rate of 10,000/ha	CTS	400 m^2 (3)	-51.6 (-76.2, -1.6)
Yu et al. 1981	S. Korea	*An. sinensis*	*Aphycypris chinensis* (presence)	CTS	2000 m^2 (2)	-92.2 (-97.3, -77.2)
				RE model for all studies		**-81.5 (-91.4, -60.2)**
Bolay & Trpis 1989	Liberia	*An. gambiae* s.l.	*Tilapia nilotica* (n=300)	CITS	150 m^2 (3)	-87.8 (-96.0, -62.4)
Kim et al. 2002	S. Korea	*An. sinensis*	*Tilapia mossambicus* at 2 fish/10m^2	CITS	300-600 m^2 (2-4)	-41.8 (-57.1, -20.9)
Kim et al. 2002	S. Korea	*An. sinensis*	*A. chinensis* at 2 fish/10m^2	CITS	300-600 m^2 (2-4)	-62.4 (-76.0, -41.2)
Kim et al. 2002	S. Korea	*An. sinensis*	*T. mossambicus* at 2 fish/10m^2 + *A. chinensis* at 1 /m^2	CITS	300-600 m^2 (2-4)	-55.1 (-72.6, -26.3)
Yu & Lee 1989	S. Korea	*An. sinensis*	*A. latipes* at 2 fish/m^2 + *T. mossambicus* at 2 /m^2	CITS	150 m^2 (2)	-73.4 (-80.5, -63.6)
				RE model for all studies		**-87.1 (-93.9, -72.7)**
<u>Copepod</u>						
Marten et al. 2000	USA	*An. quadrimaculatus*	*Mesocyclops ruttneri* (n=500)	CTS	100 m^2 (2)	-40.5 (-82.8, +105.6)

Study	Country	Predominant vector	Details of intervention (application method, rate, dose, frequency, timing, fish species)	Study design	Plot size (no. of replications*)	Relative percent difference (95% CI)
Azolla						
Rajendran & Reuben 1991	India	*An. subpictus*	*Azolla microphylla* introduced at rate 100 g/m^2 on 5th DAT[3]	CTS	40 m^2 (2)	-48.7 (-96.8, +720.4)
Rajendran & Reuben 1991	India	*An. subpictus*	*Azolla microphylla* introduced at rate 200 g/m^2 on 5th DAT	CTS	40 m^2 (2)	+45.6 (-89.0, +1826.3)
				RE model for all studies		**-10.3 (-86.4, +493.3)**
Neem						
Rao et al. 1995	India	*An. subpictus*	Neem (Nimin) at 0.063 kg ai/ha	CTS	400 m^2 (3)	-29.4 (-84.3, +217.8)
Rao et al. 1995	India	*An. subpictus*	Neem (Nimin)-coated urea at 0.063 kg ai/ha + 62.5 kg urea/ha	CTS	400 m^2 (3)	-34.0 (-74.4, +70.4)
Rao et al. 1995	India	*An. subpictus*	Neem-coated urea (Neemrich-1 80EC[4]) at 0.09 kg ai/ha	CTS	400 m^2 (3)	-25.1 (-75.4 , +127.7)
Rao et al. 1995	India	*An. subpictus*	As above + 62.5 kg urea/ha	CTS	400 m^2 (3)	-33.2 (-83.5, +171.2)
Rao et al. 1995	India	*An. subpictus*	Neem-coated urea (Neemrich-1 80EC) at 0.12 kg ai/ha	CTS	400 m^2 (3)	-27.0 (-81.5, +187.4)
Rao et al. 1995	India	*An. subpictus*	As above + 62.5 kg urea/ha	CTS	400 m^2 (3)	-32.6 (-76.6, +93.9)
				RE model for all studies		**-30.7 (-57.2, +12.3)**
Azolla and neem						
Rajendran & Reuben 1991	India	*An. subpictus*	*Azolla microphylla* at 100 g/m^2 on 5th DAT + neem cake powder 50 g/m^2 on day of transplantation (TP)	CTS	40 m^2 (2)	-53.9 (-96.5, +528.2)
Neem and water management technique						
Rao et al. 1995	India	*An. subpictus*	Neem (Nimin)-coated urea at 0.063 kg ai/ha + 62.5 kg urea/ha + water allowed to stand 2.5-3.5 cm in the week following TP + from the second week, plots were dried for 2-3 days before re-irrigation	CTS	400 m^2 (3)	-27.5 (-90.1, +430.6)
Rao et al. 1995	India	*An. subpictus*	Neem-coated urea (Neemrich-1 80EC) at 0.09 kg ai + 62.5 kg urea/ha + water allowed to stand 2.5-3.5 cm in the week following TP + from the second week, plots were dried for 2-3 days before re-irrigation	CTS	400 m^2 (3)	-43.7 (-93.3, +370.7)
				RE model for all studies		**-35.6 (-84.9, +175.2)**

*The number of plots per treatment group

[1]CTS: Controlled time series

[2]CITS: Controlled interrupted time series

[3]DAT: Days after transplanting

[4]EC: Emulsifiable concentrate

4.3.5 Rice cultivation practices

All trials experimenting with rice cultivation practices were CTS studies. Compared to continuously flooded fields, water management techniques involving drying intervals were not consistently associated with lower densities of anopheline immatures (Figure 4.3, Table 4.3). When separated into subgroups according to type of drainage, neither active (where water is removed by drainage into canals) nor passive (where water is lost through evaporation or percolation) intermittent irrigation was associated with reduced larval densities, but one-time drainage was associated with 24% higher densities (95% CI 16.6, 31.8, $p<0.0001$, 2 studies, Figure 4.3). When immature abundance was separated into developmental stages, it was revealed that although intermittent irrigation was not associated with significant reductions in early instar larvae, it reduced the abundance of late instars by a pooled estimate of 35% in four CTS studies (95% CI 43.5, 24.0, $p=0.002$, Appendix 4.7). In one Kenyan study, draining during transplanting followed by active intermittent irrigation was associated with a 35% reduction in late-stage larvae, but a 770% increase in early-stage larvae [44]. In another study, based in China, qualitative analysis showed that intermittent irrigation provided good control of *An. sinensis* larvae [180] (Appendix 4.6).

Increasing water height in rice fields was associated with 96% higher *An. freeborni* larval densities in the US (95% CI 83.0-110.0, $p<0.0001$, one study, Table 4.3). One study comparing water management systems found no association between efficient drainage systems and either anopheline larvae abundance or human biting rate [181] (Appendix 4.7).

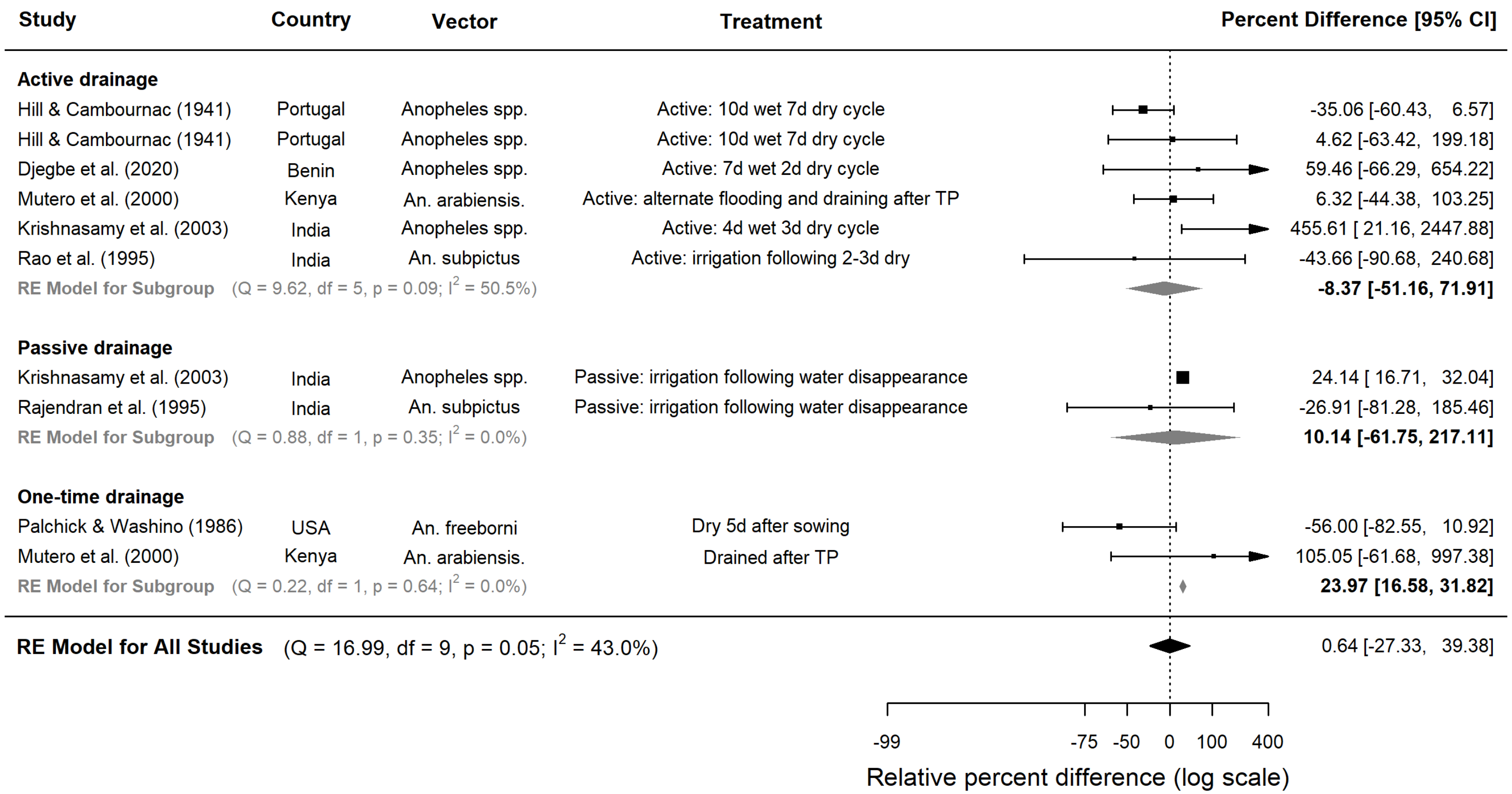


Figure 4.3. The effect of different intermittent irrigation techniques on larval densities of *Anopheles* vectors in rice fields. Seven studies were included, conducted between years 1936 and 2016. Squares represent the relative effectiveness of individual studies, where square size represents the weight given to the study in the meta-analysis, with error bars representing 95% CIs; diamonds represent the pooled effects from random effects (RE) sub-group and meta-analyses.

Table 4.3. Summary of findings of meta-analyses of the effect of rice cultivation practices on *Anopheles* larval density (the number of larvae and pupae per dip or area sampler), arranged by the type of control, study design and geographical region.

Study	**Country**	**Predominant vector**	**Comparison**	**Plot size (no. of replications)**	**Relative percent difference (95% CI)**
Intermittent irrigation					
Palchick & Washino 1986	USA	*An. freeborni*	Drained 5 DAS[1], water depth raised to 3-5 inches until 60 DAS, then to 6-8 inches for rest of season	2800-3800 m^2 (3)	+24.1 (+16.7, +32.0)
Hill & Cambournac 1941	Portugal	*Anopheles*	10d wet, 7d dry cycle*	100 m^2 (4)	-35.1 (-60.4, +6.6)
Hill & Cambournac 1941	Portugal	*Anopheles*	10d wet, 7d dry cycle*	2000 m^2 (4)	+4.6 (-63.4, +199.2)
Djegbe et al. 2020	Benin	*Anopheles*	7d wet, 2d dry cycle*	16.5 m^2 (3)	-56.0 (-82.5, +10.9)
Mutero et al. 2000	Kenya	*An. arabiensis*	Flooded before TP, drained during TP[2], flooded after TP	750 m^2 (4)	+6.3 (-44.4, +103.3)
Mutero et al. 2000	Kenya	*An. arabiensis*	Flooded before TP, drained during TP, alternately flooded and drained after TP	750 m^2 (4)	+455.6 (+21.2, +2448.0)
Krishnasamy et al. 2003	India	*An. subpictus*	4d wet, 3d dry cycle* (rotational water supply)	Varying sizes (5)	+59.6 (-66.3, +654.2)
Krishnasamy et al. 2003	India	*An. subpictus*	Irrigation to 5 cm one day after disappearance of ponded water in fields	Varying sizes (5)	+105.1 (-61.7, +997.4)
Rajendran et al. 1995	India	*An. subpictus*	2.5 cm depth maintained for the first 10-14 DAT[3]. Fields subsequently dried out and re-irrigated to 5 cm depth immediately after all standing water had disappeared (3-5d after irrigation stopped)	16.2-22.3 ha (2)	-26.9 (-81.3, +185.5)
Rao et al. 1995	India	*An. subpictus*	Water allowed to stand 2.5-3.5 cm in the week following TP + from the second week, plots were dried for 2-3 days before re-irrigation	400 m^2 (3)	-43.7 (-90.7, +240.7)
				RE model for all studies	**+0.6 (-27.3, +39.4)**
Control of water depth					
Palchick & Washino 1986	USA	*An. freeborni*	Medium: water level 3-5 inches during first 60d then raised to 6-8 inches vs shallow: water level 1-2 inches during first 60d then to 6-8 inches	2800-3800 m^2 (3)	+89.7 (+77.7, +102.4)
Palchick & Washino 1986	USA	*An. freeborni*	Deep: 6-8 inches all season vs shallow: water level 1-2 inches during first 60d then to 6-8 inches	2800-3800 m^2 (3)	+103.4 (+89.1, +118.9)
					+96.0 (+83.0, +110.0)
Water management system					
Sogoba et al. 2007	Mali	*An. gambiae* s.l.	*Hors-casier* plot sector (no technical assistance in irrigation system and therefore lack efficient drainage systems) vs. *casier* plot sector (renovated irrigation systems)	1000 m^2 (4)	+113.4 (-50.9, +827.1)
Rice variety					
Takagi et al. 1996	Japan	*An. sinensis*	Tall rice (98.5 cm) vs short rice (45 cm)	1500 m^2 (2)	+150.0 (-66.1, +1745.1)
Rice variety and plant spacing					
Victor & Reuben 2000	India	*An. subpictus* & *An. vagus*	ADT36 (short duration variety of 110 days) at 60 hills/m^2 (20x15 cm) vs. 80 hills/m^2 (15x10 cm)	40 m^2 (4)	-49.1 (-94.8, +396.5)
Victor & Reuben 2000	India	*An. subpictus* & *An. vagus*	IR50 (short duration variety of 110 days) at 60 hills/m^2 (20x15 cm) vs. 80 hills/m^2 (15x10 cm)	40 m^2 (4)	-77.9 (-97.0, +60.8)
Victor & Reuben 2000	India	*An. subpictus* & *An. vagus*	IR20 (medium duration variety of 120 days) at 60 hills/m^2 (20x15 cm) vs. 80 hills/m^2 (15x10 cm)	40 m^2 (4)	-62.0 (-95.2, +202.5)
				RE model for all studies	**-66.3 (-90.0, +13.4)**
Weed control					
Palchick & Washino 1986	USA	*An. freeborni*	Weed controlled by herbiciding vs. no weed control	2800-3800 m^2 (3)	+77.4 (+65.7, +89.9)

Study	Country	Predominant vector	Comparison	Plot size (no. of replications)	Relative percent difference (95% CI)
Agricultural insecticide					
Martono 1988	Indonesia	*An. aconitus*	Organophosphorous compound (Basudin 60 EC) used to control paddy pests (such as *Trvporvza* spp., *Leptocorsica acuta* and *Nilaparvata Lugens*) at 960 ppm	250 m^2 (2)	-76.4 (-88.8, -50.2)
Land preparation					
Djegbe et al. 2020	Benin	*Anopheles* spp.	Minimal tillage (tillage depth <15 cm) vs. deep tillage	16.5 m^2 (3)	-64.7 (-85.5, -14.1)
Djegbe et al. 2020	Benin	*Anopheles* spp.	Normal levelling vs. abnormal levelling	16.5 m^2 (3)	-12.8 (-65.2, +118.5)

*Water is applied to the field so that it is wet for X days and left for X days to dry before being irrigated again

[1]DAS: Days after seeding

[2]TP: Transplanting

[3]DAT: Days after transplanting

[4]EC: Emulsifiable concentrate

Table 4.4. Summary of findings of meta-analyses of the association between different types of rice cultivation practices and agronomic outcomes.

Study	Country	Comparison	Plot size (no. of replications)	Outcome	Relative percent difference (95% CI)
Water management techniques					
Hill & Cambournac 1941	Portugal	10d wet, 7d dry cycle*	2000 m^2 (4)	Rice yield	+15.1 (+0.5, +31.9)
Mutero et al. 2000	Kenya	Flooded before TP[1], drained during TP, flooded after TP	750 m^2 (4)	Rice yield	-7.9 (-18.0, +3.3)
Mutero et al. 2000	Kenya	Flooded before TP, drained during TP, alternately flooded and drained after TP	750 m^2 (4)	Rice yield	-9.5 (-21.3, +4.0)
Krishnasamy et al. 2003	India	4d wet, 3d dry cycle* (rotational water supply)	Varying sizes (5)	Rice yield	+3.9 (-0.7, +8.7)
Krishnasamy et al. 2003	India	Irrigation to 5 cm one day after disappearance of ponded water in fields	Varying sizes (5)	Rice yield	-0.2 (-5.5, +5,4)
Rajendran et al. 1995	India	2.5 cm depth maintained for the first 10-14 DAT[2]. Fields subsequently dried out and re-irrigated to 5 cm depth after all standing water had disappeared (3-5d after irrigation stopped)	162,000-223,000 m^2 (2)	Rice yield	+2.4 (-8.1, +14.1)
				RE model for all studies	**+0.8 (-3.8, +5.7)**
Hill & Cambournac 1941	Portugal	10d wet, 7d dry cycle*	2000 m^2 (4)	Water use	-18.5 (-30.0, -5.1)
Krishnasamy et al. 2003	India	4d wet, 3d dry cycle* (rotational water supply)	Varying sizes (5)	Water use	-7.5 (-10.5, -4.5)
Krishnasamy et al. 2003	India	Irrigation to 5 cm one day after disappearance of ponded water in fields	Varying sizes (5)	Water use	-21.0 (-23.8, -18.0)
				RE model for all studies	**-15.4 (-24.0, -5.7)**

*Water is applied to the field so that it is wet for X days and left for X days to dry before being irrigated again

[1]TP: Transplanting

[2]DAT: Days after transplanting

Studies that examined the effect of rice cultivation practices other than water management methods were scarce (Table 4.3). One study in Japan observed that varying rice plant heights was not associated with larval numbers [182]. A study in India showed that plant density, regardless of rice variety, did not affect anopheline larval densities [38]. Palchick and Washino (1986) observed that using herbicides for weed control, compared to no weed control, was associated with 77% (95% CI 65.7, 89.9, $p<0.0001$) higher larval numbers [36]. On the other hand, pesticides were associated with a 76% reduction (95% CI 88.8, 50.2, $p=0.001$) of anopheline larvae in Indonesia [183]. Different processes in land preparation seemed to affect mosquito numbers: whilst levelling had no effect, rice plots that were minimally tilled were associated with a 65% reduction (95% CI 85.5, 14.1, $p=0.02$, one study) compared to those with deep tillage [184].

4.3.6 Rice yield and water consumption

Agronomic outcomes were not measured in the eligible studies that investigated larviciding and biological control in rice fields; they were only measured in four studies assessing intermittent irrigation (Table 4.4). A meta-analysis of the four studies revealed that water management techniques alternative to continuous flooding did not significantly affect rice yield. In Portugal, however, Hill and Cambournac (1941) observed a 15% increase in yield (95% CI 0.5, 31.9, $p=0.005$) [185]. This study, combined with Krishnasamy et al. (2003), demonstrated that intermittent irrigation (active or passive) reduced water use significantly, saving around 15% (95% CI 24.0, 5.7, $p=0.002$) [186].

4.3.7 Scalability of technologies

Of 47 quantitative and qualitative studies, 13 studies (11 quantitative and 2 qualitative) included intervention readiness in their discussions (Appendix 4.8). One study showed that using MSFs seemed to be appropriate for small-scale rice farmers, whilst larvicides were not economical, especially at an individual field basis [176,187,188]. Sundaraj and Reuben (1991) stated that in order to increase acceptance, labour-saving operations must be developed [188]. Fish, on the other hand, seemed to be well-accepted as an additional source of income and protein [189,190]. *Azolla* was also popular amongst rice farmers, not only because rice yields increased, but also because weed pressure halved [191]. Neem, however, needed to be more affordable and commercially available to promote large-scale use [191].

Discussions on the scalability of intermittent irrigation were mixed: in Portugal and China, it was well-accepted and promoted by the government due to increased yield and decreased water consumption [180,185]. In India, farmers held different views: whilst convinced of intermittent irrigation based on water conservation, they doubted their own ability to organise water distribution and wanted the supervision of a government agency [192]. Moreover, its efficacy was dependent on farmer practices and a lot of effort was still required to change practices on a large scale [186]. In Kenya, intermittent irrigation could not be recommended to farmers as rice yield was not increased significantly, required more labour and had no apparent advantage on water consumption [44].

4.4 Discussion

We investigated whether rice-field mosquito larval control and/or rice cultivation practices are associated with malaria vector densities through a systematic review and meta-analysis. Forty-seven experimental studies were eligible for inclusion in the qualitative analysis and thirty-three studies were eligible for the meta-analysis. It was demonstrated that the use of fish, chemical and biological larvicides in rice fields were effective in controlling larval malaria vector densities, using density indices that included all developmental stages combined. Intermittent irrigation, however, could only significantly reduce late-stage larvae. Based on a limited number of studies, meta-analyses on other forms of larval control such as monomolecular surface films (MSFs), neem, copepods and *Azolla* failed to demonstrate any consistent reduction in anopheline numbers. Similarly, rice cultivation practices such as plant variety and density, type of levelling and pesticide application were not generally associated with reduced malaria vectors. Nonetheless, in one study, minimal tillage was observed to reduce average numbers of larvae throughout a cropping season. In another study, herbicide application increased larval abundance over a 4-week period, as did one-time drainage in a third study.

Despite their different modes of action, the use of chemical and bacterial larvicides and MSFs were all relatively effective measures of larval control in rice fields, varying between a 57% to 76% reduction in vector abundance compared to no larviciding. Their effects were highest (often reaching 100% reduction) only shortly following application but did not persist for longer than two weeks. These larvicides mostly had short residual half-lives because they were applied to paddy water which was naturally not completely stagnant: there was a small but constant process of water loss (through drainage, evapotranspiration and percolation) and replacement through irrigation. Hence, even with a residual formulation, weekly re-application would be needed for sustained control [193–196]. This would be very labour- and cost-intensive to scale-up, to ensure that larvicides are evenly distributed across vast areas (even at plot/sub-plot level) throughout at least one 5-month long rice-growing season per year [188,197]. Aerial application (including unmanned aerial vehicles), although widely used in the US and Europe, is unlikely to be a feasible delivery system for smallholders in SSA, even in large irrigation schemes [175,176,194,195].

Biological control using fish was found to be, in general, slightly more effective than (chemical, bacterial and MSF) larviciding. The degree of effectiveness was dependent on the fish species and their feeding preferences: surface-feeding, larvivorous species provided better anopheline control than bottom-feeding selective feeders [48,189]. Selecting the most suitable fish for local rice fields is not straightforward; many criteria need to be considered [48,198,199]. Generally, fish were well-received by rice farmers, perceived to contribute to increased yield by reducing weeds and pests and providing fertiliser through excrement [189,190]. This was reportedly also observed in Guangxi, China, where a certain proportion of the field had to be deepened into a side-trench where the fish could take shelter when the fields were drained. Even with this reduction in rice production area, carp rearing still increased yields by 10% and farmer's income per hectare by 70% [199]. Unfortunately, none of the eligible studies in this review had included yield or water use as an outcome. Future entomological studies need to measure these critical agronomic variables so that studies of vector control in rice can be understood by, and transferred to, agronomists. In SSA, irrigated rice-fish farming can be scaled up provided that an inventory of fish species suitable for specific locations is available and that water is consistently available in fields (an important limiting factor in African irrigation schemes) [200]. Lessons can be learnt from successful large-scale rice-fish systems in Asia, where they have served as win-win solutions for sustainable food production and malaria control [80,201].

Overall, there was only limited evidence that intermittent irrigation is effective at reducing late-instar anopheline larvae in rice fields. This finding contrasts with prior reviews, which found mixed results (regardless of larval stage) but emphasised that success was site-specific [27,48,81]. This contrast is presumably due to the inclusion criteria of our systematic review. These excluded some older studies that reported successful anopheline control with intermittent irrigation but lacked either a contemporaneous control arm, adequate replication or adequate differentiation between culicines and anophelines [80,202–206]. It seems, from our review, that intermittent irrigation does not prevent the recruitment of early instars (and in one case, may have encouraged oviposition [44]) but tends to prevent their development into late-stage immatures. This important conclusion is, however, based only on four studies; more evidence is urgently needed where future trials should consider the basic principles of modern trials with adequate replication, controls and differentiation between larval instars and species.

Generally, it is observed that drainage, passive or active, did not reliably reduce overall numbers of mosquito immatures. In India and Kenya, closer inspection revealed that soils were not drying sufficiently, so any stranded larvae were not killed [44,192]. Highlighted by van der Hoek et al. (2001) and Keiser et al. (2002), water management in rice fields is very dependent on the physical characteristics of the soil and the climate and is most suited to places that not only favour rapid drying, but also have a good control of water supply [27,81]. Moreover, repeated drainage, although directed against mosquitoes, can also kill their aquatic predators [207]. Since mosquitoes can re-establish themselves in a newly flooded rice field more quickly than their predators, intermittent irrigation with more than a week between successive drying periods can permit repeated cycles of mosquito breeding without any predation pressure. Its efficacy against malaria vectors is therefore highly reliant on the timing of the wetting and drying periods. Further site-specific research on timing, especially with regards to predator-prey interactions within the rice agroecosystem, is required to find the perfect balance.

It appears that intermittent irrigation cannot be applied during the first two to three weeks following transplanting, because rice plants must remain flooded to recover from transplanting shock. Unfortunately, this time coincides with peak vector breeding. Thus, other methods of more focussed, larval control will be needed during this period and should be explored to fill this gap. To agronomists, intermittent irrigation provides benefits to farmers, as it does not penalise yield but significantly reduces water consumption. Nonetheless, farmer compliance seems to be variable, especially in areas where water availability is inconsistent and intermittent irrigation would potentially require more labour [44,180,185]. Importantly, rice farmers doubted their ability to coordinate water distribution evenly amongst themselves, suggesting that there may be sharing issues, as in the "tragedy of the commons" [208]. Instead, they said that they preferred to have an agreed authority to regulate water [192].

No general conclusions could be made on the effect on malaria vectors of other rice cultivation practices (apart from water management) because only one study was eligible for each practice. Nevertheless, these experiments on pesticide application, tillage and weed control, as well as another study on plant spacing (not eligible since glass rods were used to simulate rice plants), do illustrate that small changes in agronomic inputs and conditions can have considerable effects on mosquito densities, not just rice yield [36,50,184,209]. Moreover, in partially- or shallowly-flooded plots, the larvae are often concentrated in depressions (usually footprints), suggesting that rice operations which leave or remove footprints (e.g., hand-weeding, drum seeders, levelling) will influence vector breeding [48].

Our study has some important limitations. First, in most trials, the units of intervention were replicate plots of rice, and success was measured as a reduction in larval densities within treated plots. This design focuses on the identification of effective and easy-to-implement ways of growing rice without growing mosquitoes, on the assumption that higher vector densities are harmful. However, from a public health perspective, the need for epidemiological outcomes is often, and reasonably, stressed [174,210]. Nonetheless, from a farmers' perspective, it is also important to consider whether the vectors emerging from their rice fields significantly contribute to the local burden of malaria and to determine how this contribution can be minimised. There is evidence that riceland vectors can and often do increase malaria transmission, since human biting rates are much higher in communities living next to rice schemes than their non-rice counterparts [62], and these additional riceland vectors may intensify transmission, leading to higher malaria prevalence in rice communities [173]. Hence, when investigating how rice-attributed malaria risk can be minimised, despite WHO opinions, we consider immature mosquito abundance[7] (as measured in the experimental rice trials) a useful indicator of potential impact on epidemiological outcomes.

Second, larval density was not always separated into larval developmental stages. This can be misleading because some interventions work by reducing larval survival (but not by preventing oviposition) and development to late instars and pupae. Therefore, an intervention could completely eliminate late-stage larvae and pupae but have little effect on the total number of immatures. This was illustrated in our meta-analyses of intermittent irrigation in Table 4.3 and Appendix 4.7 and could have been the case for some studies that failed to

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[7] Please refer to Pages 80-81 on further explanations.

demonstrate consistent reductions in overall anopheline numbers but did not differentiate between larval instars [182,191,211–213]. When an intervention reduces larval densities, density-dependent mechanisms may cause an increase in survival to adulthood, so that there might be no reduction in the number of adults emerging. Hence, we infer that when monitoring mosquito immatures in rice trials, it is important to focus on late stages and pupae rather than on early stages or all stages combined. Pupae should always be counted separately since its abundance is the most direct indicator of adult productivity[8] [214].

Third, experimental trials rarely reported the timing of intervention application or accounted for different rice-growing phases, or "days after transplantation", in the outcome. Both aspects are important to consider since an intervention may be suited to control larvae during certain growth phases but not others. This is illustrated by Djegbe et al. (2020), where, compared to deep tillage, minimal tillage could significantly reduce larvae during the early stages of rice cultivation but not during tillering and maturation [184]. In contrast, other interventions, such as *Azolla* and predatory copepods, took time to grow and accumulate, and were more effective during the later stages of a rice season [49,191,211]. This differentiation is important because it can identify components that could potentially form a complementary set of interventions against riceland malaria vectors, each component being effective at different parts of the season. Since rice fields, and hence the dynamics of riceland mosquito populations, vary from place to place, this set of interventions must also be robust. Special attention must be paid to the early stages of rice cultivation, particularly the first few weeks after transplanting (or sowing), since, with many vector species, a large proportion of adult mosquitoes are produced during this time.

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[8] Note that for interventions that target pupal stages (e.g. pyriproxyfen), pupae may not be the most direct indicator of adult productivity and thus, newly emerged adults would have to be considered instead.

Fourth, the analysis of entomological counts is often inadequate. Many studies failed to provide the standard deviation (or any other measure of error) for larval counts and could not be included in the quantitative analysis. Often, due to the extreme (and not unexpected) variability of larval numbers, sample sizes were insufficient to calculate statistically significant differences between treatments. Fifth, a high risk of bias was found across both CTS and CITS studies, including high heterogeneity and some publication bias. Study quality was, in general, a shortcoming and limited the number of eligible studies for certain interventions, including intermittent irrigation. Moreover, there are conspicuous a priori reasons for bias in such experimental trials: trial locations are frequently chosen to maximise the probability of success.

Finally, few studies were conducted in African countries, where the relationship between rice and malaria is most important because of the efficiency, and the "rice-philic" nature, of the vector *An. gambiae* s.l. [173]. In particular, there was a lack of studies on the effectiveness and scalability of biological control and rice cultivation practices. There is also very little information (particularly social science studies) on the views and perspectives of African rice farmers on mosquitoes in rice and interventions to control them [215,216].

In the future, as malaria declines (particularly across SSA), the contribution of rice production to increased malaria transmission is likely to become more conspicuous [173]. Unless this problem is addressed, rice growing will probably become an obstacle to malaria elimination. Current default methods of rice production provide near-perfect conditions for the larvae of African malaria vectors. Therefore, we need to develop modified rice-growing methods that are unfavourable to mosquitoes but still favourable for the rice. Although larviciding and biological control may be appropriate, their unsustainable costs remain the biggest barrier to uptake amongst smallholder farmers. Future investigations into riceland vector control should pay more attention to interventions that may be useful to farmers.

Supported by medical entomologists, agronomists should lead the research task of identifying cultivation methods that achieve high rice productivity whilst suppressing vector productivity. Rice fields are a major global source of greenhouse gases, and agronomists have responded by successfully developing novel cultivation methods that minimise these emissions while maintaining yield. We need the same kind of response from agronomists, to achieve malaria control co-benefits within rice cultivation. At present, only a few aspects of rice cultivation have been investigated for their effects on mosquitoes, and the potential of many other practices for reducing anopheline numbers are awaiting study. Due to the spatial and temporal heterogeneity of rice agroecosystems, it is likely that no single control method can reduce mosquito numbers throughout an entire cropping season and in all soil types and irrigation methods. Thus, effective overall control is likely to come from a combination of local, site-specific set of complementary methods, each of which is active and effective during a different phase of the rice-growing season.

4.5 Conclusions

Our findings suggest that whilst larviciding, fish and intermittent irrigation can reduce the breeding of malaria vectors in rice fields, their effectiveness is sensitive to environmental conditions and highly dependent on the timing and frequency of both intervention application and sampling. There is a lack of experimental studies on the interactions between these factors and their effects on anopheline larval densities, especially during different parts of a rice-growing season. Such studies are needed to find a robust combination of rice cultivation practices that do not exacerbate, and can potentially control, malaria vector production throughout an entire cropping season. To do this, the agricultural sector needs to take the lead, and take responsibility, for the deadly mosquitoes produced by agriculture. Therefore, long-term alliances between the agricultural and health sectors are required, not only to develop effective methods to control mosquitoes without compromising rice yields, but also to encourage intervention uptake and adoption by farmers through agricultural extension systems.

4.6 Methods

A systematic review and a meta-analysis were conducted to assess how specific rice cultivation practices and mosquito control methods affect malaria vector abundance, rice yield and water consumption. Recommendations of the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) were followed. The study was not registered with the International Prospective Register of Systematic Reviews because it did not consider outcomes from human subjects and mosquitoes are not considered animal subjects. KC and JL did the systematic search, selected studies for inclusion and extracted relevant information. Any disagreements were resolved by LT. Data were extracted by KC and a 10% sub-sample was randomly selected for validation by JL.

4.6.1 Eligibility criteria

This systematic review was concerned with mosquito populations. The intervention term encompassed a wide range of measures related to rice-growing practices (rice variety, plant density, land preparation method, crop establishment method and water management technique as well as application of fertilisers, herbicides and pesticides) and potential larval control (synthetic organic chemicals, oils and surface agents, biological larvicides, insect growth regulators, fish, nematode, *Azolla*, neem).

Studies were included if they measured effects on the relative density of larvae and pupae of malaria vectors (measured by area samplers, sweeping or standard dipping techniques) or the relative density of adult malaria vectors (measured by human landing catch, CDC light trap, pyrethrum spray catch, odour-baited traps or emergent traps). The secondary outcomes of interest were agronomic measures including rice yield (in tonnes per hectare) and water consumption (defined as the amount of used for rice cultivation in cubic metres).

Only experimental study designs were considered; (1) controlled time series trials (CTSs), with the unit of allocation being a rice plot and at least two replications per arm; (2) controlled interrupted time series studies (CITSs), with a contemporaneous control group and at least two replications per arm (Appendix 4.1). Studies were included only if they reported data collected from experimental rice fields; laboratory and semi-field studies were excluded. Studies were excluded if a control arm was absent and if the follow-up periods in each arm differed.

4.6.2 Search strategy

PubMed, Embase, Global Health, SCOPUS, Web of Science, AGRIS, GreenFILE, TRIP database, BASE, ProQuest Dissertations & Theses Global, and EThoS were searched from 5th to 10th October 2020 to identify all relevant studies, using specified search terms (Appendix 4.9). The search was restricted to published studies dated from 1900, and in English and French language. Proceedings from the following conferences were also searched: the MIM Pan-African Malaria Conferences, Pan-African Mosquito Control Association, American Society of Tropical Medicine and Hygiene, American Mosquito Control Association, Society for Vector Ecology and Agriculture for Nutrition and Health Academy Week. Reference lists of all relevant identified studies and published reviews were also searched. Authors and colleagues in the field were contacted for any additional references.

4.6.3 Data extraction

From each eligible study, the following information were extracted into a pre-designed form: country, study setting, study design, intervention(s), control group, outcome(s), sampling, sample size, and vector(s). Any statements concerning the adoptability or scalability of the intervention by rice farmers were also extracted. If relevant data was unclear or not reported, study authors were contacted for clarification.

4.6.4 Risk of bias

Risk of bias for CTSs and CITSs was assessed using the Effective Practice and Organisation Care (EPOC) tool [217]. If a sufficient number of studies were included in the meta-analysis, funnel plots were constructed and Egger's test for funnel plot asymmetry were conducted to assess risk of publication bias [123].

4.6.5 Data analysis

Analyses were structured by (1) the type of intervention, (2) outcome and (3) study design. All eligible studies were included in a qualitative analysis. If sufficient data to calculate crude effects was reported (i.e., standard deviations or 95% confidence intervals), studies were also included in a quantitative analysis. Post-intervention data were considered only up to the end of a rice-growing season, marked by harvest. Each outcome (entomological and agronomic) was combined in separate meta-analyses.

Analyses were conducted in R (version 4.1.2) [218]. For both entomological (count) and agronomic outcomes in CTSs, measures of effect (relative percent difference) were calculated by back-transforming the log-transformed ratio of means. For CITSs, relative percent differences were calculated by fitting a quasi-Poisson regression (due to overdispersion in larval counts) to pre- and post-intervention period (i.e., interruption) whilst using the control as an offset terms to adjust for trend [219]. For CTSs, means were compared between study arms. Where there were multiple measurements over several time points these were averaged. Grouped by study design, random effects models were then used to calculate pooled measures of effect and their 95% CI to illustrate the effect of each intervention on each outcome [120,220]. Heterogeneities were analysed using the I^2 statistic, and to reduce the extent of heterogeneity, random effects models were used.

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SECTION A – Student Details

Student ID Number	1602712	**Title**	Miss
First Name(s)	Kallista Hay Ching		
Surname	Chan		
Thesis Title	Rice and malaria in Africa: Seeking vector control in rice cultivation practices		
Primary Supervisor	Jo Lines		

If the Research Paper has previously been published, please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?			
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SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	Parasites and Vectors
Please list the paper's authors in the intended authorship order:	Kallista Chan, Lucy S. Tusting, Kimberly Fornace, Ellie Sherrard-Smith, Kazuki Saito, Jo Lines, Raphael N'Guessan, Alphonsine Koffi, Christian Bottomley
Stage of publication	Not yet submitted

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper.	I designed the experiment, performed all analyses and wrote the paper. Other authors assisted in suggesting analysis methods and writing.

SECTION E

Student Signature	[redacted]	**Supervisor Signature**	[redacted]
Date	12/08/2022	**Date**	12/08/2022

5 Suppressing the breeding of malaria vectors in African rice fields: measuring the distribution, composition, density, and productivity of malaria vectors in rice fields of Côte d'Ivoire

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5.1 Abstract

Background

Rice fields provide excellent breeding sites for the most efficient malaria vectors in sub-Saharan Africa, *Anopheles gambiae sensu lato.* This calls for the development of alternative rice cultivation practices that minimise mosquito production. A more thorough understanding of the ecology of riceland malaria vectors is required, particularly their temporal and spatial distribution, species composition and vector productivity.

Methods

Anopheles mosquito larvae were sampled twice weekly for four rice-growing seasons (from 2019 to 2021) in irrigated rice plots in M'bé, central Côte d'Ivoire. To assess their temporal distribution, relative anopheline densities between rice growth phases were analysed using Prais-Winsten regression. To assess their spatial distribution, associations between anopheline abundance and distance from the edge of rice plots (that underwent intensive sampling) were analysed using negative binomial regression models. The vector productivity of rice fields was calculated as the product of the mean number of pupae collected in a dip, and a scaled calibration factor obtained from a dip-quadrat zero-intercept linear regression.

Results

A total of 40,310 anopheline immatures were collected over 782 sampling days, of which 84.0% were *An. coluzzii*. Anophelines were most numerous earlier in the rice cropping season, where paddies produced 88% of pupae observed in the whole season up to four weeks after transplanting. Their productivity was lower during the later stages of the season (reproductivity and ripening phases), but still persistent. Vector abundance was greater closer to the edges of fields and when rice plants were shorter, water was clearer and without iron (III) oxide residues and predators. It was estimated that a total of 700,000

malaria vectors was produced in one hectare of rice during a growing season in the M'bé irrigation scheme of Côte d'Ivoire.

Conclusions

Rice fields can generate a large amount of malaria vectors and understanding how their phenology impacts mosquito populations is vital. This understanding helps facilitate the development of (a combination of) rice cultivation techniques that minimise mosquito production throughout a cropping season. Further work is still required to identify the optimal strategies of riceland malaria vector control. Better methods to measure the epidemiological impact of these control strategies are also needed; current reliance on vector density is inadequate.

Keywords

Malaria, *Anopheles*, vector, irrigated rice, agriculture, vector productivity, spatial distribution

5.2 Introduction

Current malaria control methods, such as insecticide-treated nets (ITNs), indoor residual spraying (IRS) and artemisinin-based combination therapy (ACTs) have been highly effective at reducing global malaria burden; Bhatt et al. (2015) estimated that they contributed to a halving of infection prevalence in endemic Africa from 2000 to 2015 [2,7]. Owing to the effectiveness of these interventions, many countries in sub-Saharan Africa (SSA) are still starting to plan for elimination, despite the recent malaria resurgence after a period of stagnation in the last half-decade.

However, further progress towards malaria elimination will be impacted by the planned expansion of rice harvested areas [221]. To reach self-sufficiency and increase food security, Ministries of Agriculture across SSA are actively promoting strategies that increase rice production, including fulfilling Africa's irrigation potential [25]. Unfortunately, because rice fields provide excellent breeding sites for *Anopheles gambiae* sensu lato, the most efficient malaria vector, their expansion may compromise malaria elimination, or lead to malaria resurgence [222]. Continent-wide studies have consistently demonstrated that greater densities (of up to 6-fold higher) of adult malaria vectors are found in rice-growing areas compared to nearby non-rice growing areas (e.g., traditional crop farming, market gardening, pastoralism, or savannah areas) [Chapter 3] [221]. By producing a large number of competent vectors, the inherent potential of a rice agro-ecosystem to increase malaria transmission is high, endangering populations living close to rice fields.

Recently published evidence suggested that as malaria declines, the associations between rice and malaria will emerge and become stronger [221]. In the future, rice fields are therefore likely to emerge as foci of remnant transmission. Currently available malaria control interventions like ITNs are not long term or complete solutions to this [223]. More effort in minimising the malariogenicity of landscapes is essential; in this instance, the creation of rice fields that are highly productive of efficient malaria vectors should be avoided. Thus, the role of the rice sector is to stop being part of the problem, and start being part of the solution. Rice-growing methods that are unfavourable to mosquito production (but still favourable to rice) need to be developed. This strategy has been historically important in elimination settings, where targeted riceland mosquito control was used in countries such as the USA, Portugal, Turkmenistan, and China to achieve malaria elimination by suppressing vector densities and malaria transmission [169,224]. Although extra effort is required, the development of rice cultivation techniques that minimise mosquito production in Africa is possible.

To achieve this, better understanding of the epidemiological significance of these modified rice cultivation practices is urgently needed [225]. It is particularly important to determine vector productivity (i.e., the absolute number of adult malaria vectors that emerges from rice fields) in order to estimate the malaria burden attributable to rice cultivation (e.g., number of cases/disability-adjusted life years/deaths per tonne of rice produced) and ultimately, the number of cases that can be averted by adopting particular rice practices. However, due to the "boom and bust" nature of mosquitoes, vector productivity is unusual and strikingly difficult to measure; previous attempts to estimate mosquito population densities are summarised by Silver (2008) [89]. In rice fields, most studies related the number of mosquito immatures from dippers or enclosures of known size (volume or surface area) to fields of known size [226–231]. Whilst some estimates took into account of the proportion of rice field with water (i.e., water-logged rate), most assumed that immatures are randomly distributed, which they are not [226,231]. Therefore, there needs to be more consideration into mosquito spatial distribution within a rice field (i.e., whether and where clustering occurs) when estimating vector productivity.

Meanwhile, a more thorough understanding of the ecology of malaria vectors in rice fields is also needed. Due to the spatially and temporally dynamic nature of paddies, several bio-environmental factors are known to affect the rate at which mosquitoes emerge from paddies, such as the "age" of the water (the colonisation of aquatic predators), the development of rice plants and the inputs of rice operations [80,222,225]. However, the impact of these factors varies from place to place and other features, such as mosquito survivorship in rice fields, still need further exploration.

In this study we aimed to measure the vector productivity of irrigated rice fields in central Côte d'Ivoire, which are representative of the inland rice-growing valleys in West Africa, with a view to estimate its epidemiological impact. Additionally, we studied the temporal and spatial distribution and species composition of malaria vectors in rice fields to aid vector surveillance in rice fields and identify target areas for control. To our knowledge, this is the first attempt to estimate the absolute number of malaria vectors produced in a spatial unit of African rice fields over a cropping season.

5.3 Methods

5.3.1 Study site

The study was carried out in experimental fields at the M'bé Africa Rice Center (AfricaRice) research station, in the Gbêkê region of the Vallée du Bandama district of central Côte d'Ivoire (7°52'31.1" N, 5°06'46.2" W). In this region, there is a dry season from November to March which is followed by a rainy season from April to October [232]. The research station is located within low-lying plains and is characterised by a slope of 0 to 1% and poorly drained, deep clayey soils [233]. The irrigation scheme covers an area of 150 hectares, of which more than 95% is used for irrigated rice cultivation (Figure 5.1). Within a 5-km radius around M'bé, there is a total population of approximately 21,000 people in 24 villages [234].

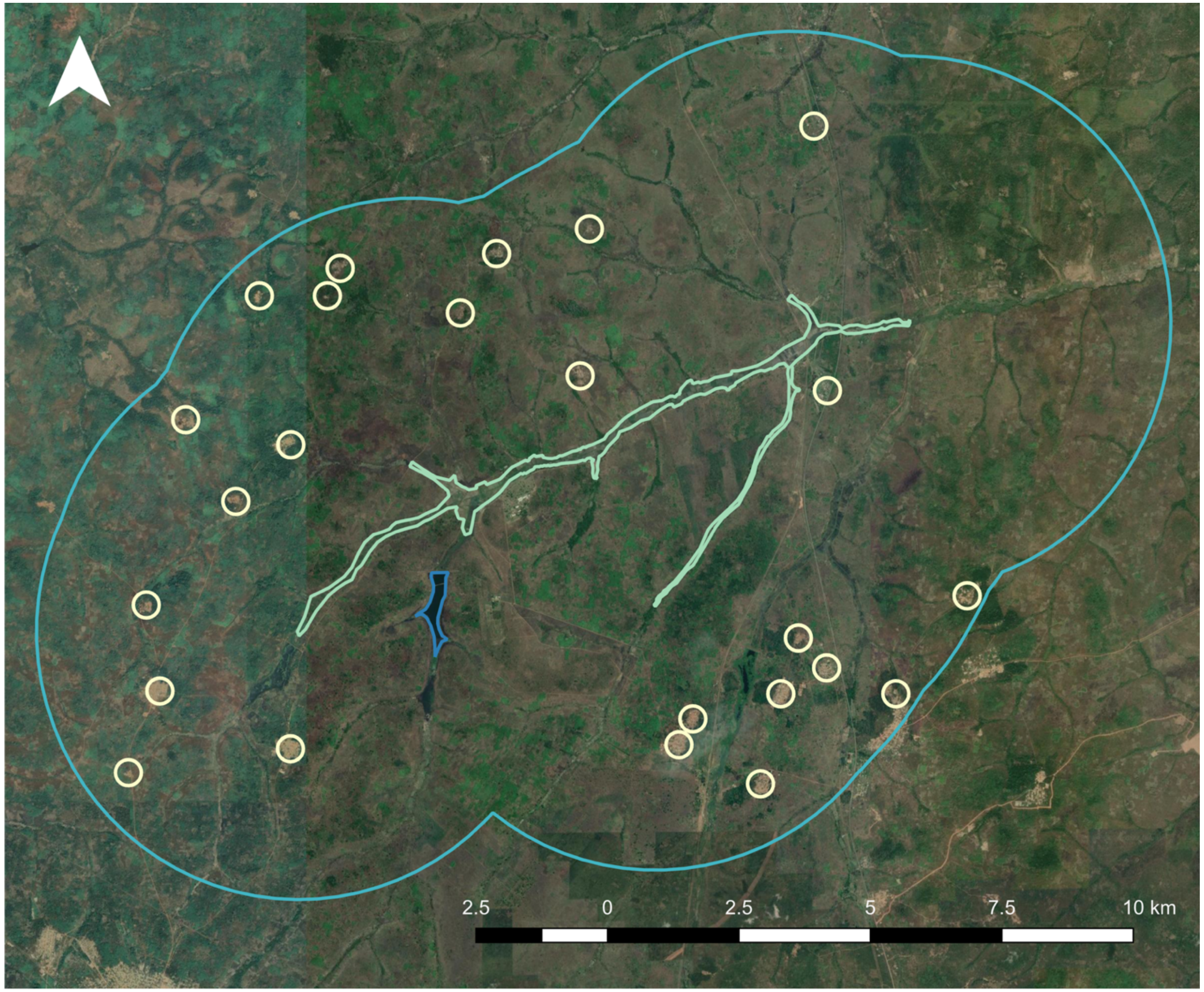

Figure 5.1. Map of the M'bé 1 irrigation scheme and its 24 neighbouring villages (with a population of approximately 21,000) within a 5-km radius in central Côte d'Ivoire.

5.3.2 Experimental rice paddies

Experimental rice paddies were established in the wet seasons of 2019 (Apr-Aug)[9] and 2021 (Mar-Jul) and in the wet-dry cusp of 2019 and 2021 (Aug-Dec). Fifteen to 20 square plots of 5 metres were established. They were hydrologically isolated from each other and from surrounding plots using plastic-covered bunds and canals to avoid water mixing. All the plots had the same “standard” growing conditions and treatments that are recommended to local farmers (by AfricaRice): use of the local seed variety WITA-9, transplanting, continuous flooding (where the water table is maintained at 2-5 cm), three rounds of weeding and herbicide application and three rounds of fertiliser applications (at transplanting, tillering and panicle initiation). Meteorological data (rainfall, humidity, and temperature) was recorded daily by the weather station at the field site.

5.3.3 Mosquito sampling and identification

All plots were exposed to the natural colonisation of *Anopheles* mosquitoes. Larvae were sampled from the beginning of land preparation until two weeks after harvesting for four rice-growing seasons (~22 weeks of sampling for each season). They were sorted into larval developmental stages: L1, L2, L3, L4 and pupae. Late-stage larvae (L3/L4) and pupae were transported to the entomology laboratory and reared into adults for morphological species identification. A 20% sample of morphologically identified members of the *An. gambiae* species complex was then molecularly identified using PCR.

Different sampling techniques were used to assess the temporal and spatial distribution of larvae and vector productivity.

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[9] Mosquito collection data from this trial was used for the power calculations detailed in Appendix 6.5.

5.3.3.1 Temporal distribution

Sampling was conducted twice weekly (every 3-4 days) using standard dipping techniques with a plastic dipper (BioQuip, 350 ml). Technicians dipped by skimming at an angle quickly through deeper waters or by lowering the dipper gently into the water at an angle to collect all water accumulated in a depression (in shallower waters). Two dips were taken every 2.5 metres at the borders of each plot so a total of sixteen dips were conducted in each plot. A total of 343 sampling occasions were conducted over the four aforementioned cropping seasons.

5.3.3.2 Spatial distribution

One to two plots were randomly selected for more intensive sampling every week. Within a 5 by 5 metre plot, two-hundred dips (2 dips within 100 0.25m^2 squares) were made within the plot. A total of 51 sampling occasions were conducted over one cropping season (Jun-Dec 2021).

5.3.3.3 Vector productivity

Our approach, like previous studies, still relied on dipping to estimate relative vector abundance, but used a separate calibration measure based on quadrats to estimate absolute abundance. Quadrats (0.25 m^2, made locally of metal, see Appendix 5.1) were placed in the centre, corners, and borders of a plot. For each sampling occasion, they were firmly inserted into the mud to ensure that no larvae could escape through the bottom. Two dips were taken from within the quadrat and the abundance of larvae at each developmental stage was recorded. Then, all water from the quadrat was “evacuated” (scooped) into buckets; bottom mud was washed with water to detach any stranded larva or pupa. The total number of larvae per quadrat was counted. The immature count from two dips within the quadrat was then calibrated against the total immature count from the quadrat to obtain a conversion factor to convert the yield of two-dips per quadrat into an estimate of the number of individuals per spatial unit. A total of 110 sampling occasions were conducted over four aforementioned cropping seasons.

5.3.4 Habitat characterisation

The following environmental variables were recorded at each sampling occasion (Appendix 5.2): rice height (from base to highest leaf), water depth, water turbidity (classed into five categories, determined through observation), type of vegetation (surface floating, filamentous algae, weeds), type of fauna present other than mosquitoes (tadpoles, large insects, arachnids, molluscs) as well as the presence of a layer of iron (III) oxide (Appendix 5.3). The build-up of iron (III) oxide occurs when there are large concentrations of reduced iron in the soil and presents itself on the water surface as a film of oil-like (sometimes with rainbow iridescence) substance.

5.3.5 Data analysis

All three datasets (temporal, spatial and vector productivity) were analysed using R version 4.1.2 [218]. Results were reported as statistically significant if the p-value was <0.05 unless stated otherwise. The abundance of *Anopheles* mosquitoes was expressed as the number of mosquito immatures per 10 dips. Rice growth phases were separated into land preparation (operations before transplanting), vegetative (transplanting to panicle initiation), reproductive (panicle initiation to flowering), ripening (flowering to harvest), and post-harvest (Figure 5.2).

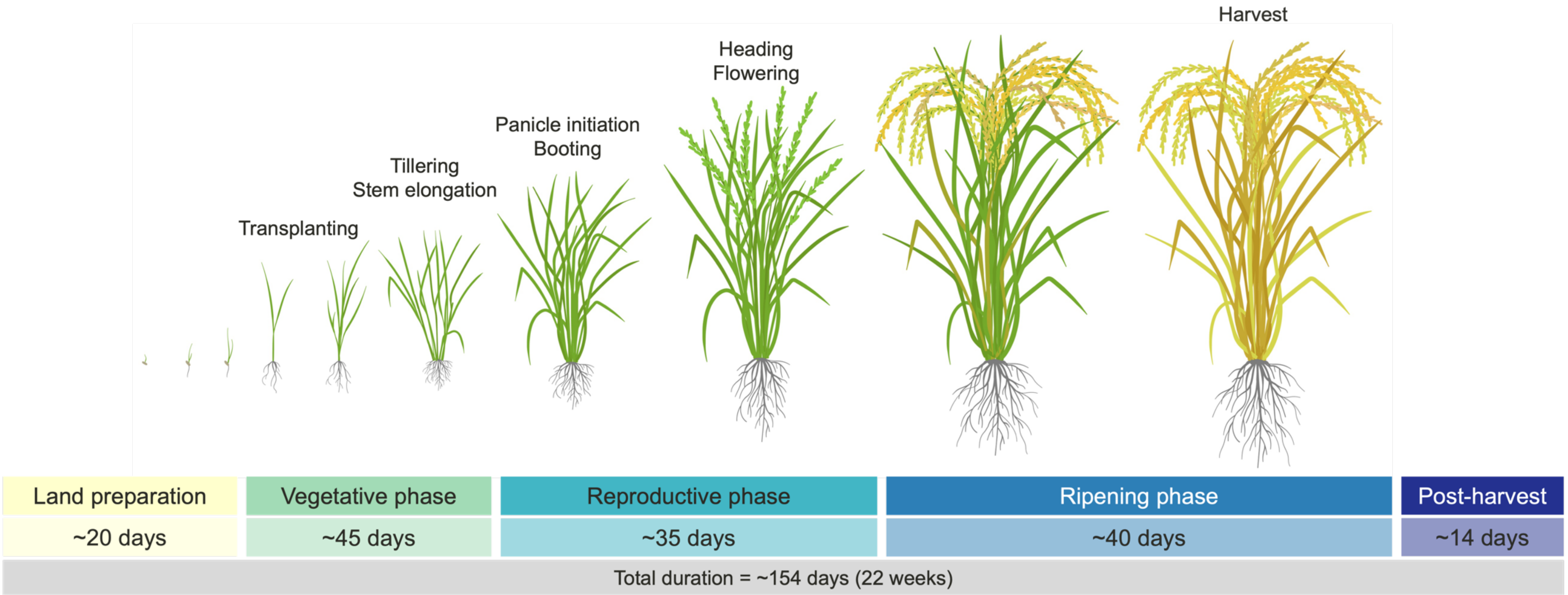

Figure 5.2. Total duration of a rice cropping season in this experimental trial: land preparation, followed by around 120 days of crop growth (estimated by M'bé local seed variety WITA-9) and two weeks after harvesting.

5.3.5.1 Temporal distribution

To compare relative densities of *Anopheles* mosquitoes (grouped by larval development stage) between rice growth phases and cropping seasons, data collected at each sampling occasion were aggregated across all plots and analysed using the Prais-Winsten method [235]. When comparing mosquito counts over time, time series methods such as the Prais-Winsten method (a form of linear regression) are necessary to account for temporal autocorrelation. To allow for multiple pair-wise comparisons between growth phases, differences in means were computed using model coefficients. To identify the weeks (during a rice season) that were most productive for malaria vectors, cumulative distributions were plotted to find the weeks after transplanting that had the steepest gradient.

5.3.5.2 Survivorship

Less than 100% of mosquitoes at each life stage will reach the subsequent stage. Previous work in rice fields suggests that about 2-7% of anopheline eggs eventually hatch into larvae and eclose from pupae to become adult mosquitoes [236,237]. To estimate the survivorship of *An. gambiae* s.l. larval populations (from first instar to adult emergence), Service's (1971) vertical method was used on the temporal dataset [226,238]. It relies on the assumption[10] that the mosquito population has a stable age distribution (i.e., the rate at which early instars appear changes slowly during the cropping

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[10] The assumption underlying the method is that the age distribution reflects larval survival alone, i.e. it is unaffected by changes in the size of the mosquito population. This assumption is valid provided that the mosquito population size changes slowly relative to the lifespan of the mosquito (approximately 2 days). In our analysis, we made the further assumption that the distribution of larval survival times follows an exponential model. Appendix 5.4, which is based on a subset of data where immatures were classified at L1, L2, L3, L4 and pupal stages (instead of at early instar, late instar and pupal stages – available for 2 of 4 seasons), suggests that this model is reasonable.

season relative to the larval mortality rate), so that the rate of mortality can be inferred from an exponential model.

Since larval stages rather than exact ages are observed, it is necessary to account for interval censoring when fitting the model. According to precipitin tests by Service (1973), mosquito immatures completed their aquatic stages (pupation time) in 11.77 days [239]. These age durations were based on *An. gambiae* s.l. populations reared in the laboratory, which generally have lower air temperatures than rice fields, so we expected shorter pupation times. During our sampling period, the meteorological station at M'bé had captured average air temperatures of 30°C. Thus, the estimates by Lyimo et al. (1992) of an average emergence time of 7.7 days for lab-reared *An. gambiae* s.l. under 30°C were used to provide bounds for each stage, where the assumed durations of instars were 0.94 days for 1st instars, 1.88 for 2nd, 1.26 for 3rd, 2.45 for 4th and 1.17 for pupae [240]. Interval censored exponential models were then fitted using the "survival" package to infer the mortality rate of *Anopheles* mosquitoes [241]. Differences in mean mortality between rice phases were inferred from confidence intervals of model coefficients.

5.3.5.3 Association between *Anopheles* abundance and environmental variables

The temporal dataset was analysed to determine the associations between different biophysical characteristics and immature anopheline abundance. The first stage entailed describing the biophysical characteristics according to growth phase; chi-squared tests were not conducted because of existing spatial and temporal correlation. The second stage entailed using multiple negative binomial regression (using the "glm.nb" function from the "MASS" package) to identify possible significant correlates of vector abundance [242]. For each putative correlate, regression was used to adjust for i) rice-growing phase, ii) rice-cropping season (i.e., wet or wet-dry season), iii) rainfall, and temperature as well as iv) plot as a fixed-effect. To account for development time, rainfall and temperature were lagged by 2 days for early instars, 5 days for late instars and 7 days for pupae. Plots were included as a fixed effect because all experimental plots were subjected to the same rice-growing conditions and because the models then estimate “within-plot” exposure effects. The models also included an interaction between rice growing phase and plot to allow for the variation in time trends in each plot. Depending on the speculated relationships between variables, each model included different potential confounders and/or excluded different mediators[11]:

- Rice height: Surface vegetation (especially *Azolla*) tended to be more abundant later in the rice season and was hence a potential confounder. In turn, rice height can affect arthropod presence. This means that arthropod presence could have been a mediator to rice height and was therefore omitted from the model.
- Water transparency, iron toxicity and surface vegetation: None of these variables had clear relationships with any of the other variables and therefore no confounders were considered for their models.
- Arthropods: They tend to be more abundant later in the rice season and because rice height also tends to be higher later in the season, it could act as a potential confounder. It was also assumed that (aquatic) arthropods also tend to be more abundant with greater water depth.
- Water height: As above, water height could lead to more arthropods and therefore arthropods were omitted as a potential mediator.

5.3.5.4 Spatial distribution

To determine whether the edges of a rice plot were consistently more productive, negative binomial regression models were fitted to data from the plots that underwent intensive sampling (i.e., 200 dips with typical distances of 0.5m apart). Whilst distance from edge was considered the main variable of

[11] Because of these potential confounders and mediators, a model selection procedure would not be appropriate; individual models with variables of interest were opted for instead.

interest, plot was included as a fixed effect to account for between-plot effects and days after transplanting was included to account for variation over time.

5.3.5.5 Vector productivity

To estimate the total number of *An. gambiae* s.l. produced per hectare during the growing season, we used the formula:

$$A = \sum_{t=1}^{T} X_t \times p_t^{An} \times p_{female} \times \gamma,$$

where:

A	Total number of *An. gambiae* s.l. produced per hectare of rice in a cropping season
X_t	Number of pupae per hectare of rice on day *t*
p_t^{An}	Proportion of late-stage larvae and pupae that are *An. gambiae* s.l. (ranging from 0.047 to 0.568 depending on growing phase *t*, found through morphological identification)
p_{female}	Proportion of late-stage larvae and pupae that are female (0.506, found through rearing and morphological identification)
γ	Per capita rate at which adults emerge from pupae (as estimated above, 1 / 1.17 days for pupae to emerge as adults = 0.85 adults per pupa per day)
T	Number of days in a cropping season (161 days, as estimated from our trials)

Because $\sum_{t=1}^{T} X_t \, p_t^{An}$ was not observed, we estimated this quantity using data on the mean number of pupae collected per two dips and average p_t^{An} in each growing phase:

$$\sum_{t=1}^{T} X_t = \sum_{s=1}^{4} \left(\sum_d \bar{x}_{s,d} \times e^{-\varphi \times d} \times Q_d \right) \times \beta \times \theta \times T_s \,,$$

where:

$\bar{x}_{s,d}$	The mean number of pupae collected per two dips in growing phase *s* in quadrats at distance *d* from edge of plot (1=land preparation, 2=vegetative, 3=reproductive, 4=ripening & post-harvest)
β	Dip-quadrat calibration factor (obtained from zero-intercept linear regression) corresponding to the expected number of pupae in a quadrant (0.25m^2) for each pupa sampled in two dips
φ	Reduction in the number of pupae for each metre from closest edge. In the linear regression described under “Spatial distribution”, we estimated 38.1%

	reduction of pupae with every meter from the edge of a rice field therefore φ = -ln(0.381) = 0.479.
Q_d	The total number of quadrats in a plot at distance *d* from edge
Θ	Scaling factor of a standard sized plot to a hectare (which is 39.1 for a plot of 256 m^2 and a hectare of 10,000 m^2)
T_s	Duration in days of growing phase *s*

The dip-quadrat calibration factor (β) was computed by fitting a zero-intercept linear regression between the square root transformed number of pupae collected per two dips (x-axis) and the square root transformed number of pupae collected per 0.25 m^2 quadrat (y-axis). A zero-intercept linear regression was used because it is assumed that the true intercept is zero i.e. when there are no mosquitoes in a quadrat, there would be no mosquitoes in a dip. The calibration factor was then calculated as the square of the resulting regression coefficient.

Error estimates for A were calculated using the formula for the variance of a product of estimates.

In addition, the human biting rate of newly emerged female mosquitoes in rice communities (HBR), that might be expected per night in a place like M'bé, was estimated from this absolute number of malaria vectors produced in one hectare of rice during a season (A). The following formula was used:

$$HBR = (A \times RI \times e^{-\mu a})/NT,$$

where:

RI	The area of the irrigation scheme under rice irrigation (95% of 150 hectares)
μ	Adult mosquito mortality rate (0.17 per day, as reviewed by Matthews, Bethel and Osei (2020))
a	Age of adult mosquito at biting (assumed to be 1 i.e., from the first day following emergence)
N	The human population size within a 5-km radius of the irrigation scheme (21,000 in 24 villages)
T	Number of days in a cropping season (161 days, as estimated from our trials)

The human biting rate of newly emerged females was therefore based on the assumptions that:

- The daily rate of emergence (0.85 per day - based on the estimates by Service (1973) and Lyimo et al. (2012)) was sustained for the 161 days of a cropping season
- These female mosquitoes were evenly distributed within an area of up to 5 kilometres from the edge of rice fields
- 83% of mosquitoes survive 1 day
- These female mosquitoes only took bloodmeals from humans and bit from the first day following emergence
- Pupal abundances and vector species composition (for each growing phase) as well as the sex ratio measured in our plots were representative of the irrigation scheme
- The average size of a farmer rice plot in the irrigation scheme is around 256 m^2 (sizes usually ranged between 100 to 500 m^2)

5.4 Results

5.4.1 Mosquito species composition

A total of 40,310 anopheline larvae were collected over 91 weeks (782 sampling occasions), of which 50.0% (n=20,162) were early instars, 40.3% (n=16,228) were late instars and 9.7% (n=3,920) were pupae. Morphological identification of late-stage larval instars and pupae found three anopheline species, where *An. gambiae* s.l. was the predominant species (84.5%), followed by *An. ziemanni* (15.0%) and *An. pharoensis* (0.5%). Molecular identification by PCR of a 20% sample of the morphologically identified specimens of *An. gambiae* s.l. confirmed that *An. coluzzii* was predominant (99.4%), as opposed to *An. gambiae* s.s. (0.6%).

An. gambiae s.l. was dominant during the early stages of rice growth, comprising over 87% of all anophelines from land preparation to the reproductive phase (Table 5.1). From ripening to post-harvest, however, *An. ziemanni* became dominant, albeit in much smaller densities. In the early rice growing season, *An. gambiae* s.l. abundance was 17-fold greater than *An.*

ziemanni abundance whereas in the late season, *An. ziemanni* abundance was 9-fold greater than *An. gambiae* s.l. abundance.

5.4.2 Temporal distribution

Over the entire rice-growing season, from land preparation to one week after harvest (around 22 weeks), peaks of *Anopheles* larvae were seen in the early stages of rice growth, from two weeks before transplanting up to six weeks after transplanting (Figure 5.3A, Appendix 5.5). However, productivity was estimated only from the observed numbers of pupae and 88% of pupae observed in the whole season were seen in up to four weeks after transplanting.

Table 5.1. Anopheline species composition in the M'bé rice irrigation scheme, Côte d'Ivoire, grouped by rice growth phases over four cropping seasons.

	Land preparation, n (%)	**Vegetative, n (%)**	**Reproductive, n (%)**	**Ripening, n (%)**	**Post-harvest, n (%)**
Weeks	**-4 to 0**	**0 to 6**	**6 to 11**	**11 to 16**	**16 to 18**
***An. gambiae* s.l.**	485 (88.7)	1906 (99.3)	873 (87.1)	42 (9.8)	0 (0.0)
An. pharoensis	7 (1.3)	2 (0.1)	5 (0.5)	4 (0.9)	0 (0.0)
An. ziemanni	55 (10.1)	11 (0.6)	124 (12.4)	383 (89.3)	14 (100.0)
Total	547	1919	1002	429	14

Cumulative frequency graphs displaying times with the steepest gradients demonstrate these "windows of peak productivity" even more clearly (Figure 5.3B and 5.3C). These times of peak productivity were relatively consistent between early (from March to July) and late (from August to December) rice-cropping seasons: the largest "steps" in pupae production were seen from weeks two to four (Appendix 5.6). Figure 5.3B also illustrates a clear shift in larval development across time: within one week, peaks (or largest "steps") of early instars advanced to slightly smaller peaks of late instars and then to even smaller peaks of pupae (e.g., in the first panel, the early instar peak in week 1 progressed to late instar and pupal peaks in week 2, Appendix 5.6).

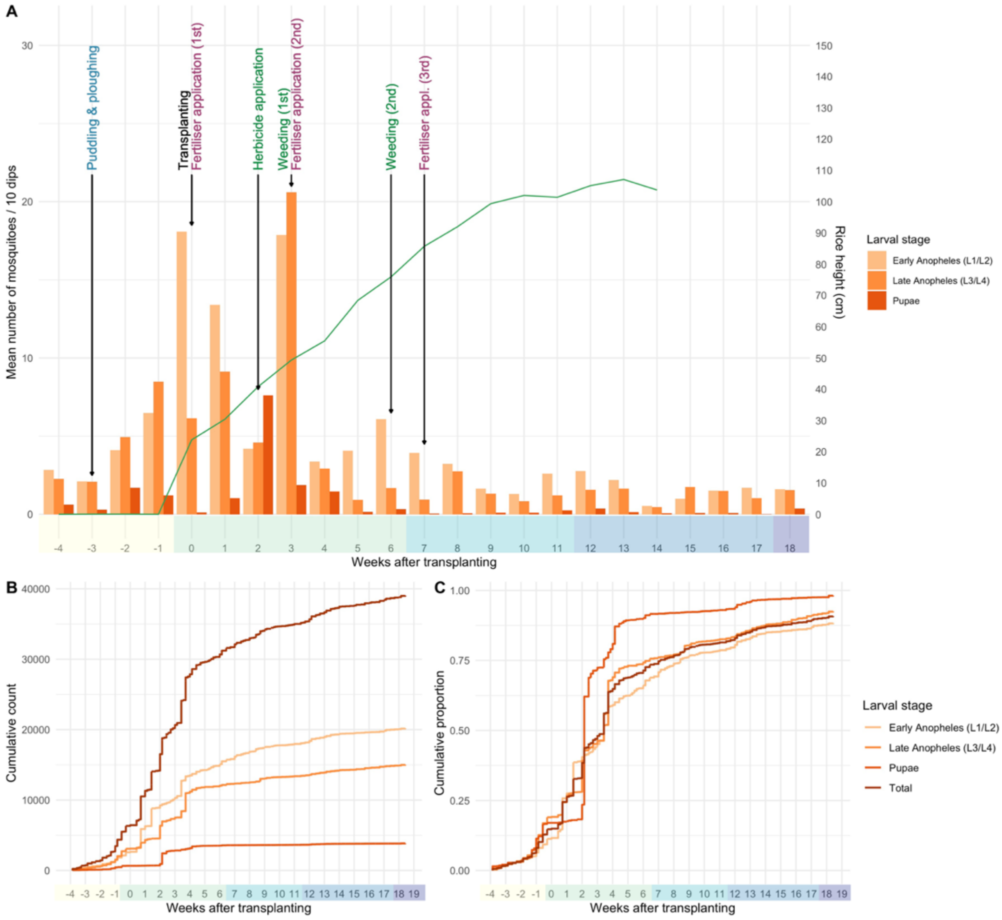

Figure 5.3. Weekly mean *Anopheles* abundance (separated by development stage) throughout harvesting time, in relation to **(A)** agronomic interventions and rice height (green line), and **(B)** cumulative count and **(C)** cumulative proportion. A total of 782 sampling occasions were conducted over four cropping seasons from 2019 to 2021.

In general, early instar densities were significantly higher during the vegetative period of rice growth, which is from 0 to around 43 days after transplanting, than other periods ($p<0.0001$, Figure 5.4A). Land preparation was another productive period for *Anopheles* larvae. These two stages together comprised 78.2% of all immatures observed in the whole season. During the later stages of rice growth, including reproductive, ripening, and post-harvest, anopheline larval density was generally low, averaging 2.17 early instar larvae, 1.08 late instar larvae and 0.15 pupae per 10 dips.

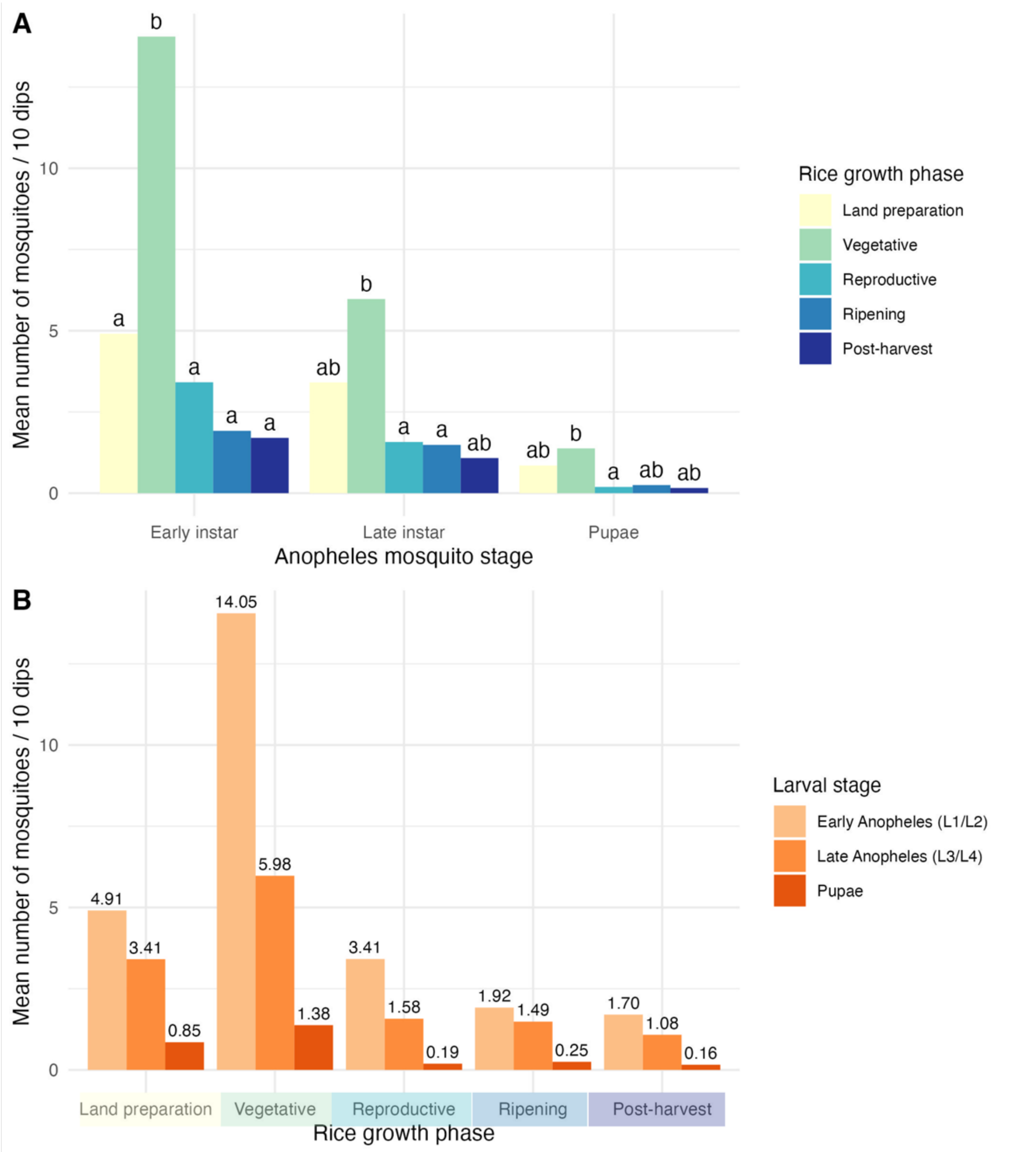

Figure 5.4. Mean *Anopheles* abundance in M'bé, Côte d'Ivoire, throughout a rice growing season, grouped by **(A)** mosquito developmental stage and **(B)** rice growth phase.

Mean mortality rate from early instar to pupal stage (i.e., average age at death) was lowest during the land preparation and vegetative phases and highest during the reproductive phase (Figure 5.4B and denoted by alphabetical labels in Figure 5.5).

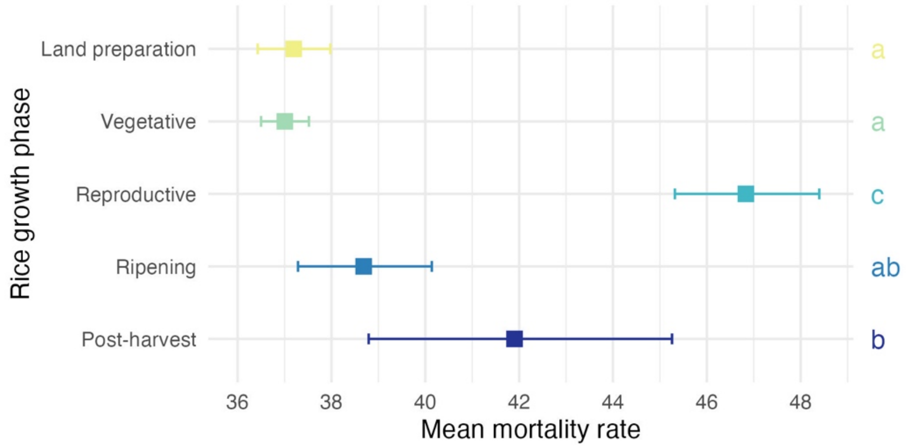

Figure 5.5. Mean mortality rate (in percentage per day, and 95% confidence intervals) of *Anopheles* mosquitoes during different rice growth phases in M'bé, Côte d'Ivoire, as estimated using methods of Service (1971) and Lyimo et al. (1992).

Biophysical characteristics tended to fluctuate during a rice-growing season (Table 5.2). Dips conducted in the later rice (ripening and post-harvest) phases were more likely to contain *Culex* larvae than earlier phases. Aquatic arthropods and weeds were also more common during land preparation and later rice phases (reproductive and ripening) than the vegetative phase. Similarly, floating vegetation was slightly more common during the reproductive and ripening phases than land preparation and vegetative phase. Water was mainly clear during vegetative and reproductive phases, but cloudier during times where rice was not grown (i.e., land preparation and post-harvest). The probability of observing iron (III) oxide as a layer in the rice water was also highest during these two latter phases (see Appendix 5.3). Water depth, as expected, was mainly between 0.1-5 cm, but there were more instances of drying (hence 0 cm) during the vegetative phase compared to reproductive and ripening.

Table 5.2. Biophysical characteristics of M'bé rice fields during different rice growth phases across four cropping seasons (in years 2019-2021).

Field charact-eristics	Land preparation, n (%)	Vegetative, n (%)	Reproductive, n (%)	Ripening, n (%)	Post-harvest, n (%)
***Culex* larvae**					
Absent	3404 (84.2)	4690 (72.5)	3152 (75.4)	2871 (62.3)	980 (66.9)
Present	640 (15.8)	1777 (27.5)	1026 (24.6)	1735 (37.7)	485 (33.1)
Aquatic arthropods					
Absent	2983 (73.8)	5178 (80.1)	3271 (78.3)	3317 (72.0)	1202 (82.0)
Present	1061 (26.2)	1289 (19.9)	907 (21.7)	1289 (28.0)	263 (18.0)
Floating vegetation					
Absent	3857 (95.4)	5592 (86.5)	2130 (51.0)	3109 (67.5)	1445 (98.6)
Present	187 (4.6)	875 (13.5)	2048 (49.0)	1497 (32.5)	20 (1.4)
Weeds					
Absent	3364 (83.2)	5984 (92.5)	3311 (79.2)	3972 (86.2)	1454 (99.2)
Present	680 (16.8)	483 (7.5)	867 (20.8)	634 (13.8)	11 (0.8)
Water transparency					
No water	340 (8.4)	1129 (17.5)	316 (7.6)	588 (12.8)	776 (53.0)
Clear	744 (18.4)	2686 (41.5)	21056 (49.2)	1418 (30.8)	8 (0.5)
A little cloudy	2105 (52.1)	2153 (33.3)	1584 (37.9)	1754 (38.1)	283 (19.3)
Cloudy	750 (18.5)	379 (5.9)	222 (5.3)	846 (18.4)	390 (26.6)
Very cloudy	105 (2.6)	120 (1.9)	0 (0.0)	0 (0.0)	8 (0.5)
Water depth (cm)					
0	348 (8.61)	1137 (17.6)	324 (7.8)	588 (12.8)	785 (53.6)
0-5	1235 (30.5)	3645 (56.4)	1724 (41.3)	2018 (43.8)	487 (33.2)
5-10	1798 (44.5)	1457 (22.5)	1118 (26.8)	1487 (32.3)	193 (13.2)
>10	663 (16.4)	228 (3.5)	1012 (24.2)	513 (11.1)	0 (0.0)
Iron toxicity					
Absent	2889 (71.4)	6119 (94.6)	3646 (87.3)	4137 (89.8)	1097 (74.9)
Present	1155 (28.6)	348 (5.4)	532 (12.7)	469 (10.2)	368 (25.1)
Footprint					
Absent	753 (73.0)	1565 (72.2)	872 (65.7)	517 (69.0)	0 (0.0)
Present	279 (27.0)	603 (27.8)	456 (34.3)	232 (31.0)	11 (100.0)

5.4.3 Association between *Anopheles* abundance and environmental variables

A correlation matrix of biophysical characteristics showed that week after transplanting and rice height as well as water depth and percentage of water were highly collinear (Appendix 5.7). The models obtained from the negative binomial regressions indicated that anopheline immature abundance generally decreased as rice grew taller, as water was more turbid and had more iron toxicity, and as there were more arthropods present (Table 5.3). Deeper waters were associated with slightly more mosquito immatures (ranging between +2.3% to +3.8% per cm across development stages). Fields with more surface vegetation were associated with more mosquito immatures (ranging between +60.3% to +109.8% across development stages).

Table 5.3. Importance of biophysical characteristics for anopheline abundance (exponentiated per ten dips) in rice fields within the M'bé irrigation scheme.

	Ratio of means					
	Early instar		**Late instar**		**Pupae**	
Rice height, cm[a]	0.969	***	0.981	***	0.969	***
Water transparency: a little cloudy[b]	0.824	*	0.525	***	0.478	***
Water transparency: cloudy	0.912		0.654	***	0.292	***
Water transparency: very cloudy	0.142	***	0.122	***	0.049	***
Water depth, cm	1.038	***	1.023	**	1.029	*
Iron toxicity (presence)	0.714	***	0.648	***	0.861	
Arthropods[c] (presence)	0.608	***	0.670	***	0.655	***
Floating vegetation (presence)	1.944	***	2.098	***	1.603	**

Ratio of means from a negative binomial regression model that included as covariates: i) plot ii) rice-growing phase, iii) rice-cropping season, iv) rainfall and v) temperature. Both rainfall and temperature were lagged by 2 days for early instars, 5 days for late instars and 7 days for pupae.
[a] not adjusted for week after transplanting because of high collinearity (r = 0.80)
[b] clear water was the comparator for water transparency
[c] additionally adjusted for rice height and water depth
* significant at the 0.05 level; ** at the 0.01 level; *** at the 0.001 level (2-tailed)

5.4.4 Spatial distribution

Maps of *Anopheles* abundance show some distinct spatial patterns, which vary slightly with each mosquito immature stage. However, in general, there were higher mosquito abundances around the edges of the plot, especially on the northern side of the plot (Figure 5.6). There was an association between mosquito number and distance from edge (Figure 5.7), where with every meter from the edge of a rice field, early instars reduced by 11.6% (95% CI -19.0, -3.4, p=0.006), late instars by 17.7% (95% CI -26.6, -7.7, p=0.0008) and pupae by 38.1% (95% CI -49.2, -24.6, p<0.0001).

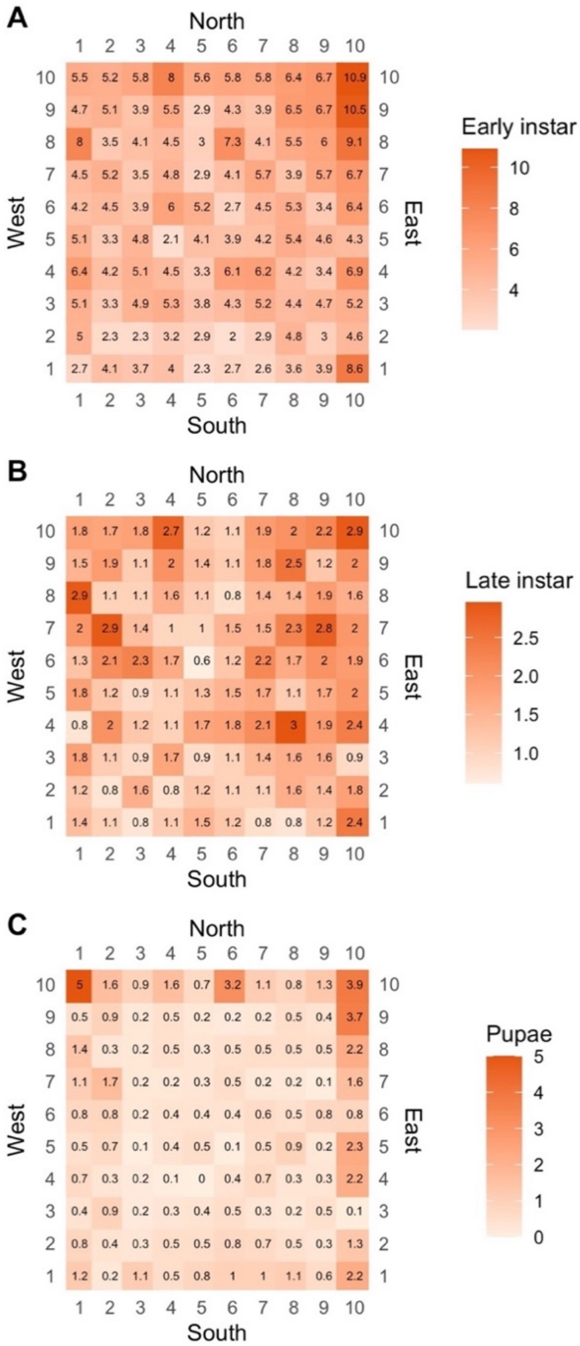

Figure 5.6. Spatial distribution of mean *Anopheles* abundance, separated by immature stage (**A**: early instar, **B**: late instar, and **C**: pupae), within a rice plot over one rice-growing season (June-December 2021) in the M'bé irrigation scheme, Côte d'Ivoire.

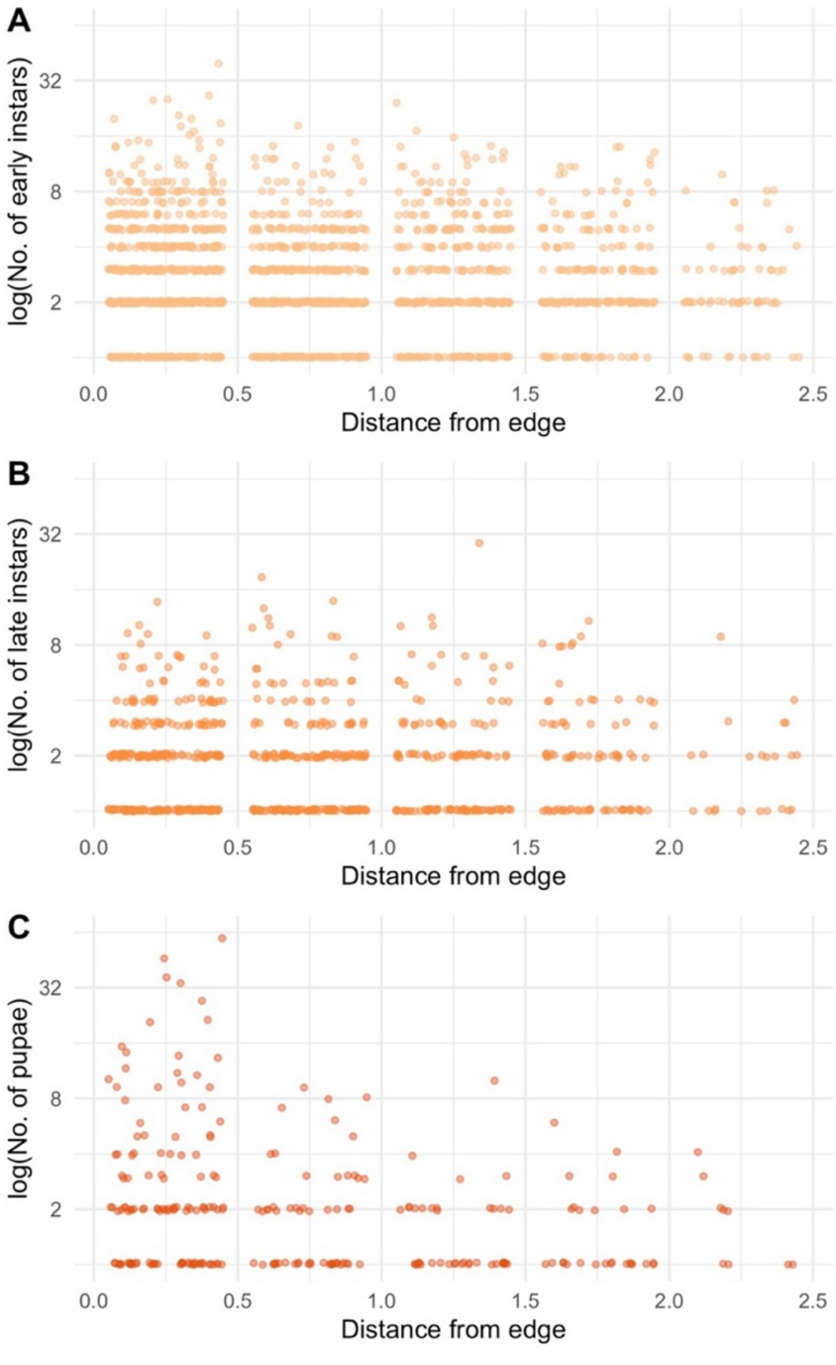

Figure 5.7. Association between distance from edge of a rice field plot and anopheline vector abundance (displayed on a log-scale), separated by immature stage (**A**: early instar, **B**: late instar, and **C**: pupae).

5.4.5 Vector productivity

The calibration factors between the number of immatures collected per two dips and per quadrat were obtained by back-transforming the coefficients in zero-intercept linear regressions (Figure 5.8 and Appendix 5.8). The calibration factors were 4.46 for early instars (95% CI 4.16, 4.76, $p<0.0001$), 6.06 for late instars (95% CI 5.57, 6.57, $p<0.0001$), and 6.95 for pupae (95% CI 6.44, 7.49, $p<0.0001$). In other words, for every pupa found in one dip, 6.95 pupae were observed in a quarter square metre quadrat.

Our estimates suggests that, over the entire rice-growing season, 723,000 malaria vectors (95% CI 593,000, 852,000) were produced in one hectare of rice (Table 5.4). Of that, 133,000 adult *An. gambiae* s.l. females were produced during land preparation (18.3%), 568,000 during vegetation (78.6%), 18,000 during reproductive (2.5%) and 4,000 (0.6%) during ripening and post-harvest periods. These estimates assumed that plots in a hectare of rice were, on average, 256m^2. The vector productivity of various plot sizes is presented in Appendix 5.9.

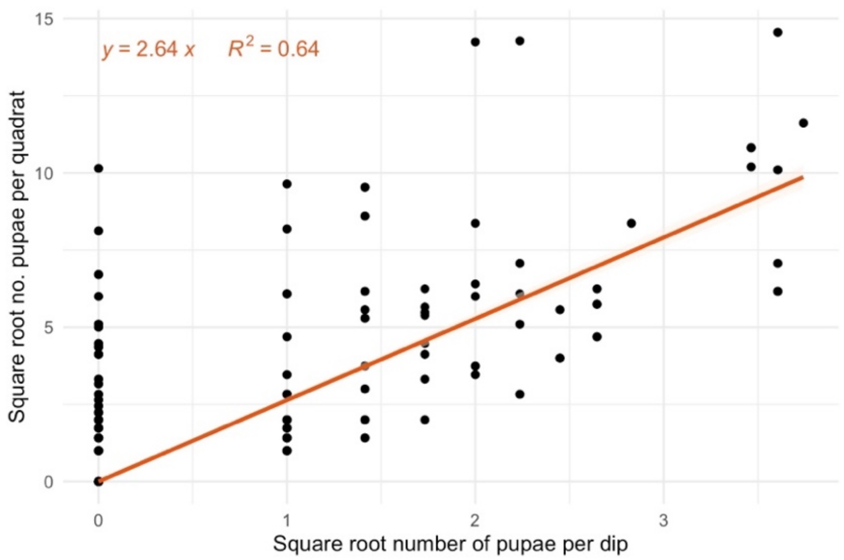

Figure 5.8. Calibration factor between two dips and quadrat counts obtained through zero-intercept linear regressions for pupae.

If the hectare that we measured is representative of the whole irrigation scheme of M'bé 1, it can be estimated that the scheme produces a total of 103 million malaria vectors during a cropping season (95% CI 84.5, 121.4 million). In relation to the 24 villages within a 5-km radius around the scheme, and based on the assumptions listed in the Methods section, it can be further estimated that a resident of the area is exposed to an average of 4,100 newly-emerged malaria vectors per cropping season, or that there are an average 25.7 newly-emerged female *Anopheles* mosquitoes per person per night during the rice-growing season (95% CI 18.6, 24.5) [243].

Table 5.4. Estimations of the absolute number of adult *An. gambiae* s.l. females produced per hectare per rice cropping season in M'bé irrigation scheme, Côte d'Ivoire.

Rice-growing phase	$\bar{x}_{s,d}$ (SE)	β (SE)	φ (SE)	Q_d	θ	T_s	X (95% CI)	γ	p^{An}_t	p_{female}	A_s (95% CI)
Description of parameter	Mean no. of pupae collected per two dips in growing phases	Calibration factor between pupae per two dips and pupae per quadrat	Exponential fold-reduction of the number of pupae with distance from the closest edge	Total number of quadrats in a plot at distance *d* from edge	Scaling factor of a plot to a hectare	Duration in days of growing phases	No. of pupae produced per hectare of rice in a growing phase	Per capita rate at which adults emerge from pupae	Prop. of late-stage larvae and pupae that are *An. gambiae* s.l.	Prop. of late-stage larvae and pupae that are female	No. of *An. gambiae* s.l. females produced per hectare of rice in a growing phase
Land preparation	0.183 (0.013)	6.952 (0.269)	0.479 (0.101)	1024	39.1	34	754 thousand (635 – 872)	0.85 adults per pupa per day	40.7%	50.6%	133 thousand (112 – 153)
Vegetative	0.443 (0.038)					43	2.31 million (1.89 – 2.74)		56.8%		568 thousand (464 – 672)
Reproductive	0.027 (0.003)					34	110 thousand (85 – 137)		38.6%		18.3 thousand (14.3 – 22.3)
Ripening & post-harvest	0.033 (0.003)					50	202 thousand (168 – 236)		4.7%		4.1 thousand (3.4 – 4.8)
Total number of *An. gambiae* s.l. females produced per hectare of rice in a cropping season (*A*)											**723 thousand** (593 – 852)

5.5 Discussion

5.5.1 Summary of results

This study investigated how irrigated rice cultivation influences the abundance and population dynamics of *Anopheles* mosquitoes during a season. Anophelines were regularly sampled in experimental rice fields that were established for four cropping seasons. They were most numerous earlier in the cropping season (in the land preparation and vegetative phases): three-quarters of total *Anopheles* pupae were sampled in the first four weeks after transplanting. As the season progressed, fewer anophelines were observed, where although *Anopheles* density did not manifest as peaks, they were still persistent during the reproductive and ripening phases. There was a species succession: *An. coluzzii* was dominant earlier in the cropping season and was replaced by *An. ziemanni* (albeit at lower abundances) later.

In general, anopheline abundance tended to be higher when rice plants were shorter, water was clearer, shallower, with surface vegetation, did not observe iron (III) oxide residues and aquatic predators. We observed larvae to accumulate at the edges of rice fields, especially the north side of the plots. It was estimated that a total of 700 thousand malaria vectors were produced in one hectare of rice during a cropping season in the M'bé irrigation scheme of Côte d'Ivoire. Assuming that all malaria vectors were evenly distributed amongst all villagers living 5 km within the scheme and were not taking bloodmeals from animals, it was estimated that each villager was potentially exposed to 26 newly-emerged female *Anopheles gambiae* s.l. mosquitoes every night during the rice-cropping season.

Our study aligns with previous work on the phenology of malaria vectors in irrigated rice fields in East and West Africa: *An. gambiae* s.l. is most abundant during the early periods of rice growth [244,245]. It also adds to the evidence that rice fields produce a large amount of malaria vectors.

5.5.2 Species composition

In this area of central Côte d'Ivoire, *An. coluzzii* was the dominant vector species earlier in the cropping season of irrigated rice and was succeeded by *An. ziemanni* later in the season. This species succession can be explained by changes in the aquatic conditions of paddies over a growing cycle [46,50,246]. It is consistent with other entomological studies in West African rice fields, that *An. coluzzii* predominated rice fields in the early phases of rice but was replaced by a variety of secondary vector species such as *An. funestus*, *An. coustani, An. rufipes,* and *An. ziemanni* [126,244,247]. The dominance of this subspecies of *An. gambiae* complex in West Africa demonstrates that rice fields are exceptional niches to specialise in, to the extent of facilitating speciation [248]. It confirms that rice fields do not promote species richness in mosquitoes, and instead promotes the monoculture of the most efficient malaria vector in Africa [46,50,149].

5.5.3 Biophysical characteristics affecting the temporal and spatial distribution of malaria vectors

The critical window for peak *Anopheles* pupae production in rice fields was the first four weeks after transplanting. Our findings are consistent with studies conducted in the Mwea irrigation scheme in Kenya and the Office du Niger scheme in Mali, which observed a four-to-eight-week window during the vegetative phase [44,181,244,245]. As observed in our study, this window of productivity is attributable to several factors which occur in the early stages of rice growth: (1) shorter rice plants, (2) clearer water, and (3) absence of aquatic predators.

It is speculated that following transplanting, when rice plants are still short, the water surface is largely left exposed, sunlit, and warm [244,249]. This creates aquatic and soil conditions ideal for *An. coluzzii* development [250]. Conversely, when rice plants have matured and reach a height of around 75 cm, they form a canopy which in turn creates shadows and cooler water conditions that are less suitable for *An. coluzzii* and are instead suitable for other *Anopheles* species that prefer habitats shaded by vegetation [222]. These speculations need to be verified by experimental studies that test the impact of different rice varieties with different canopy coverage [182,209].

An. gambiae is known to be a "pioneer" species, where it opportunistically colonises newly created bodies of water to complete several cycles of development before its predators are established. Thus, the "new", clearer water added to rice fields following transplanting (as observed in our study) present ideal conditions for the species to proliferate [222]. Their aquatic predators, on the other hand, require more time to develop (for example, it takes dragonfly eggs one to two weeks to hatch and nymphs around one month to complete development). However, once several weeks have passed and the rice water has remained relatively stable, the established array of predators will then start regulating vector populations, contributing to high mosquito mortality [91,207,239,251]. Although oviposition may continue in this "older" water, a higher proportion of them are eaten before they mature into adults [40,252]. Our study demonstrated this: mortality rate was markedly higher[12] in the reproductive phase than the land preparation and vegetative phases.

Our study is also consistent with the idea that certain aquatic conditions are preferred by gravid female mosquitoes looking to oviposit. First, it demonstrated that water depths of 4 to 7 cm were associated with the highest *Anopheles* abundances. This can be explained by the Goldilocks principle on water depth, where both shallower and deeper waters are avoided for oviposition, as the former indicates rapid drying of fields whilst the latter indicates more stable habitats colonised by predators [36,249]. Second, the negative relationship found between iron toxicity and mosquito density

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[12] Note that the mortality rate in the reproductive phase was also higher than in the ripening phase. This may be explained by the flooding schedule during the ripening phase: fields were drained 7-10 days before harvest. This drainage could have placed more pressure on mosquito predators and therefore promoted immature survival. Regardless, the mean number of pupae per dip was similarly low in reproductive and ripening phases (0.19 pupae and 0.25 pupae per 10 dips, respectively.

reinforces that the chemical substances present in the rice water act as oviposition cues. In the case of iron toxicity, it is likely that oviposition in water surfaces with iron (III) oxide films are avoided, because similar to oils and monomolecular films (which are used as mosquito control agents), these films may be detrimental to mosquitoes through their toxicity and/or suffocation impact by acting as a physical barrier to respiration [197]. Relatively neglected, the rice operations and soil conditions which enhance iron toxicity, decomposition, and other processes which may affect mosquitoes should be further investigated. Third, our study revealed that more larvae were found closer to the edge of the rice fields. This is consistent with previous findings, which suggested that mosquitoes oviposit in breeding site boundaries for enhanced protection against predators [253–255]. However, this could also have been an artifact of sampling in rice fields: compared to the centre, it is easier to sample the borders of fields without disturbing and driving larvae away.

Other than the aforementioned factors (water depth, iron toxicity and field edges), several biophysical characteristics are also associated with anopheline abundance. They were not sufficiently captured in our study but have been reported in previous literature. They include fertiliser application and aquatic vegetation. Fertilisers could promote mosquito proliferation via two proposed mechanisms. The first is that by reducing water turbidity, nitrogenous fertilisers provide extra stimulus for oviposition [37]. The second is that coupled with plant material in the paddies, fertilisers supply mosquitoes with rich nutrients and thus aid their growth and survival rate [256,257]. Our study observed peaks of early instars in weeks 0 and 3 after transplanting, which corresponds to the basal fertiliser application on the day of transplanting and the first topdressing three weeks after transplanting. However, because this study was observational, these peaks could not necessarily be attributed to fertiliser application. The presence, amount, and type of vegetation can also greatly influence *Anopheles* mosquitoes [258]. Whilst aquatic vegetation, including rice plants, can shelter larvae from predators, it can also discourage oviposition and immature development when it is densely distributed. This mixed relationship is well-illustrated by a type of floating vegetation called *Azolla*: whilst it normally acts as a source of food for mosquito larvae, its high field cover (>80%) can also act as a physical barrier for gravid mosquitoes to lay their eggs, larvae to respire or obtain sunlight, and adults to emerge [259–261]. It appears that our study only demonstrated the positive relationship, where presence of floating vegetation was associated with more early and late anopheline instars.

5.5.4 Vector productivity

Our estimation of the absolute population density of (~700,000) malaria vectors in a hectare of irrigated rice in a cropping season is not dissimilar to that of Stewart, Schaefer, and Miura (1983) on *Culex tarsalis* in rice fields, which was 1 million pupae per hectare in three months (~60% of a rice cropping season) [227]. This difference is anticipated since their estimates concern a different species of mosquito in different rice field conditions and did not take account of mosquito clustering. Its conversion to a human biting rate of 26 bites per person per night in a rice season is lower than the human biting rates previously found (between 39 to 141) in irrigated rice-growing villages approximately 100 km away from M'bé in central Côte d'Ivoire [137,147]. This is likely because our estimates represented the rice-attributable fraction of mosquito production within a village. In general, it must be taken into consideration that our estimates are based on numerous assumptions on mosquito bionomics (as listed in the Methods section). Importantly, they assumed that *An. coluzzii* were completely anthropophilic (when previous studies have observed 60% anthropophily), only bit once (when there is technically no limit to the number of bites they can inflict) and were evenly spread within 5 km of the irrigation scheme [262]. Nonetheless, these estimates are useful for the modelling of rice-attributable fraction of malaria burden (i.e., the number of cases, disability-adjusted life-years, or deaths per hectare of irrigated rice) to highlight the impact of rice cultivation on increased malaria risk.

5.5.5 Limitations

There are several limitations to this work. First, due to the labour intensity required of mosquito sampling, certain variables were crudely measured. For instance, data on potential mosquito predators were only collected at presence-absence level, when the number and types of potential predators would have been more informative, but this poses a useful future question. Similarly, water turbidity was estimated by the naked eye, when information on the chemical composition of the water would have been more useful to reveal how inputs for rice growing affect aquatic conditions and hence mosquitoes. Experimental studies in the laboratory or the field, like the study by Mutero et al. (2004), can be conducted to reveal these relationships [37].

Second, it is difficult to determine whether the amount of mosquito immatures collected could have been biased by sampling success or differences in mortality. In terms of sampling technique, larvae can evade the dipper more easily in open, deeper waters than when they are accumulated in residual pools of shallow water. Dipping is also subject to wide user differences. In terms of mortality, the assumption that larval populations were stable during each rice-growing phase could have biased the results. More effective and representative methods of sampling mosquitoes in rice fields are therefore required.

Third, age durations of instar classes used to estimate survivorship were based on laboratory-reared *An. gambiae* s.l. populations [239,240]. Although these inferences may not have had strong implications on survivorship estimates, precipitin tests (or other methods) to determine instar age durations under field conditions would have been more applicable. Based on these age durations, the estimates of riceland malaria vector productivity may be rather weak.

Fourth, evidence of the spatial distribution of malaria vectors in rice fields were based on plots of one size, in the same area and established for only one season. More accurate estimates of the association between field edge and pupal number would have been obtained if more replicates of plots of different sizes (ranging between 100 to 500 m^2) were intensively sampled across more locations within the irrigation scheme.

Lastly, these detailed results, particularly the absolution population density estimates, are not necessarily applicable to other irrigation schemes in sub-Saharan Africa since rice environments, and hence, *Anopheles* population dynamics, differ. Nevertheless, these relatively simple approaches can be applied to a wide variety of settings.

5.5.6 Implications and recommendations

It is important to understand the population dynamics of malaria vectors within a rice agro-ecosystem, especially the features of rice cultivation that provide desirable habitats for mosquitoes. This understanding helps inform effective larval control that is both temporally and spatially targeted. Temporally, options for vector control can be distinguished by two periods: the early and late rice-cropping seasons. Whilst more immediate interventions are required early in the season, when peak mosquito productivity occurs, more suppressive and sustainable interventions are required to control residual mosquito breeding which occurs later in the season [225]. For the former, larval control could be integrated into rice operations whereby the timing of their application coincides with mosquito proliferation, such as fertiliser application on the day of transplanting and three- and eight-weeks following transplanting and herbicide application around two weeks following transplanting. Tanzanian semi-field studies have demonstrated the efficacy of combining *Bti* with fertilisers on local *Anopheles* populations [263]. Although unaffordable to deploy larvicides throughout an entire rice season, it is perhaps feasible for a short period in conjunction with routine operations done by farmers. For later in the rice-cultivating season, long-term suppressive interventions such as *Azolla,* rice-fish co-culture and intermittent drying of rice fields can be considered [Chapter 4] [225]. Regardless, both sets of interventions must bear predator populations in mind, to maintain natural vector regulation. Spatially, vector control that targets and can be maintained in field borders, such as oils and monomolecular surface films, should be paid special attention.

Other than the aforementioned methods of riceland mosquito control, the potential of modified rice cultivation practices as vector control should be further explored. As reported by this study and by the workshop between International Rice Research Institute and the WHO/FAO/UNEP Panel of Experts on Environmental Management for Vector Control, even slight adjustments to growing techniques, such as rice variety, plant spacing, rice operations that enhance iron toxicity and water turbidity, can affect mosquitoes [80]. All aspects of the rice environment should be regulated to reduce mosquito production: this includes synchronising transplanting amongst farmers, preventing residual breeding in fallow fields, promoting biodiversity to avoid producing a monoculture of *An. gambiae* s.l., and creating permanent pools near main cultivating fields to encourage rapid predator establishment [147,211,244,261,264]. Ultimately, the agricultural sector needs to take responsibility for the unintended, harmful side effects of rice cultivation and collaborate with the health sector in farmer sensitisation to reduce malaria risk within rice-farming communities.

5.6 Conclusions

The recommended procedures for growing irrigated rice are generally very similar to the ideal breeding conditions for *An. gambiae* larvae: fresh, clean, sunlit, and shallow “new” water with damp mud surfaces. Understanding how rice phenology impacts malaria vector populations helps facilitate the proper timing of vector control operations, where the window of peak productivity following transplanting as well as the constant breeding throughout a cropping season must be targeted. To ensure the efficacy and applicability of interventions, long-term sustainable vector control methods that can be built into the recommended regimen of rice operations (for farmers) are desired. This in turn not only necessitates accountability by the agricultural sector but, most importantly, incentive.

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Primary Supervisor	Jo Lines		

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SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper.	I designed the experiment, performed all analyses on entomological data and wrote the paper. Other authors assisted in suggesting analysis methods and conducting analysis on agronomic data.

SECTION E

Student Signature	[redacted]	**Supervisor Signature**	[redacted]
Date	12/08/2022	**Date**	12/08/2022

6 Suppressing the breeding of malaria vectors in African rice fields: the effects of rice cultivation practices on rice yield, water productivity and greenhouse gas emissions in lowland irrigation schemes

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6.1 Abstract

Background

Rice is a rapidly expanding and intensifying crop in sub-Saharan Africa. However, rice fields consume a lot of water, produce a large proportion of the world's methane emissions, and, in Africa, produce a large number of malaria vectors. Therefore, strategies that reduce/mitigate rice intensification's negative impacts on climate change and have health co-benefits are urgently needed.

Methods

To determine the effect of different rice cultivation practices on malaria vector density, rice yield, water productivity, and greenhouse gas emissions, seven experimental field trials were conducted in central Côte d'Ivoire and eastern Tanzania. Each trial assessed the effect of different growing techniques (i.e. treatments), including the period of flooding during land preparation, method of crop establishment, timing of fertiliser application, and water management technique (e.g. alternate wetting and drying irrigation – AWD). Over four cropping seasons (April 2019 to December 2021), rice plots were arranged in a randomised complete block design with at least three replicates for each treatment. Mosquito larvae were sampled at the borders of each rice plot twice every week from land preparation to two weeks after harvesting (except for the trial on land preparation, where sampling was conducted for the duration of land preparation). Greenhouse gas emissions, conducted in two of seven trials, were measured every three days using the static chamber – gas chromatograph method.

Results

Fields that were direct-seeded (vs transplanted) were associated with more malaria vectors. Compared to continuous flooding, AWD was not effective in reducing malaria vectors in Côte d'Ivoire but did not cause any yield penalties, consistently reduced water use by 41-71% and reduced global warming potential by 41% (8.61 tCO_2/ha under continuous flooding vs 5.21 under AWD). On the other hand, compared to fields under continuous flooding, fields under AWD-15 were successful in reducing early mosquito instars by 70.6% (95% CI -88.6%, -23.9%, $p<0.0001$) in Tanzania.

Conclusions

This study confirms that there are rice-growing techniques that can minimise mosquito and methane production, whilst reducing water use and sustaining yields. Some techniques need to be adjusted and repeated across more trials (and more seasons and locations) to demonstrate its efficacy. Nonetheless, regular rice research looking to improve yield should prioritise methods that can provide climate and health co-benefits.

6.2 Introduction

Owing to growing consumer demand in Africa, cultivation of rice is rapidly intensifying and expanding in sub-Saharan Africa (SSA) [113]. To improve food security, agricultural development agencies and Ministries of Agriculture are planning to expand rice-growing areas, especially those under irrigation [25]. It has been long established that rice fields are ideal breeding sites for African malaria vectors [48]. It has recently been confirmed that compared to communities without rice fields in their vicinity, communities living near rice fields are exposed to higher risk of malaria, which causes 600,000 annual deaths in SSA [2,221] [Chapter 3]. The continent-wide intensification and expansion of rice-harvested areas is therefore likely to slow progress towards malaria control and elimination. Additional effort into providing insecticide-treated nets, anti-malarial drugs and improved healthcare services to rice communities is required. At the same time, and more importantly, methods of rice cultivation that minimise malaria vector production need to be developed and adopted. This is to ensure that agriculture no longer contributes to the malaria problem and, instead, starts being part of the solution.

On the one hand, medical entomologists have shown that numerous larval source management methods are effective in riceland malaria vector reduction. These methods range from chemical control using larviciding to biological control using fish and/or *Bacillus thuringiensis israelensis* (*Bti*) [48,265]. In China, rice-fish[13] systems and intermittent irrigation have been incorporated into routine practice [167,266]. But in other parts of the world, such anti-mosquito rice growing methods have not been adopted. These modified cultivation techniques require extra effort (costs and labour) and do not

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[13] Note that a Cochrane review evaluating the impact of fish introduction on malaria transmission was published in 2017 [174]. Due to the lack of studies that reported the primary outcomes, the review could not determine whether introducing larvivorous fish reduces malaria transmission or adult anopheline density and hence could not be recommended as a supplementary larval control measure. In China, however, larval control using fish was critical for malaria control and elimination [349].

necessarily improve rice yields. They have hence not been widely adopted by farmers, whose main priority is yield.

On the other hand, agronomists are constantly looking for improved methods of rice cultivation. This is especially so under "sustainable intensification" [267]. Rice cultivation practices are continuously evolving over time to increase productivity and minimise harmful environmental impacts. Techniques that have been innovated and increasingly adopted include soil conservation, laser land levelling, alternate wetting and drying irrigation, and precision technologies for irrigation and nutrient use efficiency [268]. Moreover, rice researchers already have established pathways to recommend such improved techniques to farmers. Thus, in order to find methods of rice cultivation that also minimise mosquito production, there is an opportunity for agronomists to take on this problem. Successful collaboration between agronomists and climate change scientists on methane emission research provides a precedent for collaboration of agronomists with entomologists to incorporate mosquito monitoring into routine rice monitoring. Whilst agronomists are experimenting with different cultivation techniques to increase yields, mosquito density could be another parameter to account for, alongside weed production, water consumption, and labour intensity.

Many rice cultivation practices can affect mosquitoes in rice fields; a few studies have demonstrated that some techniques (e.g., levelling, fertiliser application, plant spacing, see Box 1.1 for a description of these techniques) have positive or negative effects on malaria vector abundance [38,184,213]. The effects of all techniques in each aspect of cultivation (namely land preparation, crop establishment, and water, nutrient, weed and pest management) on mosquito density should be explored to find a combination of techniques (from each aspect) that minimise vector production. However, because there are so many techniques, this paper focuses on selected techniques that will either (a) be more widely used by farmers, as projected by agronomists, or (b) have significant effects on vector abundance, based on mosquito biology. Specifically, we investigate the effect of flooding during land preparation, type of crop establishment, timing of fertiliser application and types of water management techniques.

Particular attention is directed towards water management techniques for two reasons. First, because larvae depend on water availability for survival, draining continuously flooded fields would technically be the most effective form of control. Second, prior to this study, public health and agricultural researchers had independently developed water management methods to address vector production and climate change effects, respectively. On the one hand, medical entomologists had demonstrated that intermittent irrigation (method of alternately irrigating and, passively or actively, drying the field for several days) in rice fields was effective in reducing vector abundance across a variety of settings [27,81]. On the other hand, since continuously flooded rice fields use large quantities of water and generate methane, climate change agronomists had developed a method called alternate wetting and drying irrigation (AWD) (method of maintaining the field flooded during the first days after transplanting and during the flowering stage and allow the field to dry during other growing phases) that can reduce water consumption by 30%, and methane production by 40%, without any negative effects on yield [269]. Although similar, involving regular flooding and drying of rice fields, both techniques have never been combined and compared. There is therefore potential for a rice intensification strategy that can optimise both climate change and health co-benefits.

Here, we conducted field trials in West and East Africa to test the hypothesis that rice innovations that can improve yield, minimise water inputs and mitigate climate change can minimise mosquito production.

6.3 Methods

6.3.1 Study area

The study was carried out in experimental fields at the M'bé AfricaRice research station in central Côte d'Ivoire (geographic coordinates 7°52'31.1" N, 5°06'46.2" W) and the Bagamoyo International Rice Research Institute (IRRI) satellite station in eastern Tanzania (6°28'34.5"S, 38°50'15.7"E). M'bé is located in a climatic transition area (from forest to savanna), which has two seasons: a dry season from December to April and a rainy season from May to November. The research station is located within a low-lying irrigation scheme that covers 150 hectares, where more than 95% is used for irrigated rice cultivation. The Bagamoyo IRRI satellite station is located in a tropical climate zone where two rainy seasons occur from March to May and November to December. The satellite station is hosted by the Bagamoyo rice irrigation scheme, which spans 200 hectares and is owned by small-scale farmers (from the Tegemeo farmers' cooperative society). In both research stations, water is supplied by neighbouring artificial reservoirs and two crops of rice are normally harvested per year.

6.3.2 Experimental design

The size and area of plots and number of treatments varied according to the trial (Table 6.1). All treatments were arranged in a randomised complete block design. The plots were hydrologically isolated using plastic-covered bunds and canals to avoid lateral water flow. The same rice variety (WITA-9 in Côte d'Ivoire and Komboka in Tanzania) was grown with a row and plant-to-plant spacing of 20 centimetres. At both sites, 21-day old seedlings were transplanted with two seedlings per hill[14]. Total systemic herbicide (Glyphosate 360 g/L) was applied before transplanting, followed by rotary and hand weeding based on weed growth to maintain the plots weed-free. Compound N-P-K fertiliser (12:24:48) was broadcasted at a rate of 200 kg/ha immediately after transplanting. First top dressing was applied at tillering (20 DAT) with 87 kg/ha urea and second top dressing at panicle initiation (60-70 DAT) with 87 kg/ha

[14] Planting "in hills" is a term used for the method of planting seeds together (in clusters). A hill may also consist of only one plant.

Table 6.1. Description of trials conducted in Côte d'Ivoire (n=6) and in Tanzania (n=1), and their treatments.

Trial	Plot size (m^2) [No. of replicates for each treatment]	Treatments	Experimental field area (m^2)	Seasons
1: Land preparation	50 [3]	Based on the timing of flooding between the first and second puddling during land preparation: 1. 21 days 2. 14 days 3. 7 days 4. 4 days [added for 2nd season] 5. 2 days	600-750	2 (03/2021-08/2021, 08/2021-12/2021)
2: Crop establishment & water management	200 [3]	1. TP-CF: Transplanting (TP) and continuous flooding (CF) (control) 2. TP-AWD: Transplanting (TP) and alternate wetting and drying (AWD-15) irrigation 3. DS-WET-AWD: Manual wet direct broadcast seeding and AWD-15 [added for 2nd and 3rd seasons] 4. DS-WET-CF: Manual wet direct broadcast seeding and CF 5. DS-DS-CF: Line wet seeding with a drum-seeder (Appendix 6.1B) and CF 6. DS-DRY-CF: Manual line dry seeding and CF	3000-3600	3 (04/2019-09/2019, 10/2019-02/2020, 08/2020-12/2020)
3: Water management	25 [5]	1. CF (control) 2. AWD-15	250	1 (08/2020-12/2020)
4: Water & nutrient management (Figure 6.1)	25 [3]	1. CF (control) 2. AWD-15 3. AWD-15 (2): AWD-15 that starts 2 days after transplanting (DAT) 4. FD-II: Forced drainage at 25 DAT followed by intermittent irrigation (II) in the following 25 days 5. FD-II2: Forced drainage at 25 DAT followed by II with 2 drying cycles of 7 days each 6. II3: Intermittent irrigation at 3 day-wet and 3 day-dry intervals 7. Supplemental irrigation 8. CF-FD: CF with forced drainage before fertiliser application 9. CF-NONE: CF without fertiliser application 10. No rice	525	2 (11/2019-03/2020, 08/2020-12/2020)
5: Water & nutrient management	25 [3]	1. CF (control) 2. AWD-15 3. AWD-30 4. DF: Delayed flooding (7 DAT) after transplanting 5. F-BF: CF with NPK fertiliser applied before flooding during land preparation 6. F-DA: CF with NPK fertiliser application delayed at 20 DAT	500	2 (03/2021-07/2021, 08/2021-12/2021)
6: Water management	50 [3]	1. CF (control) 2. AWD-15 3. FD-II: Forced drainage at 25 DAT followed by II in the following 25 days 4. FD-II2: Forced drainage at 25 DAT followed by II with 2 drying cycles of 7 days each 5. Supplemental irrigation	750	1 (08/2021-12/2021)
7: Water management (Tanzania)	32 [3]	1. CF (control) 2. AWD-15 3. AWD-30 4. DF: Delayed flooding (7 DAT) after transplanting 5. II: Intermittent irrigation at 3 day-wet and 3 day-dry intervals	480	1 (06/2021-11/2021)

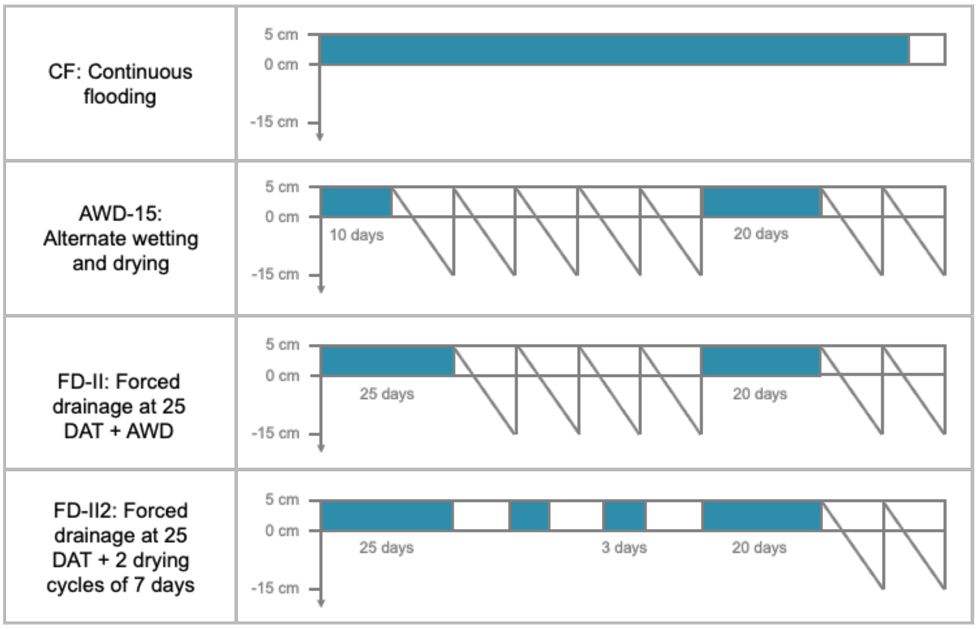

Figure 6.1. Diagram representation of some water management techniques in Trials 2-7. Triangles indicate that water is passively drained and re-irrigated only when the water table reaches 15 cm below soil level.

urea. Application of pesticide and protection against rat and bird damage was conducted when necessary.

Other than the treatment being tested, all other growing conditions were kept the same. Each treatment was applied in a randomised complete block design across three or five replicate plots per season. Trials 1, 2, 4 and 5 were established for at least two rice seasons (dry and rainy); additional seasons were performed when results from the first two trials were not consistent. Contingent on preliminary analyses of the first season, additional treatments of interest were added in the second seasons of Trials 1 and 2. Trials were conducted between April 2019 to December 2021.

6.3.3 Mosquito sampling and identification

All plots were exposed to the natural colonisation of mosquitoes. Immature mosquitoes were sampled twice per week from land preparation to two weeks after harvesting. Field technicians swapped between dipping and data entry every week. Two dips were taken at 5-metre intervals on the four borders of each plot. Upon collection, immatures were sorted into sub-families (anopheline or culicine) and larval developmental stage (L1, L2, L3, L4, or pupae). Late stage (L3 and L4) larvae and pupae were transported to the AfricaRice or Ifakara Health Institute entomology laboratories for morphological species identification [250]. Members of the *An. gambiae* and *An. funestus* species complexes were selected (up to 20% per sampling occasion) for molecular identification using PCR [270,271].

6.3.4 Greenhouse gas emissions, soil moisture and soil temperature data collection

Measurement of CH_4, CO_2, N_2O emissions from rice fields were collected for Trials 6 and 7 for one season each. It was conducted using the static chamber – gas chromatograph method [272]. Gas sampling was made by placing two static chambers on stainless-steel bases in each plot. These bases were inserted at 5 cm soil depth (3 cm above soil surface) and equipped with a water seal to ensure gas-tight closure. The size of the chamber was 40 cm × 60 cm × 60 cm (length × width × height) during tillering stage. After panicle initiation, the height was extended to 120 cm to accommodate the taller plants. Gas samples were taken between 9:00–11:00 am[15] every three days. The air temperature inside the chamber, and the soil moisture and soil temperature at a 5-cm depth were recorded for each plot simultaneously during the gas collection. The air samples were withdrawn into a sealed and pre-evacuated sample tube, then transferred into the laboratory. Gas samples were analysed for CH_4 and CO_2 by gas chromatograph flame ionisation detector and N_2O by GC-electron capture detector. The global warming potential and yield-scaled global warming potential were estimated following Islam et al. (2020) [273].

[15] Methane fluxes tend to vary diurnally. However, it was determined by Minamikawa et al. (2012) that measurements performed during mid-morning resulted in acceptable estimates [350].

6.3.5 Rice yield and water use measurements

At maturity, grain yield was determined from two 4 m^2 areas in the centre of each plot and adjusted to 14% moisture content [274].

The dates of irrigation and the number of irrigations were recorded. Field water depth in the tubes were recorded between 15:00 and 16:00 every day. A V-notch was installed at the inlet of each plot to evaluate water input [275].

6.3.6 Data analysis

Mosquito abundance was aggregated to daily counts[16] (i.e. the total number of mosquitoes dipped at each sampling occasion) and scaled to count per ten dips. To compare the abundance of *Anopheles* larvae between different rice cultivation practices (i.e. treatments), generalised linear mixed-effect models (GLMM) with negative binomial distributions were fitted using the "glmer.nb" function from the "lme4" package in R. Negative binomial models were used to commensurate the statistical distribution of daily mosquito counts. As dips conducted within a plot may be correlated, plots were accounted for in the model as a random effect. Plot position (i.e., row and column), rice-growing phase and rice-cropping season were also accounted for, but as fixed-effect explanatory factors. Plot position was adjusted for because it potentially explains some variability in the data and can provide better precision in the final estimates. Rice-growing phase was adjusted for, instead of day after transplanting (DAT), because, as illustrated in Chapter 5, it has a strong effect

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[16] It is more robust to aggregate mosquito abundance to daily counts (rather than dip counts). Dips conducted on the same day and in the same plot are more correlated to each other because of (1) correlation within a plot and (2) correlation in a day within a plot. Thus, a model for dip counts would include plot as a random effect as well as day after transplanting (DAT) as a nested random effect. But to avoid this complexity and issues with model convergence (that often occurred), mosquito counts were aggregated by sampling occasion (i.e. day) and any random effects of DAT were instead accounted for by rice-growing phase. Including rice-growing phase in the model can reduce the variance of the random effect of DAT since within a phase, there is less day-to-day variance.

in mosquito abundance and because the relationship between date after transplanting (DAT) and mosquito abundance is non-linear. Rice-cropping season was included in the model to adjust for variability between seasons. Estimated marginal means (i.e., mosquito density means that were adjusted for other factors in the model) were computed using the "emmeans" package. *Post-hoc* Tukey's Honest Significant Difference (HSD) tests were run for multiple pair-wise comparisons between treatments.

Models were fitted separately for each trial, for all mosquito developmental stages combined across all rice growth stages but were also fitted for specific immature stages (early instar, late instar, and pupae) and for specific rice growth phases (land preparation, vegetative, reproductive, ripening, and post-harvest). For Trial 1 on land preparation, mosquito densities were also fitted against treatments which were treated continuously (as a numeric variable), as days between flooding.

To compare rice yield, greenhouse gas emissions, water use (irrigation water input, number of irrigations and water productivity), and weed biomass between different treatments, analyses of variance (ANOVA) were conducted. To meet the assumptions of the analysis of variance, CO_2, CH_4, global warming potential, yield-scaled global warming potential and number of irrigations were subjected to logarithm transformations. Mean values were tested for significant differences also by using the Tukey's HSD test.

Results were reported as statistically significant if the p-value <0.05 unless stated otherwise. All statistical analyses were performed with R (version 4.1.2) [218].

6.4 Results

6.4.1 Mosquito species composition

Across the seven trials, a total of 25,767 anopheline mosquito immatures was collected, of which 18,699 were early instars, 5014 were late instar and 2054 were pupae. Only late instar and pupae were reared to adults for morphological identification.

In Côte d'Ivoire, the malaria vector *Anopheles gambiae* s.l. was morphologically identified as the predominant species (49.0%, Appendix 6.2), followed by nuisance biters *Culex cinereus* (25.3%) and *Cx. quinquefasciatus* (10.6%). *An. gambiae* s.l. comprised 85.1% of the anophelines, followed by *An. ziemanni* with 14.3%. Further tests by PCR (17.8% sub-sample) of the *An. gambiae* species complex identified that except for a few *An. gambiae* s.s. (0.4%), *An. coluzzii* comprised virtually the entire complex (99.6%). Different species predominated at different parts of the cropping season. Earlier in the season, *An. coluzzii* was predominant in the rice fields; Figure 6.2 shows that it was most abundant at vegetative phase, followed by reproductive and land preparation phases. Later in the season (ripening and reproductive phases), *An. ziemanni* was predominant.

In Tanzania (over one cropping season), 442 anopheline mosquito immatures were collected. *An. gambiae* s.l. also predominated (54.2%, Appendix 6.2), followed by *Cx. univittatus* (24.6%) and *Cx. antennatus* (14.6%). Amongst the anophelines, 93.0% were *An. gambiae* s.l. with minor amounts of *An. coustani*. Further tests by PCR identified that all *An. gambiae* s.l. were *An. arabiensis* (100%, n=185). Earlier in the season, *An. arabiensis* was predominant; Figure 6.2 also shows that it was most abundant at vegetative phase, followed by reproductive phase. Later in the season (i.e., ripening), both *Cx. antennatus* and *Cx. univittatus* were dominant.

6.4.2 The effect of rice cultivation techniques on mosquito density, rice yield, water consumption and greenhouse gas emissions

The main results for each trial are first presented in italics and bullet points. The results are then described in full detail according to each outcome (mosquito density, rice yield, water consumption, and greenhouse gas emissions). Yield-scaled mosquito density and global warming potential are also described for each treatment.

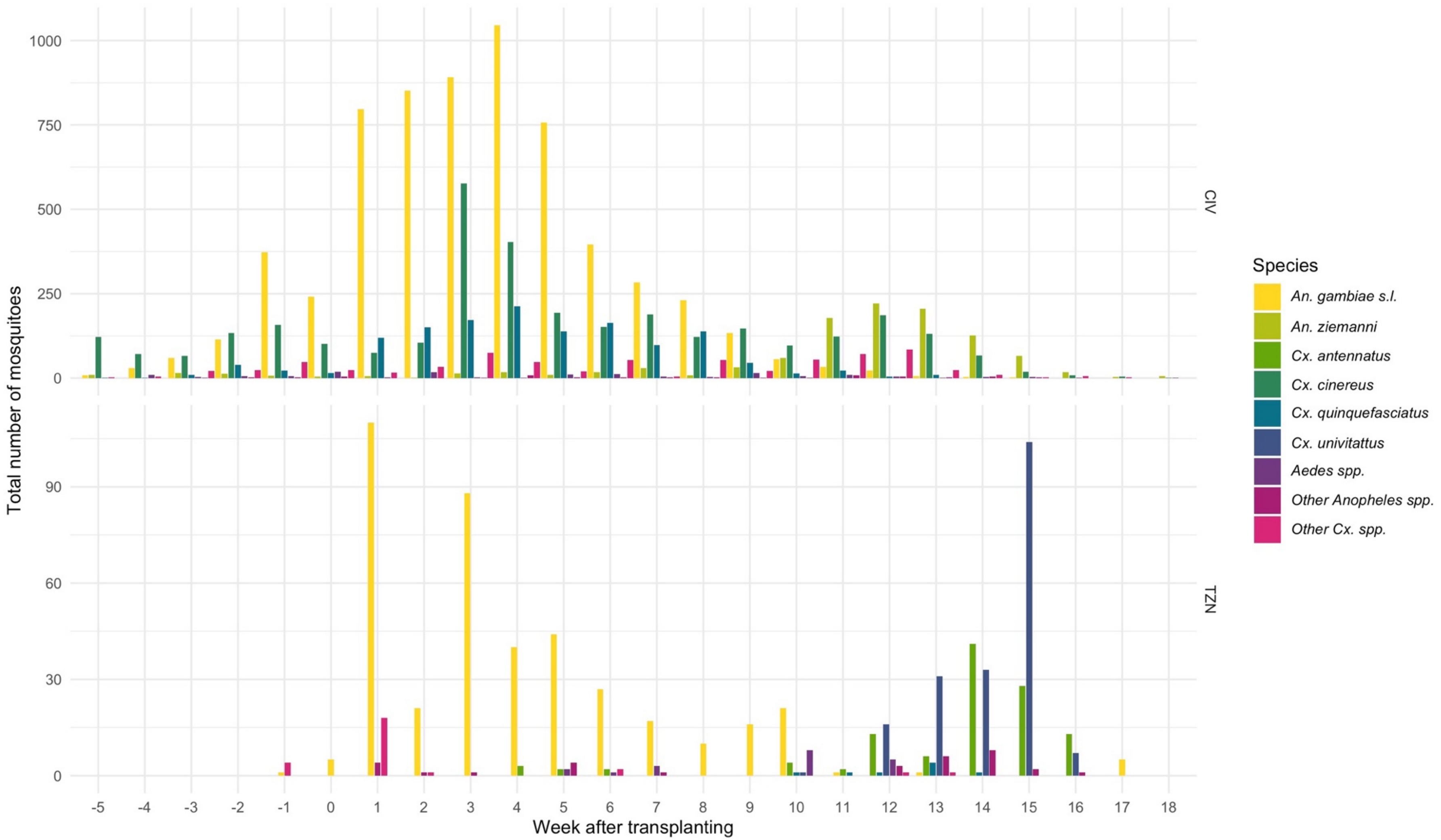

Figure 6.2. Mosquito species composition during a rice cropping season across all seven trials in Côte d'Ivoire and Tanzania.

6.4.2.1 Trial 1: Land preparation

- *The timing of flooding between primary and secondary tillage did not significantly impact* Anopheles *mosquito immature densities. However, plots with 7-day flooding produced significantly greater yield.*

The timing of flooding between the primary and secondary tillage did not significantly impact *Anopheles* mosquito immature densities, not even when looking exclusively at early or late instars or pupae (Table 6.2). There were also no trends in flooding duration and mosquito immature density.

The rice yield in plots with 7-day flooding between the primary and secondary tillage were higher than 14-day and 21-day flooding (Table 6.2). Yield-scaled early instar vector densities were lower in plots with 7-day flooding compared to 21-day flooding (Table 6.3).

6.4.2.2 Trial 2: Crop establishment and water management

- *Greater pupal productivity was observed in direct seeding with drum-seeders compared to transplanting.*

A significantly higher density of pupae was found in plots with direct seeding using a drum-seeder compared to plots that were transplanted (Figure 6.3A). Specifically, 153.5% more pupae (95% CI +36.4%, 371.3%) were found in plots that were directly seeded with a drum-seeder (Appendix 6.3). Compared to plots that were transplanted with continuous flooding, more pupae (+213.5%, 95% CI +51.9, +564.7) were also found in plots with direct seeding and alternate wetting and drying irrigation.

Different combinations of crop establishment methods and water management techniques did not significantly change rice yields at harvest nor yield-scaled mosquito density (Tables 6.2 and 6.3).

Table 6.2. Rice yield and mosquito density under different rice cultivation techniques in Trials 1 and 2. Means denoted by a different letter indicate significant differences between treatments ($p < 0.05$).

Trial / Treatment	**Yield[a] (t/ha)**	**Mosquito density (immatures/ten dips)**		
		Early instars	**Late instars**	**Pupae**
Trial 1: Land preparation				
21 days	5.3 b	9.13 a	2.50 a	1.39 a
14 days	5.1 b	8.25 a	3.41 a	1.10 a
7 days	6.0 a	8.19 a	2.78 a	0.98 a
4 days	-	5.35 a	1.98 a	1.25 a
2 days	5.5 ab	8.09 a	3.29 a	0.58 a
p-value	*0.06*	*Trend: 0.010[b]* *p = 0.588*	*Trend: -0.004* *p = 0.826*	*Trend: 0.033* *p = 0.293*
Trial 2: Crop establishment & water management				
TP-CF	5.9 a	2.84 a	0.64 a	0.07 a
TP-AWD	6.5 a	2.54 a	0.79 a	0.15 ab
DS-AWD	6.5 a	2.51 a	0.55 a	0.22 b
DS-WET-CF	6.7 a	3.23 a	0.83 a	0.16 ab
DS-DS-CF	6.9 a	3.32 a	1.06 a	0.18 b
DS-DRY-CF	6.3 a	2.80 a	0.85 a	0.12 ab
p-value	*0.49*	*p<0.001*	*p = 0.109*	*p = 0.412*

NB: TP-CF, transplanting & continuous flooding; TP-AWD, transplanting & AWD-15; DS-WET-AWD, manual wet direct broadcast seeding & AWD-15; DS-WET-CF, manual wet direct broadcast seeding & continuous flooding; DS-DS-CF, line wet seeding with a drum-seeder & continuous flooding; DS-DRY-CF, manual line dry seeding & continuous flooding

[a] Yields were based on first season only, therefore it was not measured for the 4-day treatment

[b] Trend indicates the change in mean larvae per ten dips for a 1-day decrease in timing of flooding

Table 6.3. Yield-scaled mosquito, water, and global warming potential indicators under each treatment across 7 trials. Means denoted by a different letter indicate significant differences between treatments ($p < 0.05$).

Trial / Treatment	**Yield-scaled mosquito density (immatures/t/ha)**			**Yield-scaled GWP* (t CO2/t grain)**
	Early instars	**Late instars**	**Pupae**	
Trial 1: Land preparation				
21 days	1.72 a	0.471 a	0.262 a	-
14 days	1.62 a	0.669 a	0.215 a	-
7 days	1.36 a	0.463 a	0.163 a	-
2 days	1.47 a	0.598 a	0.106 a	-
Trial 2: Crop establishment & water management				
TP-CF	0.481 a	0.109 ab	0.012 a	-
TP-AWD	0.391 a	0.122 ab	0.023 a	-
DS-AWD	0.386 a	0.084 a	0.033 a	-
DS-WET-CF	0.482 a	0.124 ab	0.024 a	-
DS-DS-CF	0.562 a	0.179 b	0.030 a	-
DS-DRY-CF	0.445 a	0.134 ab	0.019 a	-
Trial 3: Water management				
CF	0.268 a	0.103 a	0.022 a	-
AWD -15	0.262 a	0.101 a	0.030 a	-
Trial 4: Water & nutrient management				
CF	0.421 c	0.135 a	0.035 a	-
AWD-15 (10 DAT)	0.361 bc	0.112 a	0.032 a	-
AWD-15 (2 DAT)	0.316 bc	0.076 a	0.019 a	-
FD-II	0.304 abc	0.091 a	0.038 a	-
FD-II2	0.136 a	0.078 a	0.027 a	-
II3	0.278 abc	0.060 a	0.015 a	-
Supplemental	0.163 ab	0.057 a	0.032 a	-
CF-FD	0.218 abc	0.065 a	0.015 a	-
CF-NONE	0.396 c	0.082 a	0.016 a	-
Trial 5: Water & nutrient management				
CF	0.800 a	0.242 a	0.034 ab	-
AWD-15	0.787 a	0.268 a	0.085 c	-
AWD-30	0.808 a	0.266 a	0.058 abc	-
DF	1.016 a	0.272 a	0.072 bc	-
F-BF	1.160 a	0.221 a	0.052 abc	-
F-DA	0.837 a	0.174 a	0.027 a	-
Trial 6: Water management (CIV)				
CF	0.307 a	0.121 a	0.031 a	1.61 a
AWD-15	0.240 a	0.144 a	0.034 a	0.94 b
FD-II	0.593 a	0.211 a	0.034a	0.88 b
FD-II2	0.598 a	0.234 a	0.055 a	0.64 b
Supplemental	0.585 a	0.155 a	0.062 a	0.76 b
Trial 7: Water management (TZN)				
CF	3.90 b	0.950 a	0.360 a	-
AWD-15	1.43 a	0.600 a	0.175 a	4.2 a
AWD-30	3.33 ab	0.680 a	0.176 a	6.0 a
DF	1.29 a	0.629 a	0.273 a	-
II3	1.62 ab	0.739 a	0.100 a	-

*Greenhouse gas emissions were only measured in Trials 6 and 7.

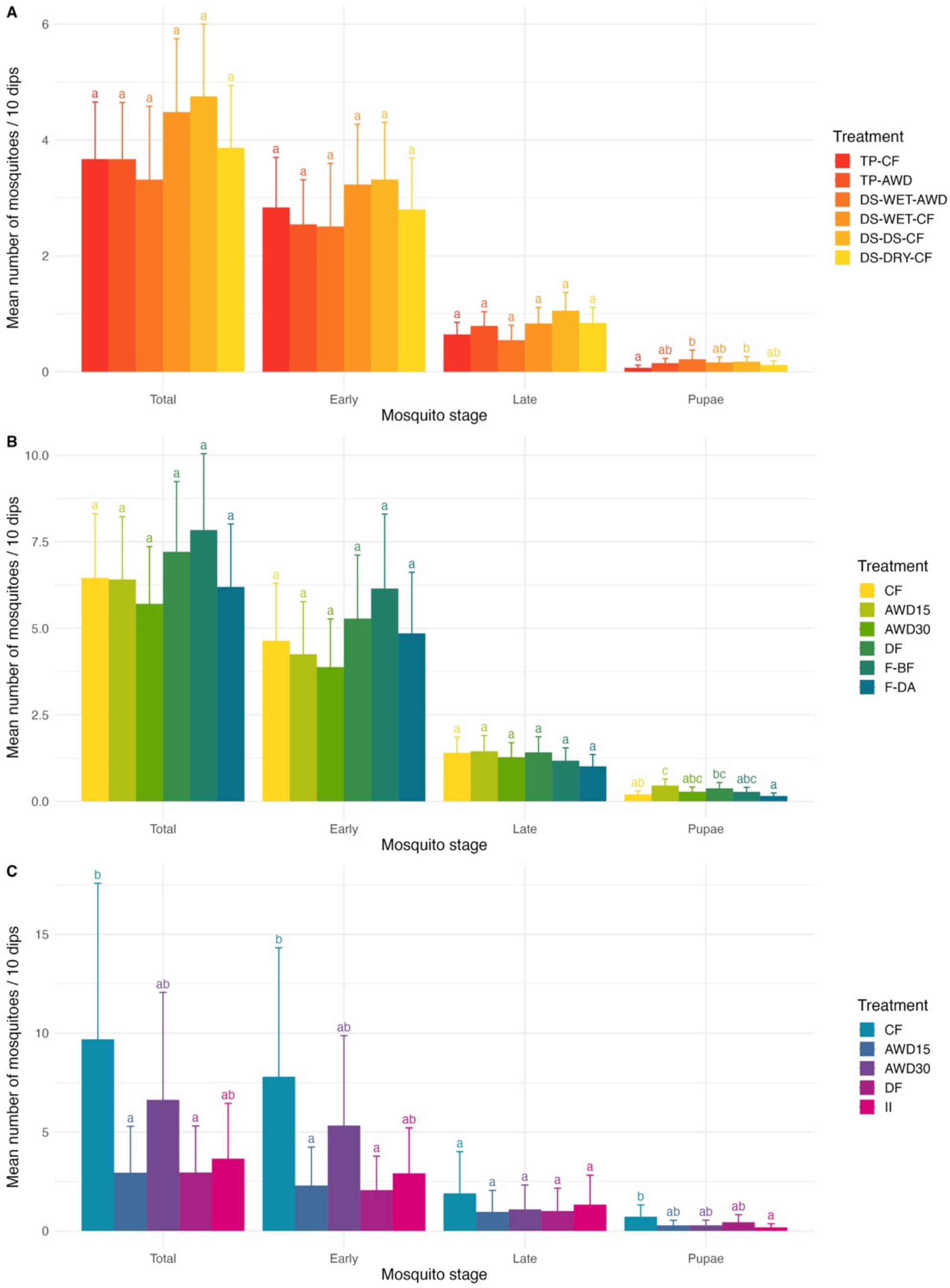

Figure 6.3. Mean *Anopheles* density per ten dips collected under **(A)** Trial 2: crop establishment and water management in Côte d'Ivoire, **(B)** Trial 5: water and nutrient management in Côte d'Ivoire and **(C)** Trial 7: water management in Tanzania. Bars denoted by different letters indicate significant differences between treatments ($p < 0.05$).

NB: TP-CF, transplanting & continuous flooding; TP-AWD, transplanting & AWD-15; DS-WET-AWD, manual wet direct broadcast seeding & AWD-15; DS-WET-CF, manual wet direct broadcast seeding & continuous flooding; DS-DS-CF, line wet seeding with a drum-seeder & continuous flooding; DS-DRY-CF, manual line dry seeding & continuous flooding; CF, continuous flooding; DF, delayed flooding after transplanting; F-BF, NPK fertiliser applied before flooding during land preparation; F-DA, NPK fertiliser application delayed at 20 DAT; II, intermittent irrigation at 3 day-wet and 3 day-dry intervals.

6.4.2.3 Trial 3: Water management

- *Compared to continuous flooding, AWD-15 saved more water, did not impose any yield penalties and did not produce more mosquitoes.*

No significant differences were seen in the number of mosquitoes between rice plots under continuous flooding (CF) and AWD-15 (Table 6.4 and Appendix 6.3).

No significant differences were seen in rice yields between either treatment. However, AWD-15 reduced the number of irrigations by 42% and irrigation water use by 61%, resulting in an increase in water productivity by 178% compared to continuous flooding (Table 6.4).

Yield-scaled mosquito density was not significantly different in plots with AWD-15 and CF. Across water management treatments, rice yield and water productivity were significantly higher in Season 2 than in Season 1, while the amount of irrigation water used, and the number of irrigations were higher in Season 2 than in Season 1 (Table 6.4).

6.4.2.4 Trial 4: Water and nutrient management

- *During the vegetative phase, compared to plots under continuous flooding, fewer (yield-scaled) early instars were observed in plots without rice growth and plots where forced drainage occurred at 25 DAT followed by two 7-day drying cycles. These observations were not seen in later stage mosquito immatures.*
- *Rice plots without fertilisers suffered a 33% yield penalty.*

Table 6.4. Rice yield, mosquito density and water use under different water (and nutrient) management methods in Trials 3, 4, and 5. Means denoted by a different letter indicate significant differences between treatments ($p < 0.05$).

	Yield (t/ha)	Mosquito density (immatures/ten dips)			Irrigation water (m3/ha)	No. of irrigations	Water productivity (kg/m3/ha)
		Early instars	Late instars	Pupae			
Trial 3: Water management							
Water Management (W)							
CF	6.2 a	1.66 a	0.64 a	0.14 a	27153 a	12 a	0.23 b
AWD -15	6.6 a	1.73 a	0.66 a	0.20 a	10544 b	7 b	0.64 a
Season (S)							
Season 1	5.6 a	-	-	-	7440 b	4.8 b	0.92 a
Season 2	4.1 b	-	-	-	11349 a	6.9 a	0.52 b
Trial 4: Water & nutrient management*							
CF	4.5 a	1.90 a	0.61 a	0.16 a	19806 a	8.5 a	0.31 c
AWD-15 (10 DAT)	4.5 ab	1.62 a	0.50 a	0.14 a	5852 de	3.5 b	0.80 ab
AWD-15 (2 DAT)	5.3 a	1.67 a	0.41 a	0.10 a	4723 e	3.3 b	1.45 a
FD-II	4.6 a	1.40 a	0.42 a	0.18 a	6892 de	4.0 b	0.70 ab
FD-II2	6.0 a	0.82 a	0.47 a	0.16 a	8569 cd	4.3 b	0.84 ab
II3	5.7 a	1.58 a	0.34 a	0.09 a	11019 bc	9.2 a	0.54 b
Supplemental	4.7 a	0.76 a	0.27 a	0.15 a	4759 e	3.2 b	1.04 a
CF-FD	5.7 a	1.24 a	0.37 a	0.08 a	11028 b	8.0 a	0.58 b
CF-NONE	3.0 b	1.19 a	0.25 a	0.05 a	12583 b	7.5 a	0.25 c
No rice	-	1.37 a	0.21 a	0.03 a	8713 bcd	7.2 a	-
Trial 5: Water & nutrient management							
CF	5.8 a	4.64 a	1.40 a	0.20 ab	21117 a	6.2 ab	0.29 c
AWD-15	5.4 a	4.25 a	1.45 a	0.46 c	12535 b	5.0 c	0.42 bc
AWD-30	4.8 a	3.88 a	1.28 a	0.28 abc	5336 c	2.2 d	1.20 a
DF	5.2 a	5.28 a	1.42 a	0.38 bc	12396 b	5.4 bc	0.47 b
F-BF	5.3 a	6.15 a	1.17 a	0.27 abc	16094 ab	5.0 c	0.33 bc
F-DA	5.8 a	4.85 a	1.01 a	0.16 a	18245 a	6.4 a	0.36 bc
p-value	*0.38*	*<0.001*	*0.032*	*<0.001*	*<0.001*	*<0.001*	*<0.001*

NB: FD-II: Forced drainage at 25 DAT followed by intermittent irrigation (II) in the following 25 days; FD-II2: Forced drainage at 25 DAT followed by II with 2 drying cycles of 7 days each; II3: Intermittent irrigation at 3 day-wet and 3 day-dry intervals; CF-FD: CF with forced drainage before fertiliser application; CF-NONE: CF without fertiliser application; DF: Delayed flooding (7 DAT) after transplanting; F-BF: CF with NPK fertiliser applied before flooding during land preparation; F-DA: CF with NPK fertiliser application delayed at 20 DAT

* Based on average of two seasons

There were no differences in overall mosquito immature densities between rice plots under various water and nutrient management techniques (Table 6.4). However, separation into larval stage and rice-growing phases revealed that plots with forced drainage 25 DAT followed by 2 drying cycles of 7 days each were associated with fewer anopheline early instars during the vegetative phase (-82.0%, 95% CI -93.6, -49.5) and during the reproductive phase (-89.2%, 95% CI -97.0, -61.3) (Appendix 6.3). Moreover, plots without any rice growth had significantly fewer immatures than plots with continuous flooding during the vegetative phase (-78.7%, 95% CI -91.5, -46.3, Appendix 6.3).

In Trial 4, CF plots without any fertiliser application produced significantly less yield compared to all other treatments (Table 6.4). Compared to plots under continuous flooding, AWD-15 (both starting at 2-DAT and 10 DAT), forced drainage and supplemental irrigation required significantly fewer irrigations and used significantly less irrigation water whilst maintaining rice yield (Figure 6.4). Thus, these treatments were significantly more water productive than CF plots (Table 6.4).

No significant differences in yield-scaled late-instar or pupae density were seen amongst treatments in Trial 4. However, compared to CF fields, significantly lower yield-scaled early instar densities were observed in FD-II2 and rainfed fields (Table 6.3).

6.4.2.5 Trial 5: Water and nutrient management

- *Water management techniques AWD-15 and delayed flooding after transplanting did not significant affect yield and increased water productivity but were associated with greater vector productivity.*

AWD-15 produced significantly greater pupal density, with an estimated 129.6% increase (95% CI +34.3, +292.4, Table 6.4). Delayed flooding also produced significantly more pupae than CF plots, with an 88.9% increase (95% CI +11.1, +220.9, Appendix 6.3).

There were no significant differences in rice yield between treatments under different water and nutrient management techniques (Table 6.4). However, compared to continuous flooding, AWD-15, AWD-30 and delayed flooding after transplanting used significantly less water (Table 6.4). Compared to plots under CF, water productivity was higher in plots under AWD-15, delayed flooding, CF with NPK fertilizer applied before flooding during land preparation and CF with NPK fertilizer application delayed at 20 DAT.

Compared to CF fields, yield-scaled pupal *Anopheles* density was greater in AWD-15 (Table 6.3).

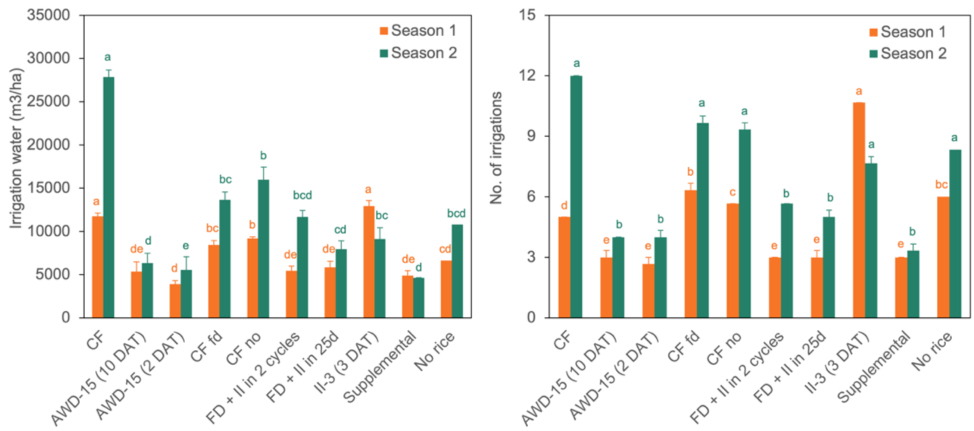

Figure 6.4. Water use under different water management techniques in Trial 4. Means denoted by a different letter indicate significant differences between treatments ($p < 0.05$).

6.4.2.6 Trial 6: Water management

- *Although forced drainage at 25 DAT followed by intermittent irrigation of two 7-day drying cycles had the lowest global warming potential and greatest water productivity, it produced more late-instar malaria vectors during the vegetative phase.*

There were no significant differences in total mosquito density across all growing phases between treatments under different water management techniques (Table 6.5). During the vegetative phase, however, compared to continuous flooding, forced drainage with intermittent irrigation (FD-II2) produced 312% more late instars (95% CI +63.2, +940.7%, Appendix 6.3).

There were no significant differences in rice yield between different water management techniques in Trial 6. Compared to CF fields, fields under AWD-15, forced drainage and supplemental irrigation used significantly less water, by 32.7 to 73.8% (Table 6.5). Compared to continuously flooded plots, water productivity was greater in fields under AWD-15, forced drainage and supplemental irrigation.

Soil dryness index (calculated as the ratio between the number of times the field was not flooded during the field visits and the total number of field visits) was significantly higher in fields under AWD-15, both types of forced drainage and supplemental irrigation than fields under CF (Table 6.5).

During the cropping season, CH_4 fluctuation varied with water management practices (Figure 6.5A). Methane fluxes increased quickly, reached a peak, and then remained low for AWD-15, forced drainage, and supplemental irrigation. However, for CF, methane fluxes remained low at the early stage of the crop growth stage, significantly increased, and produced higher peaks at latter growth stage. Compared to fields that were continuously flooded, all other water management methods produced less methane emissions (Table 6.5). The greatest reduction was seen in fields under forced drainage followed by two cycles of intermittent irrigation, at 59% reduction. With 54.5% reduction, supplemental irrigation came second and, with 46.6% reduction, forced drainage followed by AWD-15 came third. Fields under AWD-15 produced 40.5% less methane than CF fields.

The seasonal variation pattern of N_2O emissions significantly varied between water management treatments (Figure 6.5B). N_2O emissions fluxes remained lower in CF compared to other treatments (Table 6.5). Cumulated over the growing season, N_2O emission was the lowest in CF, and the highest in AWD-15 plots. There were no significant differences in carbon dioxide emissions between different water management techniques.

Global warming potential (GWP) was highest in CF fields, followed by fields under AWD-15, FD-II, supplemental irrigation and lowest in fields under FD-II2 (Table 6.5). Correspondingly, yield-scaled GWP was significantly lower in all treatments compared to CF plots, where it was lowest in FD-II2, with 60.2% reduction.

In terms of synergies and/or trade-offs between the main outcomes, GWP was negatively correlated with soil dryness index and water productivity (Table 6.6).

6.4.2.7 Trial 7: Water management (Tanzania)

- *Compared to fields under continuous flooding, lower early-instar productivity was observed in fields under AWD-15 and delayed flooding. Lower pupal productivity was observed in fields under intermittent irrigation of 3-day wet-dry cycles.*

In Tanzania, AWD-15 and delayed flooding produced significantly fewer mosquito immatures compared to CF (Figure 6.3C). At pupal stage, however, only plots under 3-day wet-dry intermittent irrigation produced significantly fewer pupae than plots under CF (-75.0%, 95% CI -90.0, -37.2, Appendix 6.3). Compared to fields that were continuously flooded, yield-scaled early instar mosquito density was smaller in fields under AWD-15 and delayed flooding (Table 6.3).

There were no significant differences in rice yield between fields under different water management techniques (Table 6.5). Compared to CF fields, both AWD and intermittent irrigation required significantly fewer numbers of irrigations and correspondingly had higher soil dryness indices. Water productivity was significantly higher under AWD-15 and AWD-30 compared to continuous flooding and intermittent irrigation. There were no significant differences in greenhouse gas emissions, GWP and yield-scaled GWP between AWD-15 and AWD-30 (Table 6.5). The lack of differences is illustrated in Figure 6.6A for methane emissions but for nitrous oxide emissions, AWD-15 avoided the two peaks produced by AWD-30 earlier in the season (Figure 6.6).

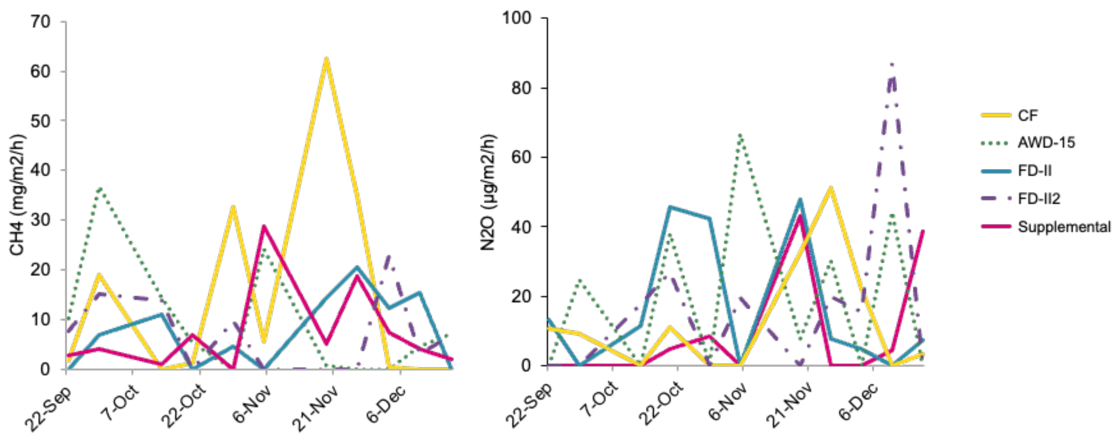

Figure 6.5. Temporal variations of CH_4 and N_2O emissions under different water management techniques during a cropping season in Trial 6 (Côte d'Ivoire).

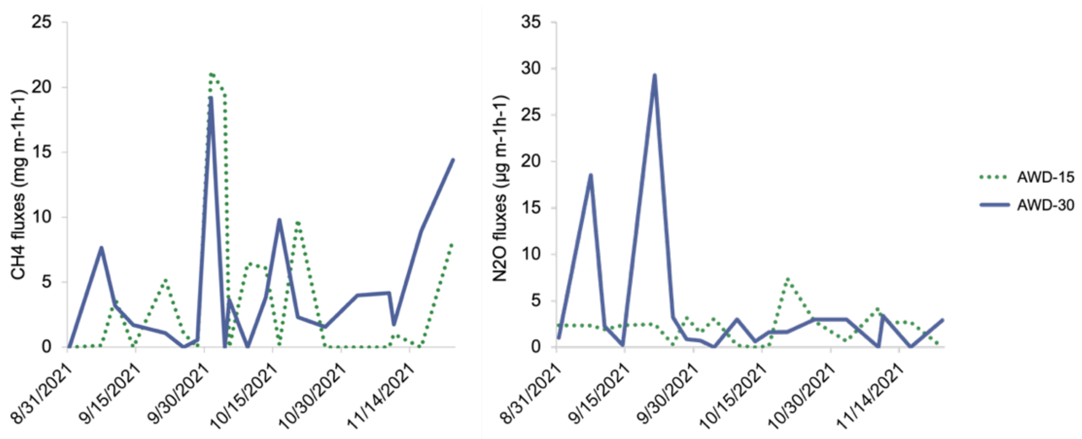

Figure 6.6. Temporal variations of CH_4 and N_2O emissions under different water management techniques during a cropping season in Trial 7 (Tanzania).

Table 6.5. Rice yield, mosquito density, water use, soil characteristics and greenhouse gas emissions under different water management techniques in Trials 6 (Côte d'Ivoire) and 7 (Tanzania). Means denoted by a different letter indicate significant differences between treatments ($p < 0.05$).

Treatment	**Yield (t/ha)**	**Mosquito density (immatures/ten dips)**			**Irrigation water (m^3/ha)**	**No. of irrig-ations**	**Water productivity (kg/m3/ha)**	**Soil dryness index**	**Soil moisture (m^3/m^3)**	**CH_4 (kg/ha)**	**N_2O (kg/ha)**	**CO_2 (kg/ha)**	**GWP (t CO_2/ha)**
		Early instars	**Late instars**	**Pupae**									
Trial 6: Water management (CIV)													
CF	5.5 a	1.69 a	0.67 a	0.17 a	2057 a	9 a	0.24 c	0 c	0.52 a	343 a	0.16 e	0.001 a	8.61 a
AWD-15	5.5 a	1.32 a	0.79 a	0.19 a	868 c	3 b	0.53 ab	0.61 a	0.49 c	204 b	0.39 a	0.001 a	5.21 b
FD-II	5.3 a	3.14 a	1.12 a	0.18 a	539 c	4 b	0.72 a	0.30 b	0.50 b	183 c	0.28 d	0.001 a	4.66 c
FD-II2	5.7 a	3.41 a	1.33 a	0.32 a	726 c	4 b	0.63 a	0.25 b	0.51 ab	139 e	0.33 b	0.001 a	3.57 e
Supplemental	5.3 a	3.10 a	0.82 a	0.33 a	1384 b	4 b	0.34 bc	0.61 a	0.48 d	156 d	0.29 c	0.001 a	3.99 d
*LSD**	*1.8*	*p = 0.022*	*p = 0.724*	*p = 0.398*	*513*	*2*	*0.20*	*0.06*	*0.008*	*6.0*	*0.008*	*ns*	*6.0*
Trial 7: Water management (TZN)													
CF	2.0 a	7.80 b	1.90 a	0.72 b	446 a	63 a	0.46 b	0.14 c	-	-	-	-	-
AWD-15	1.6 a	2.30 a	0.96 a	0.28 ab	209 ab	25 c	0.90 ab	0.62 b	-	269 a	0.17 a	-	6.8 a
AWD-30	1.6 a	5.33 ab	1.09 a	0.28 ab	166 b	21 c	1.29 a	0.77 a	-	350 a	0.29 a	-	8.8 a
DF	1.6 a	2.06 a	1.01 a	0.44 ab	468 a	63 a	0.36 b	0.18 c	-	-	-	-	-
II3	1.8 a	2.91 ab	1.33 a	0.18 a	341 ab	45 b	0.52 b	0.44 b	-	-	-	-	-

*LSD: least significant difference

Table 6.6. Correlation between rice yield, water productivity, greenhouse gas emissions and mosquito density in Trial 6.

	Global warming potential	Rice yield	Soil dryness index	Soil moisture	Soil temperature	Irrigation water	Water productivity	Mosquito density
Global warming potential	1.000	0.040	-0.660***	0.570*	0.090	0.750***	-0.600*	-0.300
Rice yield		1.000	-0.070	0.100	-0.170	0.220	-0.020	0.160
Soil dryness index			1.000	-0.880***	0.030	-0.360	0.110	0.170
Soil moisture				1.000	0.240	0.190	0.020	-0.360
Soil temperature					1.000	0.100	-0.200	-0.330
Irrigation water						1.000	-0.930***	-0.280
Water productivity							1.000	0.270
Mosquito density								1.000

* significant at $p < 0.05$, ** significant at $p < 0.01$, *** significant at $p < 0.001$

6.5 Discussion

6.5.1 Summary of results

In field trials in West and East Africa, we tested the hypothesis that modified rice cultivation practices have different effects on rice yield, water consumption, greenhouse gas emissions, and malaria vector density. We found that compared to transplanted fields, direct seeded fields did not have lower yield, but drum-seeding was associated with more vectors. Although AWD-15 was not effective in reducing malaria vectors in Côte d'Ivoire, and even led to more pupae in some trials (e.g., Trials 2 and 5), it significantly reduced early-stage malaria vectors in Tanzania. Nonetheless, AWD-15 across all trials did not cause any yield penalties and consistently reduced water use by 41-71%. Compared to continuously flooded fields, it produced less methane (by 41%) and even though it also produced two-fold more nitrous oxide, its yield-scaled global warming potential was still significantly less. In Côte d'Ivoire, forced drainage followed by intermittent irrigation produced more mosquito pupae but had higher water productivity whilst also having the lowest global warming potential.

6.5.2 Mosquito species composition

Results of the morphological and molecular mosquito identification revealed that in each study site, specific members of *An. gambiae* s.l. predominate in the early part of season.

In Côte d'Ivoire, *An. coluzzii* was observed early in the season and was replaced by *An. ziemanni* later in the season. This is consistent with other studies in West Africa, where the M molecular form of *An. gambiae* s.s. (i.e., *An. coluzzii*) was found exclusively in rice fields due to habitat segregation [248,276–278]. The succession by *An. ziemanni* in the late cropping season was observed in The Gambia, but not in Mali which saw *An. rufipes* and *An. funestus* [244,247]. Another vector that was present quite consistently earlier in the season, but in lower numbers, was *Cx. quinquefasciatus*, which is a potential arboviral vector. This was also found in studies based in neighbouring countries such as Burkina Faso and Ghana but also in Kenya [149,248,279–282].

In Tanzania, *An. arabiensis* was the only member of the species complex present in rice fields, which confirms Mutero et al.'s (2000) observations in Kenya [44]. Surprisingly, *An. arabiensis,* which was found early in the rice-growing season, was not succeeded by another anopheline but *Cx. antennatus* and *Cx. univitattus,* which can be arbovirus and lymphatic filariasis vectors. In other East African studies, *An. arabiensis* was often succeeded by *An. rufipes* or *An. ziemanni* rather than *Cx. antennatus* [46,246]. These species successions, which occur because the development of rice plants during a season changes the aquatic conditions of paddies (i.e., more shaded, cooler water), are important to take note of, especially if the latter species is also a prominent vector like *An. funestus*.

Generally, a subspecies of the *An. gambiae* complex dominates in rice fields on either side of the African continent: *An. arabiensis* in East Africa and *An. coluzzii* in West Africa. This illustrates that rice fields are exceptional niches to specialise in.

6.5.3 Land preparation

Land preparation in rice fields usually involves one round of irrigation for three to five days (pre-irrigation) before the soil is ploughed and harrowed (primary tillage). It is then followed by a second round of irrigation for two to three weeks before fertilisers are applied, and the soil is ploughed again (secondary tillage i.e. puddling) and levelled. This second round of irrigation is important for crop yields, as it is critical for seed and seedling establishment, weed control and nutrient availability. However, it is important to minimise vector production during the land preparation phase because it accounts for more than a fifth of vector productivity during a cropping season [Chapter 5].

Our study did not consistently demonstrate that flooding period during land preparation was important for larval control. This could have been due to the limitations explained in further detail later in the Discussion (namely underpowered trials). Nonetheless, based on basic mosquito biology, the duration of flooding should last no more than seven days to prevent mosquitoes from completing their development cycle. However, for rice yield purposes, it should also be long enough to create a layer of soft mud [283]. Since soil conditions vary across African rice-growing regions, more trials are required to determine an optimal duration of flooding during land preparation.

Only one other study had examined the effects of land preparation on malaria vectors and found that minimal tillage was important [184]. In Benin, Djegbe et al. (2020) observed that although levelling did not significantly affect mosquito numbers, minimal tillage (as opposed to deep tillage) was an effective larval control method. It was hypothesised that, compared to deep tillage, minimal tillage prevented mosquito development by avoiding the creation of deep depressions in the soil that allow stagnant water to accumulate [284]. The study shows that the techniques used during land preparation have an effect on mosquitoes and the effect of other techniques, such as the type of ploughing or harrowing during tillage, zero-tillage and laser land levelling, on mosquitoes should be explored.

6.5.4 Crop establishment

There has been an increasing shift from transplanted to direct seeded rice systems in sub-Saharan Africa. This is because direct seeding is less labour intensive and is considered a more sustainable strategy under adverse climatic conditions [285]. However, our study revealed that compared to transplanting, direct seeding was associated with 150-200% more *Anopheles* vectors. This can perhaps be explained by the differences in plant spacing and/or the duration that fields were irrigated and sunlit (Appendix 6.1B-E).

For the former, Freeborn (1917) had demonstrated that sparsely planted rice stands were associated with more *An. occidentalis* and *An. pseudopunctipennis* larvae than those densely planted [286]. However, an experimental trial in India observed the opposite effect: *An. subpictus* and *An.*

vagus immatures were greater in denser rice because denser rice may have been more attractive for oviposition and favourable for escape from predators [213].

For the latter, longer continuous irrigation in direct seeded fields could provide more cycles of mosquito breeding. As opposed to seeds being grown in nurseries for three weeks before being transplanted into main fields as seedlings, rice seeds are sown and sprouted directly in the main field. With three additional weeks of flooding with early-stage rice plants (seedlings), direct seeded fields could have inadvertently promoted oviposition through a longer period of "ideal" aquatic conditions (sunlit water with some vegetation) and promoted immature survival during this time due to lack of predation pressure.

The comparative effects of either explanation could be validated through further tests on plant spacing, different rates of broadcasting[17] and accounting for mosquitoes produced in nurseries. Other methods of crop establishment warrant experiments on mosquitoes, such as dry-seeded rice which does not require flooding and reduces methane emissions as well as machine transplanting which also reduces emissions by reducing cultivation time and improving water-use efficiency [287,288].

6.5.5 Fertiliser application

In modern intensive rice cultivation, fertilisers are vital to replace the nutrients that rice crops remove from the soil. Without the addition of fertilisers, crop yields and agricultural productivity would be significantly reduced.

Unfortunately, presumably due to previously mentioned issues with power and sampling, this study was not able to establish any associations between fertilisers and mosquitoes. This is evident from looking at results in Trial 4 (Table 6.4), where the observed 69.8% reduction in pupal abundance

[17] Note that both spacing and planting rate must be optimised for rice yield too.

(compared to plots with standard fertiliser application) was not significant. Nonetheless, it has been established in other field trials that there is a positive association between fertilisers and mosquitoes [Chapter 4]. Tests on different doses and types of nitrogenous fertilisers demonstrated that malaria vector abundance was greater in rice fields where higher doses and inorganic fertilisers (compared to organic manures) were applied [38]. In Kenyan fallow rice fields, more *An. arabiensis* were found in plots with ammonium sulphate than without [37]. There, it was speculated that fertilisers reduced water turbidity, making the aquatic conditions of rice fields more attractive for egg laying. A laboratory study revealed that adding chemical fertiliser into water with plant material increased the survival rate and development of mosquitoes, apparently by increasing adult emergence and by supplying the water with rich nutrients for larval growth, respectively [256,257].

Regardless, fertilisers are clearly necessary for optimal rice yield; our study showed that plots without fertilisers saw a 33% yield penalty. To overcome this, Mazigo et al. mixed fertilisers with larvicide *Bti* and showed that this approach reduced malaria vector abundance up to 67% and was also accepted by rice farmers [98,263]. Apart from this innovation, it is also important to explore the effect of different types of fertilisers, such as biochar and organic fertilisers (green manure, blue-green algae, and farmyard manure), and their application frequency and timing on mosquito vectors. Precision farming technologies that minimise fertiliser inputs, and thus can potentially reduce mosquito numbers, are of interest [289]. Since nitrogenous fertilisers potential impact greenhouse gas emissions, their effect on methane and nitrous oxide, especially, should also be investigated [290,291].

6.5.6 Water management

Akin to studies conducted in Asia, our study showed that alternate wetting and drying irrigation was associated with greater water productivity and lower global warming potential than continuous flooding in Côte d'Ivoire [34,86,292]. However, its efficacy as vector control was limited in Côte d'Ivoire. Our trials showed that even slight modifications of AWD-15, such as starting two days after transplanting rather than ten days and using the 30 cm mark as opposed to the 15 cm mark, were ineffective. On the other hand, compared to continuous flooding, AWD-15 was able to reduce 70.6% early instar *An. arabiensis* in Tanzanian rice fields.

Soil type and differences in their drainage time could explain these contrasting results. Whilst soils were heavy and largely composed of clay (>62%) in Tanzania, soils in Côte d'Ivoire were sandier (37-45%) and poorly drained [233]. In Tanzania, when clayey soils dried, they tended to shrink and crack, which facilitated strong percolation and resulted in rapid drainage within four to five days. Contrastingly, a drying cycle in Ivorian fields took around 28 days. This slower drying was highly favourable for mosquito survival. It allowed multiple generations of mosquitoes to develop with limited drying interruptions. It also allowed vector proliferation without much predation pressure: following a drying cycle, mosquitoes could re-establish themselves more quickly than their predators (which, by the time of their maturation, were killed by a drying period).

These results on AWD are consistent with previous entomological reviews on intermittent irrigation [27,81,225] [Chapter 4]. They are also similar to the results found in the systematic review and meta-analysis in Chapter 4 of this PhD thesis. Like our Tanzanian trial, Chapter 4 had shown that intermittent irrigation in rice fields provided successful late-stage larval control in some locations. But, similar to our Ivoirian trials, intermittent irrigation failed to reduce mosquito immatures in other locations. This was apparently due to poor soil drainage and uneven fields with pools of stagnant water that maintained mosquito development [27,81]. Thus, it may be most effective to pair AWD with land preparation or other rice operations that encourages levelling (rather than creates depressions e.g., operations that leave footprints) in paddies [184].

Seeing that AWD is being widely adopted across Southeast Asia because of its potential for climate change mitigation and adaptation, it is crucial to continue testing its efficacy in more sub-Saharan African locations with different types of soils, climatic zones and across more seasons. Locations appropriate for AWD can then be identified, and perhaps spatially modelled for its scaling.

6.5.7 Limitations

Our field experiments had several limitations. First, due to the high variability of mosquito populations, the variance between plots with the same "standard rice-growing" treatments in anopheline abundance was very high (~300-fold higher than within-plot variance, Appendix 6.5). Accordingly, significant differences could not be detected between various treatments despite large reductions. This is exemplified by Trial 4, where although intermittent irrigation of 3-day wet-dry cycles had reduced late instars by 45%, the p-value was 0.287. Although partially resolved by including plot as a random effect in the GLMMs, adding more plot replicates in the trial would have increased the statistical power. It was estimated from sample size calculations that sixteen plot replicates would have been required to observe significant 1.54-fold reductions in pupae[18] (Appendix 6.5). This is not only unfeasible for agronomists because of additional costs and labour but also does not add further information to their experiments. This illustrates the need for better trial design to accommodate the mosquito density outcome.

Second, mosquito sampling using dipping is labour intensive and limiting. Conducted twice a week across the entire season, it does not adequately capture mosquito density and dynamics in rice fields; with an aquatic cycle of around seven days, certain peaks in mosquito densities can be easily missed. It is speculated that older stages of mosquito immatures, especially pupae, also readily avoid capture. Moreover, certain treatments could skew sampling success using a dipper. For example, drying conditions during AWD-15 could create pools of stagnant water where larvae are concentrated and more likely

[18] By contrast, five plot replicates would have been required for early and late instar larvae. This may explain why more significant results were observed in larvae than pupae (Appendix 6.3).

to be captured when dipping, compared to the situation where the same number of larvae are dispersed over large expanses of water across a flooded paddy. Dipping is therefore subjected to wide user differences. Thus, monitoring methods that can better capture mosquito density and dynamics in rice fields are required. Methods that collect information on mosquito density more frequently, rely less on capture, and focus on accurate quantification, such as image analysis of larvae or sampling for environmental DNA[19], are options worth exploring [293–297].

Third, more parameters that help explain the effect of treatments on mosquitoes could have been included, such as the number of different types of aquatic predators and the number and size of depressions in the field (with residual water). However, the larvivorous nature of aquatic arthropods present in African rice fields must first be determined. Although soil dryness index was insightful, it was limiting as it was not as accurate as counts of potential breeding sites.

Fourth, the vector productivity of nursery beds was not accounted for. Although one-tenth of the area of the main field, its waterlogged nature can also generate mosquitoes (Appendix 6.1A). Fourth, the Tanzanian trial faced a few challenges. Due to logistical problems, tillage had occurred two months earlier than transplanting which led to poorly prepared soils. This, coupled with extensive dry spells during the dry season, led to rapid water loss and thus required excessive irrigation (almost daily) to retain water. Moreover, as the dry spells had severely reduced water levels in the river, sea water had occasionally drifted into the river/irrigation water, which led to reduced crop vigour and yield. Hence, these results may not be generalisable to other growing conditions. Nonetheless, since all plots (even the controls) were affected, the relative differences could still largely be discerned.

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[19] The capacity of environmental DNA (or other complementary methods) to differentiate between larval developmental stages must also be explored.

6.5.8 Implications and recommendations

Our study demonstrates that certain techniques practiced in rice cultivation can minimise malaria vector production. Their effects are variable according to location (and season) and hence require further trials. Strategically, this research and development task is best allocated to rice researchers, who regularly conduct such experimental rice trials when searching for improved yields. However, issues regarding trial design and sampling must first be resolved. In doing so, agronomists can then include mosquito abundance in their field trials[20] and identify techniques from each aspect of cultivation (from land preparation to weed management) that can contribute to larval control. When these individual techniques are combined, agronomists can recommend a set of rice cultivation practices that can sufficiently reduce mosquitoes throughout a cropping season.

Viewed through the lens of sustainable intensification, the climate and health co-benefits in agricultural interventions are not yet realised. Other than the techniques tested in this study, there is still a myriad of practices and technologies to explore. This includes, but is not limited to:

- rice-fish systems,
- nursery bed types,
- plant variety (e.g. shorter duration, height/canopy cover),
- pesticide application
- herbicide application,
- fertiliser application

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[20] Naturally, agronomists will prioritise rice yield. It will require extra effort to include mosquito density as another parameter in field trials. Nevertheless, it would be most ideal if mosquitoes are not excluded from the research and development task, as they have been for the last few decades.

- precision farming (e.g. drip irrigation, precision seeding, site-specific nutrient management),
- soil conservation (e.g. conservation tillage), and
- System of Rice Intensification.

Moreover, the effect of each rice input on mosquitoes needs to be better understood. Accordingly, these effects can be built into a comprehensive predictive model and amalgamated into existing models of water use, soil type, and greenhouse gas emissions to identify the most ideal combination of rice-growing practices that can reduce water consumption, greenhouse gas emissions, and vector production whilst maintaining or increasing rice yield [298]. Further to finding more "technical solutions", social science studies to encourage technology uptake by (local, small-scale) farmers should be also conducted [299].

Multi-sectoral issues are often treated as trade-offs. In this case, since rice development helps the economy, food security and farmer livelihood, it outweighs its undesirable environmental and health side-effects. This is misleading, because, as illustrated in our field trials, there are solutions that can avoid the harmful side-effects whilst still reaping the benefits. An outlook towards finding these kinds of solutions should be pursued, particularly those integrating vector-borne disease mitigation, in order to wholly fulfil the goals of sustainable intensification in agriculture. Interdisciplinary collaborations between health, environmental, and agricultural sectors must therefore be strengthened to work towards finding and implementing more win-win scenarios.

6.6 Conclusions

This study suggests that using alternate wetting and drying irrigation in rice cultivation can reduce greenhouse gas emissions and water consumption whilst maintaining rice yield in Côte d'Ivoire and Tanzania. Its efficacy against malaria vectors was variable and would require further adjustments. Rice cultivation practices other than water management techniques are equally important in contributing to mosquito density and should be explored. Overall, there is potential for a rice intensification strategy that can optimise both climate and health co-benefits.

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7 Rice farmers' knowledge and practices towards mosquitoes in irrigation schemes in Côte d'Ivoire: a qualitative study

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7.1 Abstract

Background

Irrigated rice cultivation in sub-Saharan Africa not only brings more malaria vectors to nearby communities, but also greater malaria risk. To aid the implementation of mosquito control in rice-growing communities, it is necessary to understand how farmers view their responsibility towards mosquito generation and whether they are interested in coordinating to minimise it.

Methods

Qualitative methods (observation grids, semi-structured in-depth interviews and focus group discussions) were used to reveal the perceptions of mosquitoes and their control in two irrigated rice farming communities in central Côte d'Ivoire near the M'bé and Lokapli irrigation schemes.

Results

All rice farmers viewed mosquitoes as severe nuisances, and most acknowledged that they caused *djèkouadjo* (malaria) and were less numerous during *harmattan* (dry season). Many study participants believed that mosquitoes originated from grasses and stagnant water around villages. Only those living closer in proximity (~1 km) to the paddies believed that mosquitoes came from the *bas-fonds* (irrigated lowlands). However, they did not associate mosquito production with rice cultivation. Some farmers believed that there were more mosquitoes in recent years than historically because of the dam construction but remarked on the importance of the dam (and *bas-fonds*) for their livelihood. Many farmers were not convinced that mosquito control could occur at farm-level.

Conclusion

To enhance accountability amongst rice farmers, there is a need for greater awareness on the rice-mosquito link, and emphasis that the link does not imply a trade-off between food security and health. Training should not only be directed towards farming communities, but also agricultural and health extension workers. Future riceland mosquito control methods must focus on improving productivity and address collective action problems that may occur.

Keywords

Malaria, Community perceptions, Rice farming, Irrigation agriculture, Côte d'Ivoire, Sub-Saharan Africa

7.2 Introduction

Malaria is a major health problem worldwide, where in 2020, there were an estimated 241 million cases [2]. Although nearly half of the world's population is at risk of malaria, sub-Saharan Africa (SSA) carries a disproportionately high share of over 95% morbidity and mortality. With around 7.5 million cases in 2020, Côte d'Ivoire is the 8th highest burdened country [2].

Malaria has complex associations with agriculture [221,300]. In SSA, it has major links with irrigated rice [221,300] [Chapter 3]. Rice paddies, due to their flooded nature, provide excellent and stable breeding sites for mosquitoes to thrive and proliferate [222]. Accordingly, compared to neighbouring non-rice-growing areas, communities located near irrigated rice cultivation are exposed to 6-fold higher adult malaria vector abundances and 2-fold higher malaria transmission [221]. In southwest Nigeria, it was estimated that rice farmers lose 10 days per year due to malaria, where a small proportion of farmers even indicated more than 20 days lost to malaria [301]. As a result, malaria influences agriculture too. Through the disruption of rice operations (labour loss), the inability to engage in intensive farming practices, and the high expenditures on malaria treatment, farmers achieve lower yield returns and less agricultural investments [96,302]. Thus, despite the advantages of developing water resources for agricultural purposes, these investments can have adverse effects on the health and physical, social, and economic wellbeing of households and, sequentially, their agricultural productivity [303]. This reinforces the need to control mosquitoes in agricultural communities so that farmer livelihood (and the overall development of the economy) is not hampered by malaria.

However, rice cultivation, especially irrigated rice, remains an important strategy across SSA to improve food security and keep up with ever-increasing consumer demands. Currently, there are goals in place for African countries to double rice production to 56 million tonnes by 2030 [25]. In Côte d'Ivoire, one of the priorities in the national rice development strategy involves the expansion of irrigated rice cultivation [304]. These strategies overlook the associations between rice and malaria. Whilst agricultural development agencies are actively promoting major rice expansion, health development agencies are planning for malaria elimination. This clash of equally important development goals necessitates methods of rice cultivation that can minimise mosquito proliferation. Methods of adult vector control such as the use of long-lasting insecticidal nets and indoor residual spraying near rice communities should be maintained, but they are neither permanent nor complete solutions [80]. There is instead a need for supplementary vector control methods such as larval source management, particularly through environmental management, to prevent vector production in the first instance.

Smallholder farmers constitute most of the rice production in SSA [305]. Thus, for any riceland mosquito control strategy to succeed, cooperation from all rice farmers in an irrigation scheme would be required. If a portion of rice farmers failed to adopt an intervention, mosquito production, although reduced, would not be eliminated. This is a case of the “n-person prisoner’s dilemma”, where collective participation of a new practice is a prerequisite for achieving a goal from which all individuals benefit [306]. Sometimes, individuals do not cooperate due to conflicting interests or in order to enjoy a “free ride”. This is related to a prominent and pervasive public health problem, the “collective action problem” [307]. Consequently, it is essential to involve rice farming communities in the process of designing and implementing potential control methods.

Heightened awareness on the link between rice and mosquitoes amongst farmers is also necessary. Rice farmers that are aware of this link seem to be more willing to adopt and practice farm-level mosquito control. In Rwanda, 92% of farmers recognised that rice cultivation contributed to malaria and were hence willing to spend 1-2 hours a week on larvicide (*Bti*) application [299]. Ingabire et al. (2017) also established in Rwanda that farmers that were knowledgeable about malaria, were involved in rice cultivation for less than 15 years and perceived rice farming as less profitable were more likely to contribute time to *Bti* applications [94]. Numerous studies have explored rice farmers’ knowledge, views and perspectives on malaria, its aetiology, its symptoms, and (adult) vector control practices [96,144,215,301,303,308–312]. However, these investigations were often limited to the simple acknowledgement that malaria was transmitted by mosquitoes. Except for a few studies, rice farmers' views and opinions on mosquitoes, their origin, and their links with rice were rarely investigated [92,94,95,216,313,314].

To aid the implementation of malaria vector control methods in rice communities, it is necessary to understand whether farmers are aware that their fields generate mosquitoes, whether they are concerned about it or feel any responsibility towards it and whether they are interested in coordinating to solve the problem. Thus, this study seeks to examine local rice farmers' knowledge and practices about mosquitoes and to determine if there are any existing or potential collective initiatives for malaria vector control in two rural rice communities in central Côte d'Ivoire.

7.3 Methods

7.3.1 Study area

This study was conducted in 2021 in two villages, Abokro and Bessérikro, 20-30 kilometres north of Bouaké, which is situated in the central region of Côte d'Ivoire. Abokro is a small village, deprived of electricity, of around 200 people [234]. The main economic activity of Abokro is irrigated rice farming with two cropping cycles, where they use the M'bé-1 dam and irrigation scheme. The village is situated around 1 kilometre away from rice fields (Figure 7.1). Bessérikro is a larger village of around 700 people and is part of a peri-urban town called Bamoro [234]. Its main economic activities are yam and rice cultivation. Bessérikro is situated less than 2 km away from neighbouring rice fields, but farmers' fields were usually located in another part of the Lokapli irrigation scheme, around 4 km away from the village. These two communities were purposely chosen because of their proximity to their rice growing areas, their local language (Baoulé), and their sociodemographic differences.

The two study villages are located in the equatorial transition climatic zone, where seasons are distinguished by a long rainy and long dry season [232]. The rainy season occurs from April to October, where rainfall reaches its maximum in June and September. The dry season occurs from November to March, marked by *harmattan* which is characterised by hot and dry trade winds blowing from the Sahara over West Africa. According to routine health service statistics, this study region had a malaria incidence of 166 cases per 1000 children under 5 in 2019 [315].

Figure 7.1. The two study sites and their corresponding dams and irrigation schemes.

7.3.2 Data collection methods

This study used a three-stage approach to cover different perceptions in each community: observation method, semi-structured in-depth interviews and focus group discussions.

7.3.2.1 Observation grid

Ethnographic immersions were conducted by ACKK for one month at each village. Observation grids, which are guides to remind the observer the topics of interest, were used to record information on rice cultivation and mosquitoes within domestic spaces and rice farms. The following aspects were recorded: behaviour towards mosquitoes, behaviours favouring or reducing mosquito proliferation (including mosquito control practices), sleeping habits, population movement, cultural practices with rice and cases of free-rider problems.

7.3.2.2 Semi-directed in-depth interviews

Qualitative semi-directed in-depth interviews (IDI) were administered to up to 25 rice farmers and/or their family members in each community. They were used to assess the beliefs, opinions, views, and perspectives of rice farmers on (1) rice cultivation methods, and their advantages and disadvantages, and (2) the following aspects about mosquitoes: (a) their origin, (b) their occurrence, (c) the severity of the problem in terms of nuisance and/or disease transmission, and (d) behaviours or practices perceived to favour or reduce their proliferation. The atmosphere and non-verbal behaviour made during each interview were also recorded. An interview guide is presented in the Appendix 7.1.

Participants were selected based on the level of compliance as well as observations noted during the ethnographic immersions. No distinctions were made with respect to gender nor age; any community members above 18 years old could be enrolled for interviews.

7.3.2.3 Focus group discussions

Three (more or less) homogenous groups of ten rice farmers, separated into women, men, and youth groups, from two villages were assembled for focus group discussions (FGDs). First, information on mosquitoes, as revealed by the IDIs, and views and perspectives of the general population's responsibility in mosquito production were discussed. Second, if a link between rice cultivation and mosquito production was correctly established, discussions on the existing collective practices were conducted as a community to solve this issue, the strengths, and weaknesses of said practices and reasons for their success or failure. Participatory action research (PAR) tools were used to aid focus groups in their exploration to improve existing collective practices against riceland mosquitoes. Alternatively, if a link between rice cultivation and mosquito production had not been established, PAR tools were used to raise awareness about the link and aid the groups in identifying actions that must be carried out collectively to solve the problem. The main PAR tool used was mapping, where the focus group describes the (physical features of the) territory they use and the resources they use for livelihood activities [316]. A supervising moderator was present to help direct discussions in case some topics were not well covered; probes for the FGDs are presented in Appendix 7.2.

7.3.3 Data analysis

Both IDIs and FGDs, conducted in the local language (Baoulé) were audio recorded with permission from interviewees. Recordings were then transcribed, translated to French and sequentially to English, and thematically analysed using NVivo (version 12). A coding framework was developed based on themes which emerged from the data, where the data from each participant were coded by the first and second author and discussed with the other co-authors. Key themes and their examples were then presented in vignettes and direct quotes.

7.3.4 Ethical considerations

The research protocols and procedures were ethically reviewed and approved by two bodies: The London School of Hygiene and Tropical Medicine (LSHTM Ethics Online ref: 22796) and Le Comité National d'Ethique des Sciences de la View et de la Santé du Ministère de la Santé et de l'Hygiène Publique de Côte d'Ivoire (The National Committee of Ethics of Life Sciences and Health from the Côte d'Ivoire Ministry of Health and Public Hygiene, IRB000111917). Informed written consent was obtained from each study participant. Study participation was voluntary, and each respondent was free to withdraw from the study at will at any time. Confidentiality was maintained by making the data accessible only to the members of the research team.

7.4 Results

A total of 43 participants were recruited: 25 in Abokro and 18 in Bessérikro. There were no refusals to participate. Quotes from participants are cited with fictitious initials to maintain anonymity.

7.4.1 Rice farming: characteristics and experiences

In both villages, all rice farmers (except one in Abokro) cultivated rice in irrigated lowlands, where 6 of 43 had their plots close to dams. Rainfed rice was cultivated near the river by the one exception in Abokro as well as four other farmers (3 in Abokro, 1 in Bessérikro) who conducted it alongside irrigated rice. Two-thirds of the participants cultivated other crops alongside rice, such as yams, cashews, maize, and market gardening (cucumbers, tomatoes, and okra).

Rice plots were an average size of 1.5 hectares in Abokro and 0.9 hectares in Bessérikro. They had been cultivating rice for an average of 11.8 years, ranging between a few months to 38 years, and usually conducted farm work with a mix of family and/or contract workers. Almost everyone grew the WITA-9 variety, but some also planted GT-11 and C-26. Farmers had previously tried other varieties such as BOUAKE-189 and ORILUX-6 but switched to current varieties because they were more resilient to insects, diseases, and the dry season (harmattan) and so produced greater yield (higher profitability). Some farmers mentioned that the variety they chose to grow also depended on seed availability and market demand. Two farmers from Abokro also stated that researchers from the neighbouring rice research institute AfricaRice "*advised [them] to stop using older varieties and recommended WITA-9*" instead.

When asked whether rice farming was difficult, only one participant disagreed: "*rice work is the work that nourishes the child – it brings money and allows [him]* *to send children to school*". Some respondents reasoned that it was not too difficult depending on whether one had enough means, i.e., money to purchase products and hire labour and machinery for ploughing. Most farmers (n=32) said that rice farming was difficult because of its costs and many requirements: machinery for ploughing, water control which has its extremes during dry and rainy seasons), inputs (herbicides, fertilisers, pesticides), and labour for transplanting and weeding (Figure 7.2). One farmer suggested that working with rice was a gamble:

> *"Yes, [it is difficult]. Sometimes, you do it and you come out with nothing. Other times, you win a little". - MAK*

Most farmers indicated ploughing as the main issue, as machinery availability was limited; the walking tractor often broke down and belonged to other villages of another ethnic group which tended to prioritise their own communities. Farmers were then resorted to using a *daba* (a traditional hoe) which tended to complicate transplanting and reduce yield. Some also stressed that the unpredictable nature of acquiring a tractor messed with the timing of nursery establishment and often required do-overs.

Amongst the rice farmers, the second most frequently cited issue was water availability. The dam used by farmers in Abokro was operated by AfricaRice, and so farmers lacked control and there was sometimes resistance in opening water channels during the dry season. In Bessérikro, respondents complained about water scarcity during the dry season due to poorly maintained canals; overgrown with grasses, these canals blocked water flow to rice fields farther away from the dam. Some disclosed that this issue created arguments between farmers whilst others pointed out that the president of the rice cooperative should organise these regular collective cleaning sessions. Many farmers did point out the imbalance in effort towards cleaning. The following quotations are illustrative:

"The water problem is like witchcraft. Even at midnight, I am still there…I block the water [flowing to other plots] so that the water goes to my fields". – MJLK

"There are some people who are difficult, who open their pipes and never close them. There are others who don't maintain their canals, so they are full of weeds. Every year we will clean up but when you mention it to them, they don't listen to you". – NKJP

"The president of the cooperative has to give orders for a time to clean the canals. We inform everyone but some do not go. You who are [far away from the dams] clean up properly for yourself but those who are close to the dam don't do it, so you must leave your fields and go to theirs to clean it for them…we don't love each other – it is wickedness!" – SNR

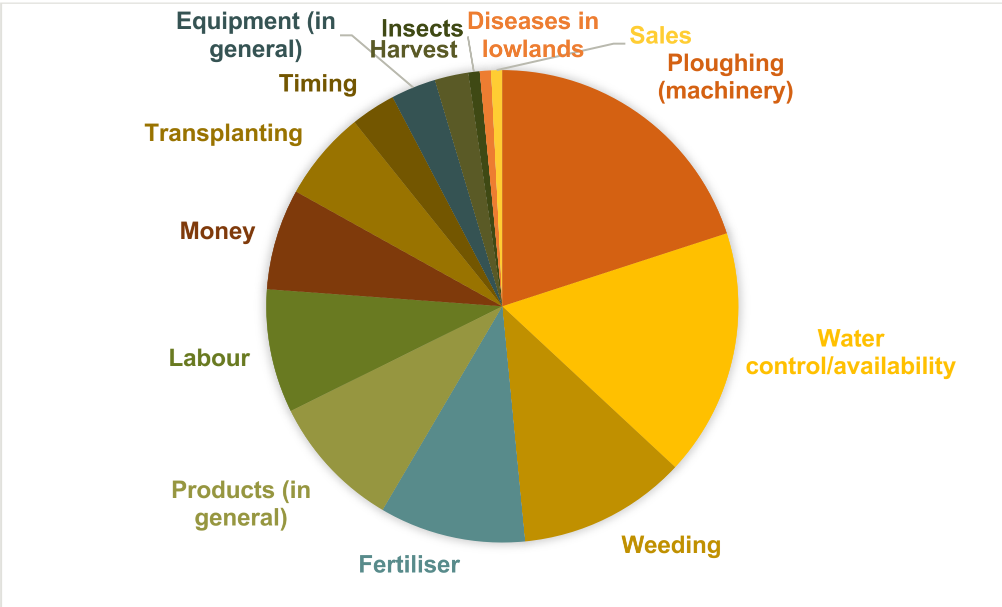

Figure 7.2. The perceived difficulties in rice farming.

In terms of the perceived disadvantages of living near rice fields, there were some differences between villages. Whilst most respondents from Abokro cited mosquitoes (14/25), sometimes together with the cold (6/25), many (10/25) also thought that despite them, it was an advantage to be closer to their workplace, saving on transport expenses. This was pointed out by one participant (MRCL):

> *"Living next to the* bas-fonds[21] *and the rice fields, our village has never lacked mosquitoes. We are always under attack. Even all the villages nearby call us the mosquito village, but the good thing is that we have easy access to the rice fields".*

A few farmers also cited that living near *bas-fonds* was inconvenient because domestic animals (goats and chickens) spoiled the rice fields and wild animals (rodents and birds) disturbed the village. When some respondents were prompted about health, the majority did not think that living near rice fields led to more illnesses whilst a few did, specifying Guinea worm disease[22], mosquito-borne diseases, and cancer. Only 4 of 25 participants from Abokro stated that it was not bothersome living next to rice fields.

On the other hand, in Bessérikro, more than half the farmers (10/18) declared that living near rice fields was not troublesome, many of whom said it would be more convenient. A third of the farmers still cited mosquitoes as a problem and a few mentioned that living near the *bas-fonds* could bring illnesses such as Buruli ulcer, malaria, and African trypanosomiasis. Two farmers did not perceive it as a danger to health, where MORY said:

> *"If it made us sick, we who have been in the* bas-fonds *since a long time would all have died".*

[21] *Bas-fonds* can be loosely translated to shallows, lowlands or wetlands but there is no English equivalent that gives the sense of low-lying valleys that are wet and marshy. They are suitable for, and often include, areas for rice cultivation and market gardening. Please refer to Appendix 7.3 for an image of a *bas-fonds.*

[22] Note that, as of 2013, Côte d'Ivoire was certified free of Guinea worm disease [351].

7.4.2 Mosquitoes: knowledge, attitude, and perceptions

7.4.2.1 Problems caused by mosquitoes

When asked if their village had any mosquitoes, all participants replied "*yes*", where a third of them added comments to the effect of "*in abundance / numerous / too much!*" and a few exclaiming or laughing in disbelief that the interviewer even asked such a question. Many farmers expressed without further prompt that mosquitoes were a significant problem mainly because they cause nuisance. They specified that mosquitoes disturbed sleep (which caused fatigue, weakness, and illnesses) through noise and/or bites, forced villagers to wear long sleeved clothing, jackets, and boots as personal protection, and prevented evening activities such as going outside, trade, and studying for children. Three respondents also mentioned that mosquitoes necessitated mosquito net use, which in turn were too uncomfortable or inconvenient to use. Most farmers (33/43) stated that mosquitoes, alongside nuisance, can lead to diseases such as *djèkouadjo* (malaria[23], n=26), zoonotic diseases (n=4), diabetes (n=1), AIDS (n=1) and anaemia (n=1), which led to treatment costs, hospitalisation, and death. When asked to rate mosquitoes against other common insects such as flies and bedbugs, mosquitoes were consistently ranked the worst by all respondents. The following quotations demonstrate what a few participants think of mosquitoes:

> *"When you don't use a mosquito net, they even go into your ears. You then hit yourself – can you sleep this way?" – KKP*

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[23] *Djèkouadjo* is the local folk name for malaria. A study conducted in another part of central Côte d'Ivoire observed that the main symptoms of djèkouadjo were fever, loss of appetite, headache, yellow eyes, yellow urine, and vomiting [215]. The main reported causes were the sun, mosquitoes, and God.

“If there were no mosquito nets, I would leave the village, that's the solution. I am so afraid of mosquitoes that if there were no nets, I would leave the village.” – NBL

“Yes, [mosquitoes are annoying] because we are not really free! We do not live peacefully. My body can’t stand the heat, I don’t like the heat too much, but if you don’t use mosquito nets, the mosquitoes will start biting you and you’ll get malaria”. – MRCL

“Mosquitoes are worse than working in rice”. – KOH

During the ethnographic immersion, it was recorded that villagers often complained about mosquitoes in the evening, where children sometimes cried because the mosquitoes were irritating them. Villagers also frequently went to bed early or ordered their children to sleep early (under the mosquito net) to avoid catching malaria. In the field, farmers were observed to rush home in the evening to evade the mosquitoes. Some farmers even claimed that the mosquitoes in the field were larger than those in the village.

7.4.2.2 Perceived origin of mosquitoes

Almost everyone across the two villages speculated that mosquitoes originated from neighbouring grasses or bushes (n=35), followed by wastewater (n=21) (Table 7.1). Following these two sites, the most common speculations differed by village. In Abokro, farmers attributed mosquitoes to *bas-fonds*, stagnant water, and the edge of water bodies (*bas-fonds*, dams, and rivers). Farmers referred to the *bas-fonds* mainly when they brought up their experience working in the fields in the evening:

“Even at night, you can’t go to the bas-fonds*, everywhere is full of mosquitoes”. – CGK*

“When you go to the field at night, the way the mosquitoes bite you are different from when you are in the village. In the village, they don’t bite you like that”. – NKJP

"Rice is a type of grass too – all the mosquitoes are in [the plants]. When you are there, there are mosquitoes hitting you, it's like you're getting stoned!" – SNR

In Bessérikro, mosquitoes were more often attributed to stagnant water, garbage, and rivers; *bas-fonds* were only mentioned once. In general, across both villages, several participants acknowledged the fact that mosquitoes can migrate, namely from the *bas-fonds*, dams, and rivers as well as grasses, wet and dirty places. Conversely, a few participants said that mosquitoes could not migrate from dams because they were too far away or that mosquitoes only came from within the village.

Table 7.1. The perceived origin of mosquitoes, enumerated by number of mentions in in-depth interviews.

Ranking	Abokro	Bessérikro
1	Grasses/bushes (20)	Grasses/bushes (15)
2	Wastewater (11)	Wastewater (10)
3	*Bas-fonds* (10)	Stagnant water (9)
4	Stagnant water (8)	Garbage (6) and river (6)
5	Edge of water bodies (6)	Dam (4)
6	Garbage (4)	Mangoes (3)
7	Forest (3) and dam (3), dark (3), humid (3)	Forest (2) and God (2)
8	River (2), animals (2)	*Bas-fonds* (1)
9	Agricultural ponds (1), ploughing (1), and God (1)	

Similar patterns in both villages could be seen from mapping conducted in FGDs (Table 7.2). In Abokro, the *bas-fonds*, dams and wastewater were perceived to be main mosquito breeding sites, whereas in Bessérikro, whilst rivers were identified as main breeding sites, rice fields often ranked lower.

Most participants were aware that mosquitoes developed in water. Rare exemptions included participants believing that mosquito development occurred in grasses (sometimes at the edge of water) and that mosquito development was only favoured by water or humidity.

Table 7.2. The perceived origin of mosquitoes as ranked in FGDs.

Ranking	Abokro			Bessérikro	
	Women	**Men**	**Youth**	**Women**	**Men**
1	*Bas-fonds*	Dam	*Bas-fonds*	Dam	River
2	Dam	Wastewater	Shower water	River	Rainy season
3	Toilet & shower water	*Bas-fonds*	Forest	Toilet & shower water	Grasses/ bushes
4	Dirty water	Stagnant water	Grasses/ bushes	Grasses/ bushes	Stagnant water
5	Garbage	Grasses/ bushes	Toilets	Garbage	Rice fields
6	Grasses/ bushes	Rainwater	Small water collections	Mangoes	Dry season
7			Garbage	Totems*	Mangoes
8			Dirty clothes	Small water collections	Toilet water
9			Maize	Rice fields	
10			Totems*		

*Totems refer to divine retribution from God for the disobedient who have trivialised ancestral practices and prohibitions.

None of the respondents stated that there is a link between mosquitoes and rice cultivation. When probed, the majority (n=30) of the participants did not believe that a link existed. About half (n=14) of them specified that mosquitoes were linked to *bas-fonds* but not to rice itself (since rice fields are a part of the *bas-fonds*). One farmer believed that mosquitoes could not survive in rice fields because agricultural insecticides are regularly sprayed. Many farmers also explained that mosquitoes were present even when rice was not being cultivated in the *bas-fonds*. Similar comments were gathered across all five FGDs; mosquitoes came from water in the *bas-fonds* but not the rice (since sometimes there is no water in the rice fields, or since there are still mosquitoes in urban areas where there are no rice fields). 11 farmers agreed that mosquitoes were associated with rice, where one mentioned that mosquitoes were associated with ploughing, another with transplantation and some thought that since it resembled grasses, rice could attract mosquitoes. The following statements capture the beliefs of a few farmers on the link between rice and mosquitoes:

"I say it is the water at the bottom of the rice that attracts mosquitoes, but the rice [plant] will not attract mosquitoes". – GNM

"Rice can't bring mosquitoes. When they came to raze this forest, we weren't making rice yet, the mosquitoes were already there at the time. They have always been here, but they were not as numerous. Rice cannot bring mosquitoes because even when you harvest, mosquitoes are still around. We know that rice does not cause mosquitoes. There are times when there is no rice in the bas-fonds, but the mosquitoes are still there. So, rice can't be a problem." – SYP

"If you stir the rice, you will see the mosquitoes fly away. Going to the fields at night – it is not worth it. If you want a tonne [of mosquitoes], you can find it". – MORY

"We can't also say that we don't cultivate rice anymore because the mosquitoes are going to kill us. What are we going to eat? …Thanks to the fields, we can harvest a little rice for children to get educated and a little for us to eat. It's not easy (laughs)!" – MRCL

7.4.2.3 Occurrence of mosquitoes

When asked about the timing of mosquitoes during a 24-hour period, almost all respondents said that mosquitoes started arriving to the villages between 18.00 h and 19.00 h in the evening and were present until dawn and morning. Some participants observed on day-biting behaviour that occurred inside homes or "*sitting in a dark area*".

When asked about the timing of mosquitoes annually, all farmers discerned that mosquitoes were not as numerous from December to February (harmattan) but were in abundance from March through to October (the rainy season). For the latter, respondents often also associated mosquitoes with heat, grass, and the mango season. Whilst many participants speculated that burning bushes during harmattan kept mosquitoes away, many also thought that the wind and cold did. Overall, however, none of the respondents said that there was a period within a year without mosquitoes:

> *"A time without mosquitoes does not exist! Even during the harmattan period, they decrease but they are there. When you sit down, there are two or three that bite you."* – *SYP*

When asked about the historical differences in mosquito abundance in the past few decades, 23 participants said that there were more mosquitoes today (that "*when they were younger, they could sleep without mosquito nets"*), 11 said there were more in the past and 5 said that there were no differences. Of the 23 rice farmers that believed that there were more mosquitoes currently, 9 attributed it to the construction of a nearby dam, 5 to the presence of more grass, garbage, and wastewater and 3 to the loss of traditions (that since Christianity, the community no longer followed ancient laws and therefore the village is now paying the price). Two farmers also referred to the fact that there was more mosquito control in the past such as aerial insecticide spraying, and mosquito traps placed around the village. The following quotations summarise their thoughts:

> *"I myself cannot understand. Our parents told us that the mosquitoes weren't around too much before. But we do not understand why there are so many mosquitoes. You see, before the neighbouring villages said that mosquitoes were abundant in Abokro but nowadays there are mosquitoes in all these villages. Mosquitoes are everywhere now. Our [matriarchal] village, which is far from here, there are mosquitoes there. Except for houses that are in town where there is light and an air conditioning system. When the house is air-conditioned, they can't stand the cold and they go out to go elsewhere."* – *REMI*

> *"They used to have a time, but now they don't have a time, mosquitoes bite us all the time."* – *KOH*

“People before respected the [traditional] prohibitions, but now no one respects them.” – RLND

“[The mosquitoes] were there before, but when the dam was created, it seemed to attract them more. Otherwise, they were here before, it is not because of the dam that they are here.” – GERO

With regards to the dam, a few farmers deliberated the dam’s importance for their livelihood:

“It was when they set up a dam that [the mosquitoes] became a lot… Dams can bring a lot of mosquitoes. Though without it, we can't eat. How are we going cope? We just have to live with [the mosquitoes]!” – MENA

“Yes, you can say it's because of the dam because before the construction of the dam, the mosquitoes were there, but they didn't tire us that much. Because of the dam, nowadays, they reign. But, as we say, it is the dam that gives us food – in the past, we were making yams, but they failed. When they created the dam, we have used it to make rice and it gives us food.” – TCHD

“The dam can also cause [more mosquitoes] because it is the water that causes mosquitoes to bite people. But since it is something that feeds us, we cannot say that we will stop [using the dam] because of the mosquitoes.” – KOH

“Mosquitoes have been here a long time. We don't know what sent them. When they made the dam, the mosquitoes multiplied. Now they sting much compared to the past… Because the dam is close to us and we are on the edge of this big bas-fonds there and there are also small bas-fonds around the village and when the rainy season comes and the water stagnates everywhere, mosquitoes come in force. It's the stagnant water everywhere that matters, but

without water we can't do anything. If we don't want the water to stagnate too, what are we going to eat? It is because of the shallows that mosquitoes have the strength". – Man from Abokro FGD

However, one or two farmers believed that recent mosquito proliferation was not because of the dam:

"They made the dam in 1997-98 so, I can't say it is because of the dam. Because the mosquitoes started to tire us long before we started the dam." – NKJP

Of the 11 respondents who said that there were more mosquitoes in the past, 4 rationalised it with the current distribution of mosquito nets, 3 with the fact that the nets are now impregnated with insecticide and 3 with how the village had grown in size so mosquitoes are spread across more community members, or their house was no longer at the edge of the village. Overall, a quarter of the participants also observed that there were no years without mosquitoes. These two quotations summarise their thoughts:

"Mosquitoes abound! A year without mosquitoes – it hasn't happened yet. They are here all the time!" – KRST

"Never!! Since I arrived, there has never been a year where the mosquitoes have not come. They are still around. The period which we have a little respite is during harmattan. But to say that they are totally gone – that does not exist." – SYP

7.4.3 Mosquito control practices

7.4.3.1 Household-level

At the household level, everyone said that they used mosquito nets. Many even observed that compared to other practices, they were the most effective against mosquitoes. Insecticidal sprays, *Timor* or *Rambo*, were popular (n=24) but many farmers claimed that they could only "*calm mosquitoes down a bit"* and bought them only if they had extra money. The effectiveness of these sprays (often in comparison to nets) is expressed in the following statements:

> *“Timor is also good because it drives away mosquitoes automatically... It is the mosquito net that is more effective, because even if you pump the Timor they will come back. But with the mosquito net, they do not have access for entry”. – LKG*

> *“Yes, [Timor] calms [the mosquitoes] down a bit, they go and then afterwards they come back again. But before the product we use to treat cotton there when you spray it, it kills certain insects. But now it just drives them away – it doesn’t kill them anymore”. – KKP*

> *“The mosquito net comes first. Because with it, and even when you don't have Timor, you can go back to sleep easily. But if you only have Timor, it's over for you...(laughs).” – KKM*

To avoid mosquitoes in their home, 12 participants also used coils (*Moskito*), seven regularly cleaned their house and yard and five ensured that their house was well constructed without many window, door, and roof openings. One farmer in Abokro suggested that having electricity for light and air conditioning would help control mosquitoes. There were slight differences in household mosquito control between the two villages. In Abokro, most farmers used both nets and insecticidal sprays, and sometimes coils, but at the same time, were not convinced of the sprays’ efficacy. In Bessérikro, most participants only used a net and cleaned their home.

From the ethnographic immersion, the field observer noticed that during evening activities, villagers regularly swatted their legs with their hands or a piece of cloth, put their feet in bags, shared mosquito coils amongst each other or used a torch to kill mosquitoes. House screening was also installed in some homes, using tarpaulins or traditional cloth.

7.4.3.2 Village-level

At the village level, the majority of the participants (n=30) and discussions from the focus groups asserted that the village needed to be cleaned to control mosquitoes. Specifically, is entailed removing or burning grass, removing garbage, and removing stagnant water and wastewater (from toilets, showers, and cooking) by making pits and closing their opening using slabs or by building proper showers. A few participants indicated that village bush clean-ups should be organised by the youth president or that they would require machinery for ease. Compared to Abokro, these sanitation programmes had been less successful in Bessérikro due to disputes over the distribution and ownership of land. Most farmers therefore resorted to conducting clean-ups individually and occasionally (rather than once a year), using machetes or herbicides.

Following village clean-ups, the most common answer was that there were no solutions (n=11), where a few farmers claimed that cleaning did not effectively reduce mosquitoes. Five farmers suggested spraying the village with insecticides but made it clear that it was only a temporary solution and labour intensive. A few participants said that the government needed to help by sending insecticidal products or aerial spraying ("*the entire country even*"). In an FGD between female rice farmers in Abokro, it was established nothing had been done to reduce mosquitoes because the village had "*no money to hire a manager from town to reduce mosquitoes".* The following quotations encapsulate many participants' suggestions:

> *"The village has to be clean, when the village is clean and they don't know where to land, where to breed, there won't be many of them there". – KASS*

> *"We have to get together and decide to resume [cleaning] in the village because of the mosquitoes." – KANG*

> *"What we can do is respect each other – so that we can all work together to make the village clean… But there are many stubborn people that do not respect the laws of the village". – IVN*

> *"I don't see what we can do to reduce mosquitoes… Maybe it's products [the state is] going to send us, otherwise I can't see." – TCHD*

> *"Because even if we spray the insecticides, we can't spray the whole village." – PIT*

Several participants from IDIs and FGDs also explained that the community had not actioned on reducing mosquitoes because they were paying penance for breaking traditional laws.

7.4.3.3 Farm-level

At the rice field level, a third of the respondents did not observe links between mosquitoes and the *bas-fonds* or rice fields and hence did not reach this topic of conversation. Regardless, most farmers (n=17) were not convinced that anything could be done to reduce mosquitoes in the *bas-fonds,* sometimes referring to personal protection (through long-sleeved clothing) or village-level control:

> *"Over [in the field], we can't do anything because the terrain is vast. There I do not see a solution because it is not possible to work until late… I say we can't do anything because we [farmers] are not in contact every day. But we who are in the village together are always together so we can do something". – CGK*

> *"We can't do anything because rice is like grass, [mosquitoes] hide in it, so if we don't stop growing rice, we can't do anything to reduce mosquitoes." – KOH*

> *"At the level of the bas-fonds, we do not have any solutions yet because we do not see a solution, since if the bas-fonds themselves are not there, it is difficult to eat. Currently rice feeds people – yams are no longer successful, and it is thanks to the*

bas-fonds that we can eat. Maybe it's the dam, but if we stop the dam, we can't eat". – Young person from Abokro FGD

Seven farmers proposed insecticide application but similar to household-level observations, many were doubtful of how effective and manageable (i.e., daily application) chemical control would be. One statement that encapsulates this was by MORY:

"No, [we can't do anything]! there is no solution for this, I can't buy Rambo to spray the rice… I also can't take the mosquito net to cover the rice!".

A few farmers, in both IDIs and FGDs, said that if they were shown or given the appropriate insecticides by the state, that they would do it:

"No [we cannot reduce mosquitoes in rice fields], except if the state shows us drugs to reduce them". – KRST

"Frankly if we can do something there, I don't know, maybe you can show us, and we will do it." – KYS

"If someone presented a solution to us, it would be good – on our own, we cannot find the solution". – Young person from Abokro FGD

One-off suggestions, that were always underlined with doubt, included upland rice cultivation and using drip irrigation and greenhouses:

"I think that if we have a possibility of cultivating rice on the plateau or with ramps and pipes connected to water. Once watered, the earth is wet, but the mud does not stay because the sun is beating down. The water disappears but the humidity remains. Apart from that, if we always cultivated in the bas-fonds, I don't think we can reduce the rate of mosquitoes". – MRCL

"Maybe in a greenhouse… Except that...if [rice is grown] in the open air, it's inevitable…Or the rice on the plateau but, for one, I haven't mastered it and then two, I don't think our land is fertile enough". – DKB

7.5 Discussion

This study aimed to investigate rice farmers' views, knowledge and practices towards mosquitoes and to search for potential collective riceland vector control initiatives in two rural communities in central Côte d'Ivoire. Most respondents found rice farming complicated because of its prerequisite costs for labour, machinery, inputs, and water, but not necessarily because of the nuisance or diseases caused by mosquitoes. Nonetheless, rice farmers were very familiar with mosquitoes and acknowledged that they caused *djèkouadjo* (malaria) and were less numerous during *harmattan* (dry season). Many farmers believed that mosquitoes originated from grasses or bushes and wastewater within and around villages. Only rice farmers living closer in proximity to the paddies thought that mosquitoes originated from the *bas-fonds*. Despite this, respondents did not identify a link between rice cultivation and mosquitoes; some specified that the rice plant itself did not bring more mosquitoes. Most respondents believed that there were more mosquitoes in recent years than historically because of the construction of the dam, as well as the occurrence of more bushes, garbage, and wastewater. Still, respondents deliberated the importance of the dam and *bas-fonds* for their livelihood and hence, were not convinced that there were solutions to control mosquitoes at farm-level.

Rice farmers in these two Ivoirian communities were knowledgeable about mosquitoes. They were aware that they transmitted malaria, resembling findings reported in rural rice-farming communities in other parts of sub-Saharan Africa, including another area of Côte d'Ivoire, Ghana, Benin, Rwanda, Kenya, and Tanzania [92,94,215,216,313,317]. Respondents accurately recalled mosquito biting patterns, identifying peak times within a day (from dusk until dawn) and a year. As remarked in other studies, farmers stated that there was a year-round presence of mosquitoes but significantly fewer during the drier periods of a year (i.e., *harmattan* in West Africa) [92,317]. This corresponds to the fact that *harmattan* is a period where rice is not grown because of the harsh, drought-like conditions, but this study could not directly confirm this because interviews did not explicitly ask the times of rice inactivity. The familiarity of mosquitoes amongst rice farmers alongside the distance of their homes from paddies was correlated with the mosquito control adopted at a household level: farmers from Abokro, which was ~1 kilometre away from the rice fields, used mosquito nets and insecticidal aerosol sprays whilst farmers in Bessérikro, which was more than 2 km away from rice fields, often used only a bed-net and cleaned vegetation around their homes. Farmers in Bessérikro also rarely combined bed-net usage with insecticidal sprays or mosquito coils. Otherwise, these methods of vector control have been observed in numerous studies on rice farming communities: it is apparent that since Essé et al.'s study in 2002, mainstays of vector control in central Côte d'Ivoire have not changed [215,314,317].

Perceptions on historical and current mosquito density were mixed amongst rice farmers in both villages. Whilst the majority attributed recent mosquito increases to nearby dam construction in the last 20-30 years, many participants also attributed them to the presence of more village wastewater and loss of traditions. Traditional/mystic factors (i.e., God or ancestors) were often the believed causes of diseases such as malaria, but this is the first known instance that mosquitoes were also viewed as divine retribution for disobeying or trivialising ancestral practices and prohibitions [215]. These opposing perceptions of increased mosquito incidence could be explained by two reasons. First, before wetlands are converted into irrigation schemes, they also generate many mosquitoes [42,138]. Thus, rice communities may not have detected a significant difference in mosquito densities before and after wetland conversion/rice cultivation and hence did not attribute it to recent developments in agriculture. Second, recent efforts to universally distribute insecticide-treated nets could have been a confounding factor; many participants rationalised that they felt more mosquitoes in the past because of increased protection today from insecticide-treated nets. Conversely, net usage could also have "flattened" mosquito biting peaks, shifting biting to early evening or late morning and causing an increased perception of their presence.

In this study, most rice farmers believed that mosquitoes came from the bushy environment and residual pools of stagnant water in villages. When we sought for statements about the link between rice and mosquitoes, most respondents instead acknowledged that the *bas-fonds* (and not the rice fields) contributed to mosquito proliferation. This was surprising because numerous qualitative studies in both East and West Africa have revealed that rice farmers are aware of the impact of irrigated rice cultivation on mosquito production, although some place emphasis on the open canals rather than the rice fields [92,94,95,216,312,313,318]. In Mlozi et al.'s (2006) study in Tanzania, farmers reported that continuous mosquito breeding was favoured by the following factors: growing rice in bunds (which retained water for long periods of time), poor drainage, and spacing between plants [319]. The findings in our study are similar to those of Benin. Djegbe et al. (2020) found that despite 94% of rice farmers recognising stagnant water as breeding sites, only 4% correctly identified rice fields as potential contributors to mosquito production [317]. This means that when spreading awareness or re-educating farmers on this topic (particularly when there are clear mitigation measures to promote), there is a need to distinguish between the different types of stagnant water and the types of mosquitoes[24] associated with them [320]. More attention towards the most productive types of stagnant water for malaria vector breeding is required. Specifically, the differences between *bas-fonds* and rice fields (which is a subset of *bas-fonds*) must also be emphasised. Whilst water bodies in wetlands

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[24] A simple classification scheme can be used to distinguish the types of mosquitoes and their associated breeding sites. Farmers can be trained that, for example, clean and sunlit water (puddles, pools and rice fields) are suitable for malaria vectors like *An. gambiae* s.l. whereas polluted water (pit latrines, wastewater) are ideal for nuisance biters like *Culex* mosquitoes.

or lowlands are responsible for some *Anopheles* production, it must be highlighted that water from within rice fields are the bigger contributors of malaria vector breeding.

The main limitation of our study was the use of double translations (from Baoulé to French and French to English). The questions that were asked in interviews were not back-translated, which could have helped researchers compare translations for quality and accuracy. This can be problematic as nuance is important in these circumstances. This issue could have been reflected in questions regarding where mosquitoes came from, as it may probe for where mosquitoes fly from, rather than where they may breed. Another limitation of this study was the potential participant bias in asking rice farmers about their views and perspectives on mosquitoes. Although questions were framed open-endedly in order to minimise such bias, farmers could have denied acknowledging links between rice cultivation and mosquitoes (and malaria) based on morality or social acceptability.

Our study illustrated that when farmers were uninformed of, unconvinced of, or indifferent to the link between rice cultivation and malaria, many believed that that living with mosquitoes was inevitable. Other farmers viewed the problem as a trade-off between their livelihood and malaria and preferred to suffer the health consequences. Hence, the majority of participants doubted that there was a solution, mainly deeming their existing control methods (environmental management, mostly referring to weeding operations) too ineffective, temporary and/or labour intensive. This was also seen in Kenya, where rice farmers did not apply known vector control methods (e.g., draining stagnant water and clearing vegetation along water canals) due to perceived lack of effectiveness and lack of time to apply [314].

Conversely, once farmers were aware of the rice-mosquito link, they were more motivated to change cultivation practices to minimise mosquito production. This was observed in a handful of respondents from this study who suggested adopting drip irrigation, upland rice cultivation or rice cultivation inside greenhouses. In another qualitative study in Tanzania, farmers seemed not only to take responsibility towards the problem but were highly motivated in solving it [319]. They expressed dissatisfaction towards the government (including agricultural extension workers) for failing to provide them the necessary education to grow rice without intensifying malaria transmission, such as such as use of *Azolla*[25] and intermittent irrigation. In another study in Tanzania, fertiliser-*Bti* mixtures were well-received by rice farming communities who were aware of their impact on mosquitoes. Farmers perceived that the reductions in mosquito densities in their farms (following *Bti* application) enabled extended working hours and that there was a reduced risk of contracting malaria within their household [98]. In this setting, rice farmers were keen to scale up the intervention in terms of area and intervention; they did not think that it was challenging to prepare and apply the mixture (where some reported increased yields) and were willing to contribute to paying for the mixture [98]. It appears that different communities perceive this rice-mosquito issue differently and a variety of approaches can motivate farmers to take up modified rice-growing and mosquito-minimising methods but in general, improvements on rice yield is the largest determining factor.

Since farmers were unaware of the significance of the rice-malaria link and therefore did not propose many methods of rice growing that could minimise mosquito production, this study was not able to uncover direct instances of collective action problems in riceland mosquito control. However, collective action problems were salient in two observed affairs: bush or weed clearing operations at village-level as mosquito control[26] and at field-level to allow

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[25] *Azolla* is also known as the mosquito fern. It acts as a biological fertiliser for rice plants and can potentially control mosquitoes breeding in rice fields.

[26] Throughout rural Africa, bush-clearing in the vicinity of houses is by far the most common community-based malaria control effort. Almost everyone accepts, as a fact, that it is not only good for neatness and hygiene but specifically effective for malaria control. By contrast and based on mosquito biology, medical entomologists state with equal confidence that bush-clearing does not and cannot work. Some evidence of its efficacy has been illustrated in a small trial by Ribbands (1946) [352].

equitable water distribution for rice cultivation. In both instances, communities, in theory, co-operated in “sanitation programmes” to achieve a “common good”. However, in practice, these programmes were often never launched, due to lack of initiative in their leaders, or were unsuccessful. They did not succeed either because of insufficient communication amongst community members (especially amongst rice farmers from different villages who shared an irrigation scheme) or “free-riders”. Individuals then often resorted to

a) Act in their own self-interest but not achieving community goals (e.g., cleaning around their own peri-domestic area),

b) Compensate for the “free-riders” (e.g., removing vegetation in the canals for those who did not), or

c) Defect further (e.g., farmers visiting the *bas-fonds* at night to divert water to their fields).

Ultimately, farming communities are unable to reach their common objectives. Particularly with regards to water shortage problems in rice cultivation, these collective action problems have led to farmer conflict [321]. Both cases of n-person prisoner’s dilemma are illustrative of potential issues in organising farm-level riceland mosquito control: community control may not be achieved if leaders do not show initiative, free-riders exploit the system, and/or if farmers in a rice cooperative are difficult to assemble when they come from different villages.

It is demonstrated that technical solutions (i.e., modified rice-growing methods that minimise mosquito production) must be designed with collective action problems in mind [322,323]. Perhaps lessons could be learnt from the rice sector’s approach in climate change mitigation. Like malaria vectors, greenhouse gases are harmful emissions that are produced as a side-effect of rice irrigation. In both cases, this happens with little or no awareness on the part of the farmers. Yet, rice-development agencies have been able to scale-up a modified rice cultivation practice, alternate wetting and drying irrigation, amongst farmers in Southeast Asia [324]. Their strategy to facilitate collective action is worth learning from.

7.6 Conclusion

Rice farmers in this part of central Côte d'Ivoire were generally not aware of the link between rice cultivation and malaria vector production. When clear mitigation measures based on agricultural techniques are ready to be adopted, education and training about the rice-mosquito link should not centre around rice farming communities only, but also agricultural and health extension workers. This can come in the form of farmer field schools and training on integrated pest management combined with integrated vector management, as trialled in Sri Lanka [325–327].

First, they should be taught that whilst certain types of stagnant water do generate mosquitoes, specific types (such as rice fields) particularly encourage mosquito proliferation due to the ideal aquatic conditions they present (fresh, clean, sunlit, shallow water with some vegetation) [Chapter 5]. These conditions are most apparent during the first few weeks after transplanting occurs [222]. Second, as pointed out by Djegbe et al. (2020), farmers and extension workers should be able to recognise mosquito larvae [317]. Third, since farmers did not seem fully informed that mosquitoes could travel from dams or rice fields farther away, they should be taught on migration (i.e., that mosquitoes can fly far distances to find bloodmeal sources). Fourth, regular cleaning of canal vegetation should be emphasised, not only in order to maintain a continuous flow of water (which is unattractive for malaria vector breeding), but also to even out water inequalities amongst fields. Fifth, it is important to emphasise that this association between rice and malaria does not suggest inevitable trade-offs between food security and human health. Instead, this association encourages the agricultural sector to take into account of the malaria vectors produced by rice. It encourages the development of modified methods of rice cultivation that can produce good yield, can minimise mosquito proliferation and can eventually be recommended as "good crop husbandry" to farmers [319]. Finally, there needs to be additional effort to avoid free riders in collective actions such as riceland vector control.

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8 Discussion

This chapter gives a synopsis of the main research findings from each chapter (3-7):

1. Nowadays, rice areas bring more malaria, and, in the future, they will become a problem in elimination settings
2. Peak vector productivity occurs in the first few weeks of the rice-growing season
3. Modified rice cultivation practices, including alternate wetting and drying irrigation, can control malaria vectors in some settings
4. Rice farmers in central Côte d'Ivoire were not aware of the rice-mosquito link but were willing to participate in interventions

It also describes the strengths, weaknesses, and conclusions of this work. The implications of these findings for the African agricultural and health sectors, alongside the future directions for riceland malaria vector control in sub-Saharan Africa, are discussed:

- More evidence on the effect of rice cultivation on malaria risk
- The agricultural sector should take the lead
- Better rice-field mosquito sampling methods
- Complementary sets of interventions to achieve riceland mosquito control throughout a cropping season
- Promising interventions: exploring all steps of rice cultivation
- Advocating collective participation of rice farmers

8.1 Overview

Rice fields are a major breeding site for many species of mosquitoes, including vectors of diseases such as malaria. In sub-Saharan Africa, they are capable of producing high densities of the most efficient malaria vector, *Anopheles gambiae* s.l. [48]. This is concerning because it suggests that human populations living near rice fields might be exposed to greater malaria risk.

However, it was established in the early 2000s that the extra malaria vectors in rice-growing areas did not necessarily increase malaria incidence in humans – this phenomenon was named the "paddies paradox" [62,63]. In light of the recent changes (last twenty years) in the malaria situation in sub-Saharan Africa, specifically the increased equity in intervention coverage and significant reduction in malaria transmission, this phenomenon required a re-assessment (Chapter 3) [2,7,78]. Moreover, as African countries reach pre-elimination conditions, it is predicted that rice fields (especially irrigated and rainfed lowland rice) will become hotspots for malaria. This has been observed in other elimination settings where the dominant malaria vectors bred in rice fields: targeted vector control was required to achieve malaria elimination in numerous countries like Portugal, Turkmenistan and China [169,171,172,266].

However, rice production in sub-Saharan Africa has been on an upwards trajectory; rice is the fastest growing food staple in the region [20]. Due to population growth, urbanisation, and changes in consumer preferences, demand for rice has been growing at more than 6% per year [25]. Africa has more than 100 million hectares of inland valleys, of which currently less than 10% is being cultivated for rice production [26,328]. Thus, the expansion of rice, particularly irrigated rice, has been heavily promoted by African Ministries of Agriculture and international development donors to secure food and nutrition across the region [329]. Unfortunately, this increased development in rice-growing areas can generate great numbers of malaria vectors.

In a rice community setting, core malaria interventions, such as insecticide-treated bed-nets (including PBO-synergist nets), antimalarial drugs and health services, are already working at full stretch but can only deliver a partial and incomplete solution. Whilst many gaps in coverage remain to be filled in rice (and non-rice) communities, the objective should not be to recreate poverty-related inequities between rice and non-rice villages (as occurred in the paddies paradox). Rather, the objective should be to promote equity: not only equity in interventions but also equity in exposure to malaria vectors. There is currently too much complacency about the inequities that rice creates. Therefore, there is a need to consider the potential role of alternative methods of vector control that can reduce the amount of additional mosquitoes produced by rice [330]. These include larval source management (LSM), especially in the form of environmental management; there needs to be less reliance on insecticide-based vector control due to its growing resistance [331].

Medical entomologists have been investigating methods of LSM within rice fields since the 1930s and found both positive and negative effects [210]. Narrative reviews written in the 1990s indicated that despite numerous studies, there were still major knowledge gaps in understanding what works, when and where [48,80]. This is still the case. Moreover, since these reviews were done in the 1990s, they are now out of date. Thus, an updated systematic evaluation of the effectiveness of riceland LSM is required (Chapter 4). Whilst insecticide-based interventions (e.g., chemical and biological larvicides) are of interest, it is particularly beneficial to determine whether rice cultivation practices can be altered to reduce malaria vector densities. For this reason, a more detailed understanding on the spatial and temporal distribution of malaria vectors in rice fields under “standard” operations is necessary (Chapter 5).

The key point is that rice cultivation is a man-made process, and its cultivation methods can be modified with the purpose of avoiding the production of mosquitoes [4]. Agriculturalists need to take more responsibility of their harmful side effects, even if these effects were unintended. Rather than being part of the problem, they can become part of the solution by working closely with medical entomologists to find rice cultivation practices that minimise vector production (Chapter 6). Amongst others, the main challenge is to advocate these recommended rice-growing methods for adoption amongst rice farmers. Accordingly, prior understanding of how small-scale rice farmers view mosquitoes and their responsibilities towards mosquito production must also be explored (Chapter 7).

8.2 Synthesis and discussion of findings

This thesis aims to explore the relationship between rice cultivation, malaria vectors and malaria in sub-Saharan Africa and to identify modified rice cultivation practices that prevent mosquito breeding in rice fields. The key findings are discussed below.

8.2.1 Finding 1: Nowadays, rice areas bring more malaria, and, in the future, they will become a problem in elimination settings

In 1995-2005, reviews of the evidence found the "paddies paradox" in sub-Saharan Africa; the phenomenon that irrigated lowland rice was associated with more abundant malaria vector mosquitoes, but not more malaria infection [62,63]. It was hypothesised that rice brought not only more mosquitoes, but also economic and infrastructural development: better housing and health services, more household resources to buy bed-nets and antimalarial drugs. Thus, residents of rice villages were, at that time, much better able to protect themselves against malaria. The idea that rice in Africa brings more mosquitoes but not more malaria has been a critical assumption underpinning investments in rice development in Africa over the last 20 years [72–74]. Many individuals in the development sector, having observed these discussions, even assumed that the extra rice-generated mosquitoes were harmless [personal communication, AfricaRice and Medical Research Council Unit at The Gambia staff].

Given recent changes in the malaria situation in sub-Saharan Africa, we re-assessed, in a systematic review and meta-analysis (Chapter 3), the associations between rice cultivation and malaria. It revealed that since 2003, residents living near irrigated rice villages in Africa are exposed to more intense malaria transmission and have a higher prevalence of malaria infection than residents of non-rice villages. This is probably because, over the last twenty years, there has been massive scaling-up of coverage with effective anti-malaria interventions. Large-scale surveys have repeatedly shown that population coverage with interventions (especially insecticide-treated nets) is remarkably equitable than before [78]. This presumably reduced the differentials between rice and non-rice villages in the ability of residents to protect themselves against mosquitoes and malaria (Figure 8.1). Moreover, twenty to thirty years ago, there was very intense malaria transmission in much of the rural lowlands of East, West and Central Africa [62]. In these conditions, infection prevalence is not very sensitive to further increases in transmission because most infectious bites fell upon already-infected people [332]. Findings of previous reviews were consistent with this when they concluded that the paddies paradox occurred only in areas of “stable malaria transmission” (areas where people are living in highly endemic areas and exhibit a high level of malaria immunity) [62,63]. More recently, because of the general reduction in transmission, many more communities are now exposed to intermediate levels of transmission and population prevalence is more sensitive to changes in transmission [7]. As well as these changes, there was an open question that remained from the previous points of the paddies paradox: are the additional rice-generated mosquitoes harmless?

Our meta-analysis found that although rice fields allow the extreme proliferation of *An. gambiae* s.l. in sub-Saharan Africa, lower sporozoite rates were found within each vector in rice areas compared to non-rice areas. This is consistent with earlier discussions that raised the hypothesis that density-dependent effects were operating under the paddies paradox, where, despite and perhaps because of extremely high mosquito abundance, vectorial capacity was reduced [71,148]. Three possible mechanisms can reduce vectorial capacity, concerning longevity, feeding frequency and zoophily:

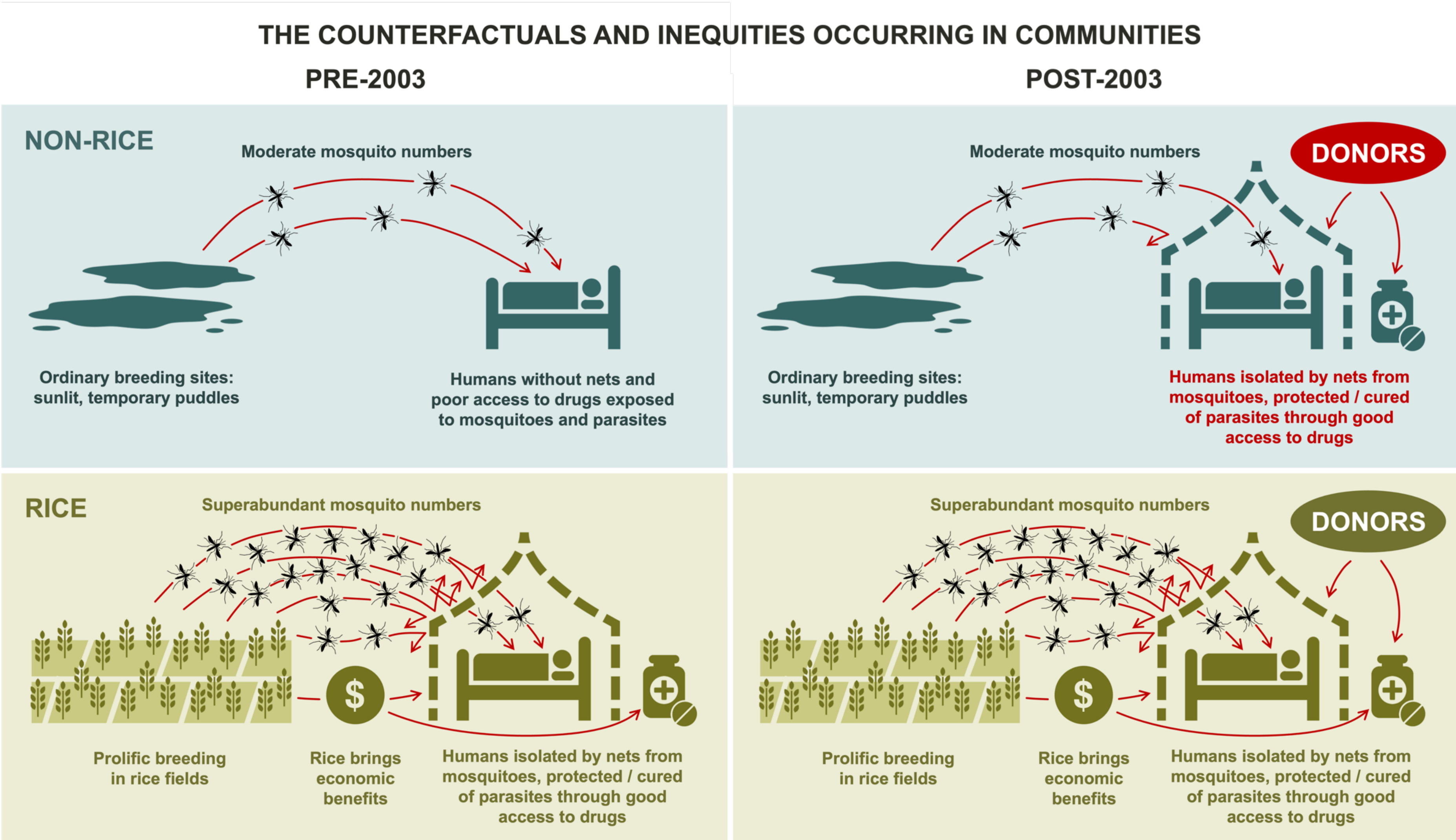

Figure 8.1. Diagrammatic representation of the counterfactuals and inequities occurring in a non-rice (blue) versus rice (green) community.

- High larval density environments lead to increased food competition, thus creating weaker adults with shorter longevity and reduced biting frequency, and hence, lowered efficiency in malaria transmission. This was observed in many settings[27] [114,116,333].
- High adult mosquito abundance (i.e., high mosquito nuisance) can increase host defence behaviour, where higher bed-net usage in rice farmers decreases the chances of mosquitoes finding a bloodmeal and thus, reducing survivorship.
- High adult mosquito abundance, leading to higher bed-net usage, can force zoophilic behaviour. Both high bed-net compliance and high cattle-feeding behaviour have been observed in many rice-growing areas [66,69,150,314,334].

There is also a possibility that the migration of older mosquitoes away from the highly productive rice fields could explain the greater sporozoite rates found in nearby non-rice communities. Nonetheless, higher malaria transmission was still seen in rice areas. This suggested that density-dependent effects do occur (the other vectorial capacity parameters, such as feeding success and longevity, of a rice-produced mosquito was reduced), but not enough to compensate for the additional mosquitoes. It indicates that the rice-produced vectors are not, and never were, harmless (as was suggested before) and other factors being equal, the additional mosquitoes do bring additional malaria [72–74].

This finding suggests that density-dependent effects in entomological parameters are likely to have played a small role in determining the relationship between rice and malaria. Instead, malaria context is the key factor. In the past (before 2000s), when large areas were under intense, “saturation” transmission (Appendix 1.1), true heterogeneities in infection prevalence between

[27] Note that it has also been observed that the survival rate of adult *An. gambiae* is not size-dependent and there is evidence that in smaller, pre-gravid *An. gambiae*, malaria parasites had a higher probability of establishing themselves [115,353].

communities were concealed. The harm of the mosquitoes was counter-balanced and potentially outweighed by the protection from the socio-economic differentials between rice and non-rice villages. Inequities in wealth and healthcare allowed rice farmers to be better "protected" and at a lower risk from malaria infection. But now, with a large proportion of the population-at-risk free from "saturation" of transmission and, with interventions equitably-distributed in both rice and non-rice villages, between-village variations in transmission are revealed in the form of higher infection prevalence in the rice villages. The higher malaria risk seen in rice communities indicates that we can no longer take the "paddies paradox" for granted.

Our meta-analysis further suggests that the magnitude of the additional malaria risk attributable to living in a rice-cultivating village depends on baseline levels of malaria transmission. The additional risk tends to be more conspicuous (and probably larger) in settings with lower background levels of transmission. Hence, as malaria continues to decline in the future in Africa, the contribution of irrigated lowland rice production to malaria risk is likely to become more conspicuous and probably stronger. Rice fields are likely to emerge as foci of residual transmission and become more conspicuous as an obstacle to elimination as we have seen previously in Europe and Central and East Asia. Therefore, rice fields will become more strategically important for national malaria programmes.

8.2.2 Finding 2: Peak vector productivity occurred in the first few weeks of the rice-growing season

Rice paddies can be inhabited by many different mosquito species throughout all stages of plant development (from transplanting to harvest). The vector species present and their abundance tend to change according to the changes that occur in a rice agro-ecosystem. The field experimental studies conducted by our team in central Côte d'Ivoire aimed to determine the population dynamics of anopheline mosquitoes in rice fields (Chapter 5). We observed peak *An. coluzzii* productivity to occur in the first six weeks after transplanting, during which period around three-quarters of total *Anopheles* mosquitoes were produced per season. This peak productivity can be attributed to the aquatic conditions during early rice stages where there is exposed, sunlit, clear, shallow, “fresh” (without many predators), clean (without iron (III) oxide residues) water with fertiliser and some vegetation highly suitable for oviposition and larval development. Our study found that a larger proportion of vectors survived to adulthood during the early stages of rice development (i.e., land preparation and vegetative phases), most likely because the rice water had not yet been colonised by mosquito predators [40]. As the season progressed, the development of rice plants changed the aquatic conditions of paddies, which led to lower *An. coluzzii* productivity and a species succession. Plant maturation established a closed canopy that shaded the water surface, which was more suitable for *An. ziemanni* and less suitable for *An. coluzzii* [44].

According to literature included in the systematic review (Chapter 4) and our own field trials (Chapter 6), other than the natural progression in plant development, many rice management practices that occur earlier in the cropping season were found to affect vector densities. They do so both positively and negatively. On the one hand, rice operations that encourage residual pools of water, such as manual weeding and transplanting, create aquatic and soil conditions for *An. coluzzii* development. Fertiliser application and weeding during the early stages of the rice season coincide with increases in larval abundance. On the other hand, rice operations that introduce chemical substances like iron (III) residues can inadvertently inhibit mosquito development. Our field trials demonstrated that more anopheline immatures tended to accumulate in the edges (and corners) of rice fields, possibly to protect themselves against predators.

Our experimental study estimated that a total of 103 million malaria vectors (95% CI 84.5, 121.4 million) could be produced in a (typical small-scale) rice irrigation scheme of 142 hectares in a cropping season. This figure can be used to approximate the epidemiological value of the additional mosquitoes produced by rice, which can highlight the extent of the potential impact of rice cultivation on increased malaria risk.

8.2.3 Finding 3: Modified rice cultivation practices, including alternate wetting and drying irrigation, can control malaria vectors in some settings

Rice crops can be cultivated using many different techniques. Both our systematic review and meta-analysis (Chapter 4) and our own experimental field trials (Chapter 6) demonstrated that many of these techniques can affect mosquito vector densities. The effect of these techniques (and their mechanisms) on riceland malaria vectors are summarised in Table 8.1 and presented in more detail in Appendix 8.1.

In terms of alternate wetting and drying irrigation, our field trials have demonstrated that, in Tanzania, it was associated with 71% reduction in early instars (95% CI -88.6, -23.9), and, in Côte d'Ivoire, it produced 41% less methane (95% CI -44.6, -36.4; and thus 39% lower global warming potential [95% CI -45.4, -33.6]) and consumed 58% less water (95% CI -65.0, -52.3; 21% higher water productivity [95% CI 33.3, 70.8) than continuously flooded fields. These trials need to be repeated for more seasons and in more locations for further confirmation.

Rice management practices that can affect mosquito numbers and require further investigation include (Appendix 2):

- Rice-fish co-culture,
- Type of levelling during land preparation,
- Plant variety,
- Plant spacing during crop establishment,
- Type and timing of weeding
- Type and timing of fertiliser application (including neem)
- *Azolla*

Other than these cultivation techniques, our systematic review and meta-analysis also revealed that chemical and biological larvicides were associated with 60 to 82% reduction in malaria vector immatures. Monomolecular surface films and copepods produced mixed results and should be further explored.

Table 8.1. Rice operations and their effect on malaria vector densities. Further details, including percent increase/decrease and comparisons, can be found in Appendix 8.1.

Aspect	Technique	Effect on malaria vector densities	Mechanism
Land preparation	Minimal tillage	Fewer	Discourages the creation of deeper soil depressions that accumulate in stagnant water
Crop establishment	Direct seeding (vs. transplanting)	More	Plant spacing in directly seeded fields was sparser and/or because irrigation at the early stages of rice growth was longer and more continuous in directly seeded fields which may have allowed more extensive breeding
Water management	Intermittent flooding of rice fields (active or passive drainage)	Fewer (late-stage)	Intermittent (rather than continuous) flooding with wetting periods that are shorter than the aquatic cycle of mosquitoes can prevent larval development. Its efficacy is highly dependent on the type of drainage and local conditions, such as the physical characteristics of the soil and the climate. It is most effective in places that favour rapid drying.
	Active drainage: intermittent irrigation of 3-day wet and 3-day dry cycles	Fewer (in one field trial)	
	Passive drainage: alternate wetting and drying irrigation at 15 cm	Fewer (in one field trial)	
Weed management	Herbicide application (vs. none)	More	Herbicides tend to remove vegetation that may have originally inhibited oviposition and/or created cooler, shaded aquatic conditions undesirable for *An. gambiae* s.l. mosquitoes
Nutrient management	Fertiliser application (vs. none)	More	Nitrogenous fertilisers tend to reduce water turbidity, which increases attractiveness for gravid females, and also tend to provide more nutrients, which aid mosquito development
Pest management	Pesticide application (vs. none)	Fewer	Active ingredients in pesticides may also kill mosquito larvae.

8.2.4 Finding 4: Rice farmers were not aware of rice-mosquito link but were willing to participate in interventions

Any potential riceland mosquito strategy must have the cooperation of all rice farmers in an irrigation scheme to be effective. Thus, a better understanding of how smallholder rice farmers in sub-Saharan Africa view and perceive their contribution to mosquitoes is required. It is especially important in terms of the level of awareness amongst farmers: whether they feel any responsibility towards mosquito production and if they are interested in coordinating to minimise mosquitoes in a rice setting.

The qualitative study (Chapter 7) found that whilst rice farmers in central Côte d'Ivoire generally did not consider living near rice fields disadvantageous, they perceived mosquitoes to be a significant problem. Similar to previous literature, respondents detailed that mosquitoes brought nuisance (disrupting their sleep and constraining evening activities) and malaria, which required treatment costs and sometimes hospitalisation [92,94,215,216,313,317]. However, most farmers believed that neighbouring bushes and wastewater were the biggest contributors[28] of mosquito production. Although some farmers believed mosquitoes came from *bas-fonds* (irrigated lowlands) and that recent increases in mosquito density were attributable to nearby dam construction, they did not establish a link between rice fields and mosquitoes. Some participants were in fact adamant that rice cultivation did not attract mosquitoes because mosquitoes were numerous even when rice was not being cultivated in the inland valleys. This was surprising because previous qualitative studies found that rice farmers in other parts of sub-Saharan Africa were aware of their impact on mosquito production [92,94,95,216,312,313,318,319].

[28] Note that this was a severe limitation of the study. The question of "where mosquitoes come from" could have been better phrased to probe for mosquito breeding, rather than mosquito migration. It remains unclear how farmers had interpreted this question.

When probed about agricultural links with mosquitoes, many rice farmers highlighted the importance of dams and irrigated lowlands for their livelihood and were convinced that there were not many options for mosquito control at village- or field-level. A few respondents proposed riceland insecticide application but were doubtful of its efficacy and ease, similar to findings from previous literature [314]. Other rice farmers were willing to participate in rice field-based interventions on the condition that appropriate solutions were recommended or that insecticides were provided by the government. As established in other studies, farmers that were aware of the rice-mosquito link tended to be more receptive towards the need for solutions [94,98,299,319]. Although collective action problems within farm-based riceland mosquito control were not identified, instances in environmental village- and field-level clean-ups indicate that n-person prisoner's dilemma may occur. This dilemma, where participants are faced with the decision between cooperating with each other for the "common good" or defecting (following "selfish" interests), must be addressed when designing community-based vector control in rice fields [322,323].

8.3 Limitations and lessons learnt

The limitations of each component study have been discussed in their respective chapters. This section briefly reviews the major limitations and lessons learnt in the project as a whole.

8.3.1 Revisiting the paddies paradox

Our systematic review and meta-analysis (Chapter 3) was based on observational studies, which are predisposed to biases and confounding. Selection bias in exposure and control groups may therefore have occurred, where there may have been low comparability in rice and non-rice growing communities; these communities may have been systematically different in their characteristics even before the introduction of rice cultivation schemes. Moreover, the sub-group analysis of the seven studies conducted after 2003 included three studies that were conducted by the same research group and in the same area of central Tanzania [152,154,161]. Additionally, only four studies had reported clinical malaria incidence, which is important to measure the burden of illness caused by malaria.

The observational studies included in our review were also prone to confounding because important factors associated with rice cultivation and malaria such as socioeconomic status, housing conditions, and access to health care, were not always accounted for; very few studies had reported adjusted effect measures. Nevertheless, because rice cultivation entails economic benefits that protect against malaria, the relevant effect measure (i.e. adjusted or unadjusted) depends on whether the “true” or combined effect is of interest. A number of other factors were also not considered in the review: variation in seasons (wet vs dry, seasonal vs perennial), landscapes of study sites and characteristics of rice cultivation (type of rice grown, number of cropping seasons, size of rice irrigation schemes, and distance of rice-growing communities from their fields). The review therefore does not reflect whether and how these factors could have impacted malaria transmission and prevalence in rice communities. For example, it did not capture the fact that rice irrigation in Mali had altered the malaria transmission pattern from seasonal to perennial [70].

The Grading of Recommendations, Assessment, Development and Evaluations framework determined that summary estimates from the meta-analyses had very low ratings on the quality of evidence, with serious imprecision, inconsistencies, indirectness, and risk of bias. Nevertheless, the study was interested in identifying the direction of effect (of rice on malaria risk), and our finding that rice is associated with more malaria is concerning.

8.3.2 Mosquito monitoring in rice fields

It is important to design a mosquito monitoring programme in rice fields that can eventually be adopted by agronomists. However, as illustrated in Chapters 4, 5 and 6, sampling mosquito populations was challenging (please refer to

Appendix 8.2 for a brief comparison of trial design, mosquito collection methods and analyses across studies included in Chapter 4).

First, the high variability in mosquito numbers within rice plots means that the level of accuracy and precision achieved in our riceland mosquito sampling programme was lower than desired. It was difficult to detect significant difference between plots. Results in Chapter 6 demonstrated that although some changes were observed under certain treatments, there was insufficient power to reveal statistically significant differences. Our sample size calculations estimated that in order to observe a significant 1.54-fold reduction (as seen in previous water management trials) in mosquito immatures between plots under continuous flooding and alternate wetting and drying irrigation, nine plot replicates would have been required for larvae and sixteen plot replicates for pupae (Appendix 6.4). These sample sizes are unfeasible to agronomists: they are inconvenient and entail additional costs and labour. Rice researchers only require three replicates to obtain significant results from agronomic outcomes and will not use limited resources to establish nine to sixteen replicates to accommodate mosquito monitoring [274].

Second, there was little standardisation in how the dipper was used for collecting mosquito immatures. The technique used depended on the water depth in the field: in deeper water, the dipper was skimmed at an angle through the water in a swift motion before it overflowed, and in shallow water, the dipper was lowered gently at an angle to allow water to flow into it. Moreover, the technique used by field assistants also varied depending on skill and experience. These differences in sampling techniques create inherent biases in mosquito collection. Furthermore, it is speculated that dipping tends to be biased against certain instars, especially late instars and pupae that can readily avoid capture (i.e., dive quickly and remain in the bottom of the water for long periods of time) [89]. This is a severe limitation when assessing the proportional distribution of mosquito developmental stages (explained in more detail later).

Considering both limitations, sampling strategies that better capture mosquito density and dynamics (by reducing noise) and that can be easily adopted by agronomists are needed. This may entail designing experimental rice trials with further subdivision of plots, and hence more replicates, for mosquito monitoring purposes.

8.3.3 Measuring the efficacy of a riceland mosquito intervention

Finding an intervention that could be used on the large scale to reduce mosquito breeding in rice fields is a process with several different stages, each with its own appropriate indicator. The eventual aim is to show that the intervention has epidemiological benefits in the local human population, by protecting them from malaria. However, such "effectiveness" evidence can only be produced by large and expensive (usually clustered) randomised controlled trials (RCTs), and to justify this kind of expense, we first need to know that the intervention to be tested is efficacious in entomological terms.

In other words, we must show, using entomological measures, that (a) it is reasonable to select this intervention, rather than the alternatives, for larger-scale testing, and (b) there is reasonable hope that large-scale implementation might have epidemiological benefits. What would be the appropriate indicators for efficacy in entomological terms? In our studies, we were mostly comparing variant cultivation practices to see which ones were associated with more or less abundant mosquito breeding. In other words, we have assumed that a reduction in mosquito numbers is the main causal mechanism by which the entomological effects of an intervention might lead to epidemiological benefits (i.e., we assume that in order to have the potential to control malaria, an intervention must have the capacity to reduce the number of adults coming from rice fields). We think that other causal mechanisms, whilst imaginable in theory, are unlikely to be useful in practice. Therefore, an intervention that lacks this capacity at the level of the rice-plot is not worth further consideration.

Still, there is a critical limitation to using simple mosquito immature density as a measure of efficacy for this purpose. Compared to samples of newly emerged adult abundance, or even pupal abundance, counts of larval numbers do not factor in survival and mortality between the first instar and pupal emergence.

Some trials included in Chapter 4 measured the total number of immatures without differentiating between instars. In effect, this gives first stage larvae and pupae equal weight as an indicator of adult productivity. This is misleading and biologically inappropriate. Our trials in Chapter 6 differentiated between stages, which can infer how an intervention affects immature survivorship (i.e., by reducing larval survival or preventing oviposition and development to late instars and pupae).

Despite primarily using mosquito immature densities in Chapters 4 and 6 to determine the efficacy of rice-field mosquito control, it is still important to apprehend the epidemiological importance of malaria vectors produced by rice fields. In order to advocate for more research on vector-reducing agricultural interventions, it would be more desirable to have some estimate (however approximate and dependent on big assumptions) of the "rice-attributable malaria burden". In other words, some attempt to answer the question "what proportion of malaria morbidity and mortality is due to infections delivered by mosquitoes from rice fields?" When trying to frame such a question, it is important to be clear about which indicators are being compared to … In Chapter 5, I have not tried to make such an estimate, but attempted to make a first step in the estimation process; I tried to estimate the absolute number of vector mosquito females coming from a hectare of rice in a growing season. In order to go further, one would have to make many assumptions related to mosquito bionomics: emergence rates, mortality, flight range, and biting behaviour. They were also approximated based on the species composition measured in our trials; our trials were not necessarily representative of the M'bé 1 irrigation scheme and/or other schemes.

8.4 Implications of findings and recommendations for future studies

African ministries of health are considering how to eliminate malaria, while ministries of agriculture are actively planning the expansion and intensification of irrigated rice production. Both of these objectives are desirable, but our findings (in Chapter 3) indicate that the latter might interfere with the former, as rice cultivation brings increased malaria risk. To reconcile these two goals, African countries urgently need to develop and promote methods of growing rice without growing malaria vectors.

8.4.1 More evidence on the effect of rice cultivation on malaria risk in Africa

More evidence on the effect of rice cultivation on malaria risk in sub- Saharan Africa is needed. The studies included in Chapter 3 were purely observational. Therefore, studies of different designs are required. They include longitudinal studies comparing malaria risk before and after the introduction of rice crops (e.g., Koudou et al. 2010 and Diakite et al. 2015 [147,157]). Numerous national malaria control programme staff from sub-Saharan Africa have declared that the effects of rice cultivation on district-level malaria indices are obvious, but such information remains to be formally collected [personal communication, senior staff in the Nigeria National Malaria Elimination Programme]. Study designs to be conducted also include, if possible, intervention studies [339]. They also include geospatial analyses[29] of the links between current (or changes in the) distribution of rice and malaria indicators.

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[29] Mosquito flight range spans an average of 0.8-1.5 km, so the bulk of the effect of rice cultivation is seen within that distance [355,356]. It may be tempting to use georeferenced household data collected from the Demographic and Health Survey (DHS) and the Malaria Indicator Survey (MIS) programmes. However, to maintain respondent confidentiality, GPS coordinates have been displaced: rural clusters are displaced to a distance of up to 5 kilometres with a further randomly-selected 1% of rural clusters displaced up to 10 kilometres. This displacement obscures the effect of rice cultivation and is therefore not useable.

Note that geospatial analyses using DHS and MIS data have been conducted. However, most studies only identified vague associations with agriculture, which in the context of vector control, is not informative [357,358]. Some associations did not account for the links between rural areas and malaria and therefore may also have been a result of residual confounding.

Given the complex relationship between vector abundance, malaria transmission and malaria prevalence, future studies should include all entomological and epidemiological indicators[30] to provide a clearer picture of the rice-malaria story. Studies need to measure more direct estimates of malaria risk, such as clinical malaria incidence too. They also need to address questions of equity by including potential confounding factors associated with rice cultivation like socioeconomic factors, housing, bed-net coverage, and use of antimalarial drugs.

Further work to estimate the rice-attributable burden of malaria in Africa is also required. Whilst Chapter 5 was an adequate start, it would have been more ideal to model our estimates against human biting rates (and malaria transmission intensity) measured in rice and non-rice communities. It would also be informative to determine the significance of rice fields as breeding sites, by comparing their vector productivity with other breeding sites in a rice-growing area, similar to the methodology by Cairncross et al. (1988), or by regressing mosquito densities against the surface area of rice compared to other land-use characteristics around villages [340].

8.4.2 The agricultural sector should take the lead

When informed of the main finding in Chapter 3 (that rice brings increased malaria risk), scientists (both agricultural and public health) often interpret it in two ways. First, that there is an inevitable trade-off between health and food security. At one extreme, it is even perceived as a reason to delay the expansion and intensification of rice in sub-Saharan Africa. Second, that the health sector needs to provide more anti-malaria interventions, namely insecticide-treated nets and antimalarial drugs. However, neither response is appropriate. First, it is unrealistic to stop growing rice: food security is vital for a growing African population. It is also unacceptable to continue contributing to

[30] Human biting rate, sporozoite rate, entomological inoculation rate, malaria infection prevalence, malaria case incidence

malaria transmission. Second, the currently available set of malaria interventions can only provide a partial and temporary solution. Moreover, if more interventions are delivered to rice farming communities, the health sector would inadvertently be promoting inequity. The problem of malaria is not going to be solved by the agricultural sector, but it does seem that rice makes the problem worse. The role of the agricultural sector is therefore to stop being part of the problem, and start being part of the solution.

As illustrated in Chapters 4 and 6, there are solutions: modified methods of rice cultivation can minimise the number of mosquitoes emerging from rice fields. It seems likely that with further research, methods that will not only reduce mosquitoes but also improve yield and be attractive to rice farmers can be developed. Achieving such agriculture-health co-benefits should become a prioritised element within rice development research in Africa. This can perhaps follow the example of reducing greenhouse gas emissions from rice fields; climate change agronomists addressed the problem by successfully developing methods to address this challenge [269]. This is encouraging for the health sector, since, compared to mosquito populations, greenhouse gas emissions are equally (if not more) variable and difficult to measure. Furthermore, as indicated in Chapter 6, it might even be possible to develop methods (like alternate wetting and drying irrigation) that can minimise both methane emissions and mosquitoes.

The best approach to identify riceland mosquito control is to integrate the objective in the work of rice research. Rice cultivation practices are constantly evolving; agronomists are always researching and developing improved methods of producing rice. The monitoring of mosquito breeding could be therefore added to the research agenda in order to find rice-growing techniques that boost yield whilst minimising vector production. Accordingly, this research and development task must be led by rice experts, aided by technical input[31] from malariologists. It will require the agricultural sector to stop ignoring the undesirable side-effects (mosquitoes) of rice cultivation and take responsibility of their actions. The sector is already moving towards "sustainable intensification" (SI)[32], which was initiated by concerns with agricultural development having serious consequences on the planet's ecosystems, biodiversity, water and climate [267,268,341]. Perhaps reducing vector-borne disease risk can be built into one of the objectives of SI and more agriculture-health co-benefits can be realised.

8.4.3 Better rice-field mosquito sampling methods are urgently needed

Agronomists will not take on the research and development task of identifying rice cultivation practices that minimise mosquito production unless better designs and methods of monitoring mosquito larvae in rice fields are available.

The main barrier to uptake is that compared to experimental designs conventionally used in agriculture, remarkable variability in mosquito numbers requires much more replication (per treatment) in field trials than agronomists might like. Moreover, due to the "boom and bust" nature of mosquito populations in rice fields, sampling must occur regularly, at frequent intervals and for an extended period of time[33]. Sampling twice a week at three- or four-

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[31] It would be most ideal to bring together a network of rice-malaria researchers i.e., an expert working group. Perhaps, taking after climate change researchers, the role of health (or One Health) agronomists should be built into the curriculum

[32] Sustainable intensification refers to agricultural practices that increase productivity on existing land and improve livelihoods and improving resilience to shocks and stresses related to climate change. It consists of using different methods and approaches, ranging from traditional management

[33] As highlighted in Chapter 4, many experimental trials did not necessarily follow mosquito populations for an entire rice season. Importantly, they did not account for different rice-growing phases, which is important to distinguish to identify components of a complementary set of rice-field mosquito control interventions. Other than

day intervals is barely sufficient to illustrate the aquatic life cycle of mosquitoes. The design of a riceland mosquito sampling programme must therefore be improved to reduce plot-to-plot variation[34].

Another barrier to uptake is that current methods of counting larvae (i.e., dipping and manually counting mosquitoes twice a week for an entire season) are too crude (because of several biases), labour-intensive and demanding. There are some promising ideas for new methods of monitoring mosquitoes, including using environmental DNA and image analysis, and these need to be developed [296,297]. Regardless, the most ideal sampling method should provide a better understanding of mosquito population dynamics, by measuring variables that affect mosquito densities but were crudely measured in this study (Chapters 5 and 6). Potential confounders and mediators (intermediate outcomes that are on the causal pathway) include the number and types of mosquito predators, the chemical composition of the rice water, and number of size of depressions in the soil. Moreover, to increase uptake amongst rice researchers, the monitoring method could incorporate variables that agronomists regularly measure, such as water depth and quality and soil conditions. These requirements may call for video monitoring (tracking and movement analysis) of mosquitoes [342,343], which may be made possible through a device that remains in the rice fields throughout a rice season.

8.4.4 Complementary sets of interventions are required to achieve riceland mosquito control throughout a cropping season

The task to minimise the production of (adult) malaria vectors throughout an entire rice-growing season, which lasts 4-6 months, is demanding. It is impractical to assume that one type of intervention is capable of consistently

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monitoring the mosquito production of main rice fields, the productivity of nursery beds and fallow fields should also be monitored.

[34] Lessons could be learnt from how agronomists managed greenhouse gas measurement in rice trials.

controlling mosquitoes in a temporally and spatially dynamic environment such as rice fields. On the one hand, as illustrated in Chapter 5, rice fields tend to produce malaria vectors throughout the cropping season, but peak production occurs in the first 5-6 weeks following transplanting. On the other hand, Chapters 4 and 6 revealed that many interventions are effective at reducing riceland anopheline production. These interventions can be separated into two categories of interventions: (a) interventions that do not kill larvae at the moment of application but are more suppressive and sustainable throughout the season and (b) interventions that are lethal to larvae almost immediately.

The first class of interventions are less immediately effective (e.g. 30% reduction), but their residual effect lasts longer. These interventions include modified methods of rice cultivation (e.g., intermittent irrigation, minimal tillage) and rice-fish co-culture. The second class of interventions are mostly chemical or biological insecticides, where although they have good immediate effectiveness, they do not have much residual effect. Frequent re-application would be needed for longer-term control, which in practice is not a long-term solution as it would be too expensive and demanding in terms of logistical effort and discipline.

The first class of interventions, because of its slower efficacy, cannot prevent the peak of malaria vectors produced in the first few weeks after transplanting. However, they may help reduce breeding later in the rice-growing season, when the rate of production of adult mosquitoes is still going on, albeit at lower levels because of the presence of predators (which are important in controlling mosquito populations). The second class of interventions, because of its immediate effectiveness, would be applicable to control the early peaks of vector production. Thus, effective cover for the whole of the season might be possible using a combination of these two classes: short-term insecticidal interventions during the initial 4 to 5 weeks post-transplantation, and suppressive interventions later.

8.4.5 Promising interventions: the need to explore all steps of rice cultivation

Work conducted in Chapters 4 and 6 suggests that it would be feasible to develop rice cultivation techniques that can minimise mosquito production whilst sustaining yields. They demonstrate that alternative rice-growing methods (including duration of flooding during land preparation, type of crop establishment, water management technique and fertiliser application) can all have moderate effects on mosquito numbers. Most of these effects have not been adequately investigated. There needs to be more detailed work on how different variations of rice cultivation practices can affect larval abundance (Appendix 8.3)[35]. This includes expanding the idea of mixing *Bti* with fertilisers (invented by the Tropical Pesticides Research Institute in Tanzania) [263]. Rice inputs are applied according to a cropping calendar (e.g., herbicides are usually added shortly before and after planting). The inputs whose application timing correspond with peaks of mosquito production could be mixed with larvicides to prove vector control.

Other than the typical procedures of rice cultivation (land preparation, crop establishment, and water, nutrient, weed, and pest management), other aspects of rice cultivation, such as *Azolla*, neem, and fish should also be explored. Practices that do not receive much recognition but may also affect mosquito numbers and are hence worth investigating include crop rotation, system rice intensification (SRI), and ducks[36]. Similarly, integrated pest and

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[35] This research and development task needs to be led by agriculturalists. Rice cultivation is a five-month-long task, that entails many inputs and operations. Medical entomologists are not aware of the *sine qua non* (or "non-negotiables") of every technique i.e., aspects that cannot be changed if rice yield were to be maintained.

[36] Wet crop-dry crop rotation can help eliminate mosquito breeding sites: it was observed in China that the rotation of rice fields with dry fields (over winter) significantly reduced anopheline densities [266,359]. In Kenya, alternate wetting and drying under the SRI regime was also observed to eliminate mosquito larvae[360,361]. Integrated rice-duck farming is adopted in many East Asian countries, where ducks did not only control weeds and insect pests very effectively (increasing rice yields by about 10-

vector management could be explored. Essentially, there should be an emphasis on interventions that exhibit transectoral benefits (increased food security, climate change mitigation and adaptation, income generation, malaria control).

Overall, a multi-centre approach to this research and development task is obligatory: suppressing mosquitoes will require different methods in different places since mosquitoes are sensitive to variations in rice agroecosystems (e.g., climate and soil conditions).

8.4.6 Advocating collective participation of rice farmers

More social science studies investigating the views and perspectives of rice farmers on mosquitoes and potential rice interventions are required. Additionally, once appropriate mitigation practices have been identified and are ready for implementation, education and training amongst farming communities about the links between rice, mosquitoes and malaria are mandatory. Since smallholder farmers constitute most of the rice production in sub-Saharan Africa, awareness and cooperation from all farmers in the same irrigation scheme or same wetland are necessary for any riceland mosquito control strategy to succeed. Thus, methods to advocate collective participation of all farmers must be explored.

From the farmer's point of view, the one aim is to maximise the crop of rice while minimising inputs in order to maximise profit; the rice is a private good. The mosquitoes that are produced by the same cultivation process, on the other hand, are a public harm. For this reason, making the extra effort to avoid growing mosquitoes is a sensible decision for a farmer only if their personal benefit from the reduction in mosquitoes from their own fields outweighs this effort, or if (nearly) all the other farmers join in. Therefore, an approach to

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20%), but they also provided an additional source of income for farmers [362,363]. In China, ducks were also seen to forage on mosquito larvae, achieving 92 to 99% mosquito control [266].

identify rice-growing methods that will not only reduce mosquitoes, but also improve yields will help maximise farmer incentive. This reaffirms the need for the agricultural sector to take the initiative to include mosquito monitoring in their research.

8.5 Conclusions

This thesis attempted to understand the linkages between rice and malaria in sub-Saharan Africa and identify potential methods of malaria vector control in rice fields. Despite identifying key knowledge gaps and areas where further research is required, this thesis has contributed to understanding how rice impacts malaria risk and identified promising interventions against malaria vectors produced in rice fields.

In sub-Saharan Africa, rice does not only bring more malaria mosquitoes, but also more malaria risk. Using current cultivation methods, the additional vectors from rice fields will always be a harmful unintended side effect of rice-growing. Conversely, the suppression of mosquito breeding using modified rice cultivation methods would be a highly beneficial intended side effect. It is therefore fundamental that the agricultural sector takes accountability for their contribution to the malaria problem in Africa and recognise that they can alleviate it. The development of irrigated rice in Africa should continue but accompanied by a comprehensive programme of research to develop anti-mosquito methods of growing rice. This would ideally involve identifying methods of growing rice that simultaneously maximise the yield of rice and minimise mosquito production (as well as environmental impact). This can be made possible by developing better sampling techniques and experimental designs so that mosquito monitoring can be built into the procedures used by rice researchers when they are looking to improve methods of rice production. It is hoped that in this way, agricultural and health researchers can work together to make gradual incremental progress towards methods of rice cultivation that reconcile the goals of achieving food security and striving for malaria elimination in sub-Saharan Africa.

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10 Appendix

Appendix 1. Chapter 1 Supplementary Material

Appendix 1.1. The concept of "saturation" transmission

The frequency at which people are bitten by infectious mosquitoes is called the entomological inoculation rate (EIR). When infectious bites are rare, people normally have time to recover from one infection before being exposed to the next. As new inoculations become more frequent, the resulting infections sometimes overlap; another infection begins before the host has fully recovered from the previous one. When transmission reaches intermediate levels, the majority of infectious bites fall on people who are already infected, and most people are therefore infected most of the time.

In other words, when transmission intensity is low-to-moderate, the prevalence of infection in humans is sensitive to, and useful as an indicator of, differences in transmission intensity (for example, in between-village and between-year comparisons). However, at high transmission levels – when EIRs exceed 25-50 infectious bites per person per year – the true prevalence is already close to 100% and cannot increase much further (Figure I). Thus, there may be substantial differences between nearby villages in the intensity of transmission measured as EIR, but only small differences in observed malaria prevalence. An example of this is demonstrated in the entomological results reported by Magesa et al. (1991) and the epidemiological data from the same villages reported by Lyimo et al. (1991) [344,345].

Certainly, in these situations, the observed prevalence is much lower (typically in the range of 50% to 70%). This is partly because of acquired immunity, which tends to be better at suppressing blood infections than at eliminating them completely. It is also because, at these levels of exposure, people who have easy access to anti-malarial drugs tend to use them often. This further complicates the relationship between prevalence (and other epidemiological indicators) and transmission intensity.

All this means that, in these saturation conditions, conventional epidemiological indicators (especially parasite prevalence) are relatively insensitive to further increases in transmission intensity, and are therefore not ideal as a means of detecting whether a given factor tends to make transmission more intense.

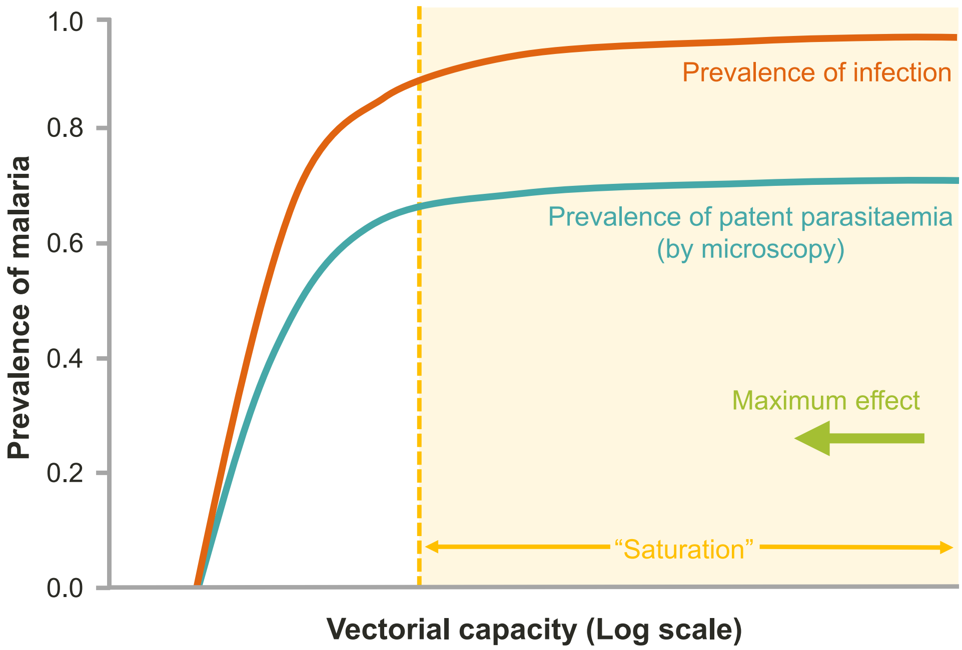


Figure I. An equilibrium between vectorial capacity and malaria prevalence. The relationship between vectorial capacity, prevalence of patent parasitaemia (by thick film) and true prevalence of parasitaemia (if a perfectly sensitive diagnostic test existed). This is hypothetical and adapted from the Garki model [346,347]. Prevalence rate levels off far below 1 because immunity is allowed for, which suppresses a large proportion of infections to detectable levels. Hence, a large proportion of people spend a large proportion of their time permanently infected but drifting above and below the level of detectable parasitaemia. At high transmission intensities (where the plateau is reached), local, moderate increases in transmission are not detectable. However, at lower transmission intensities (when prevalence increases with vectorial capacity, before the plateau), true epidemiological heterogeneities are revealed.

Appendix 3. Chapter 3 Supplementary Material

Appendix 3.1. Search strategy in multiple databases (last search date = 18 September 2020)

Search Set	MEDLINE	EMBASE	Global Health	Web of Science
1	Exp malaria/	Exp malaria/	Exp malaria/	TS = malari*
2	Exp anopheles/	Exp Anopheles/	Exp anopheles/	TS = anophel*
3	Exp plasmodium/	Exp plasmodium/	Exp plasmodium/	TS = disease vector$
4	Malaria.tw.	Malaria.tw.	Malaria.ab.	TS = mosquito*
5	Malari*.tw.	Malari*.tw.	Malari*.ab.	TS = plasmodium
6	Anophel*.tw.	Anophel*.tw.	Anophel*.ab.	1 or 2 or 3 or 4 or 5
7	Mosquito*.tw.	Mosquito*.tw.	Mosquito*.ab.	TS = rice
8	Entomolog*.tw.	Entomolog*.tw.	Entomolg*.ab.	TS = “rice field$”
9	Parasitemi*.tw.	Parasitemi*.tw.	Parasitemi*.ab.	TS = “ricefield$”
10	Parasitaemi*.tw.	Parasitaemi*.tw.	Parasitaemi*.ab.	TS = “rice cultivat*”
11	Plasmodium.tw.	Plasmodium.tw.	Plasmodium.ab.	TS = “rice grow*”
12	1 or 2 or 3	1 or 2 or 3	1 or 2 or 3	TS = “rice padd*”
13	4 or 5 or 6 or 7 or 8 or 9 or 10 or 11	4 or 5 or 6 or 7 or 8 or 9 or 10 or 11	4 or 5 or 6 or 7 or 8 or 9 or 10 or 11	TS = “rice irrigat*”
14	12 or 13	12 or 13	12 or 13	7 or 9 or 10 or 11 or 12 or 13
15	Exp oryza/	Exp rice/	Exp rice/	6 and 15
16	Exp agriculture/	Exp agriculture/	Exp oryza/	
17	Rice.tw.	Exp “irrigation (agriculture)”	Exp agriculture/	
18	Rice field$.tw.	Rice.tw.	Rice.ab.	
19	Ricefield$.tw.	Rice field$.tw.	Rice field$.ab.	
20	Rice cultivat*.tw.	Ricefield$.tw.	Ricefield$.ab.	
21	Rice grow*.tw.	Rice adj4 cultivat*.tw.	Rice adj4 cultivat*.ab.	
22	Rice padd*.tw.	Rice adj4 grow*.tw.	Rice adj4 grow*.tw.	
23	Irrigat*.tw.	Rice adj4 practice$.tw.	Rice adj4 practice$.ab.	
24	15 or 16	Rice adj4 technique$.tw.	Rice adj4 technique$.ab.	
25	17 or 18 or 19 or 20 or 21 or 22 or 23	Rice adj2 padd*.tw.	Rice adj2 padd*.ab.	

26	24 or 25	Rice adj2 irrigat*.tw.	Rice adj2 irrigat*.ab.	
27	14 or 26	15 or 16 or 17	15 or 16 or 17	
28		18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26	18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26	
29		27 or 28	27 or 28	
30		14 and 29	14 and 29	

Appendix 3.2. Characteristics of observational studies included in the quantitative, semi-quantitative and qualitative* analyses (n=53)

Study	Country	Year	Setting (irrigation scheme)	Primary vectors	Transmission / LLIN coverage / IRS coverage	Study design	Study size	Age group	Recruitment of participants / Method of mosquito collection	Control group(s) (distance from rice)	Follow-up	Outcomes included				
												Entomological			Epidemiological	
												HBR[a]	SR[a]	EIR[a]	PR[a]	MI[a]
Chandler et al. 1975	Kenya	1971	Rural (Ahero)	*An. gambiae* s.l.	-	Cohort	7-22 catches per (3) village	N/A	HLC (inside and outside)	Grassland country (10 km)	12 months	Yes*				
Chandler et al. 1976												Yes*				
Audibert et al. 1990	Cameroon	1979	Rural	-	-	Repeated cross-sectional	4611	2 – 9	Random selection of clusters	Non-rice growing	N/A				Yes	
Carnevale & Robert 1987	Burkina Faso	1980	Rural (Vallée du Kou)	*An. gambiae* s.l.	-	Cohort	-	N/A	-	Savannah (10 km)	9 months	Yes*	Yes	Yes*		
						Cross-sectional	2322	2 – 9	-		N/A				Yes	
Coosemans et al. 1984	Burundi	1981	Rural (Rusizi)	*An. gambiae* s.l.	-	Cross-sectional	4-5 trap-nights per (9) village	N/A	HLC (inside and outside)	Market gardening, other crops e.g. cotton, banana, maize, yams	N/A	Yes*				
							-	0 – 20	-		N/A				Yes*	
Couprie et al. 1985	Cameroon	1981	Rural	*An. gambiae* s.l.	55% prevalence	Cross-sectional	924	2 – 9	-	Next to lake, no rice cultivation	N/A				Yes	
Coosemans 1985	Burundi	1982	Rural (Rusizi)	*An. gambiae* s.l.	-	Cohort	8-19 houses per (2) village	N/A	HLC (inside)	Cotton (15 km)	12 months	Yes*				
						Cross-sectional	3692	0 – 5	-		N/A				Yes	
Robert et al. 1985	Burkina Faso	1983	Rural (Vallée du Kou)	*An. gambiae* s.l.	-	Cohort	176 captures across 4 villages	N/A	HLC (inside)	Savannah (20 km)	12 months	Yes*	Yes	Yes*		

Study	Country	Year	Setting (irrigation scheme)	Primary vectors	Transmission / LLIN coverage / IRS coverage	Study design	Study size	Age group	Recruitment of participants / Method of mosquito collection	Control group(s) (distance from rice)	Follow-up	Outcomes included				
												Entomological			Epidemiological	
												HBR[a]	SR[a]	EIR[a]	PR[a]	MI[a]
Mukiama & Mwangi 1989	Kenya	1984	Rural (Mwea)	*An. gambiae* s.l. & *An. pharoensis*	-	Cohort	2 houses fortnightly per (4) village	N/A	Based on permission of owner / PSC, CDCLT and exit traps	Periphery of rice area (5 km)	12 months	Yes*				
Josse et al. 1987	Cameroon	1985	Rural	*An. gambiae* s.l.	-	Cross-sectional	2375	2 – 9	Sampling random clusters	Non-rice growing area	N/A				Yes	
Boudin et al. 1992	Burkina Faso	1985	Rural (Vallée du Kou)	*An. gambiae* s.l.	-	Cross-sectional	2120	0 – 14	Voluntary participation	Savannah	N/A				Yes	
Githeko et al. 1993	Kenya	1989	Rural (Ahero)	*An. arabiensis*	-	Cohort	3 houses weekly per (2) village	N/A	HLC (inside and outside)	Sugar belt (6 km)	13 months	Yes*	Yes	Yes*		
Githeko et al. 1996	Kenya	1989	Rural (Ahero)	*An. gambiae* s.l.	-	Cohort	2-3 houses monthly per (2) village	N/A	PSC	Sugar belt (6 km)	13 months	Yes*				
Faye et al. 1993a	Senegal	1990	Rural	*An. gambiae* s.l.	-	Cohort	2 houses monthly per (3) village	N/A	HLC (inside and outside)	Traditional agriculture (5 km)	17 months	Yes*				
Faye et al. 1993b					-	Cross-sectional	1149	0 – 9	-		N/A				Yes	
Gbakima 1994	Sierra Leone	1991	Rural	Not reported	-	Cross-sectional	1106	All ages	Voluntary participation	Undeveloped swamps (5 km)	N/A				Yes	
Thomson et al. 1994	The Gambia	1991	Rural	*An. gambiae* s.l.	-	Cohort	1 house weekly per (16) village	N/A	PSC and exit traps	Non-rice growing	7 months	Yes*	Yes	Yes*		

Study	Country	Year	Setting (irrigation scheme)	Primary vectors	Transmission / LLIN coverage / IRS coverage	Study design	Study size	Age group	Recruitment of participants / Method of mosquito collection	Control group(s) (distance from rice)	Follow-up	Outcomes included				
												Entomological			Epidemiological	
												HBR[a]	SR[a]	EIR[a]	PR[a]	MI[a]
						Cross-sectional	1465	1 – 4	Compounds randomly selected, up to 30 children recruited from each 10 villages		N/A				Yes	
Faye et al. 1995	Senegal	1992	Rural	*An. gambiae* s.l.	-	Cohort	156-168 trap-nights	0 – 10	HLC (inside and outside)	Traditional agriculture (5 km)	26 months	Yes*	Yes*			
						Cross-sectional	985	0 – 10	-		N/A				Yes	
Githeko et al. 1994	Kenya	1993	Rural (Ahero)	*An. gambiae* s.l.	-	Cohort	41-65 trap-nights	N/A	CDCLT	Sugar belt (6 km)	-	Yes*				
Ijumba et al. 2002a	Tanzania	1994	Rural (Lower Moshi)	*An. gambiae* s.l.	-	Cohort	2 houses fortnightly per (3) village	N/A	CDCLT	Savannah (8 km) and sugarcane irrigation (15 km)	12 months	Yes*	Yes	Yes*		
Ijumba et al. 2002b						Cross-sectional	2951	1 – 4	All children enrolled		N/A				Yes	Yes
Marrama et al. 2004	Madagas-car	1994	Rural	*An. arabiensis* & *An. gambiae* s.s.	-	Cohort	8-16 captures monthly per (3) village	N/A	HLC (inside and outside), PSC, CDCLT	Natural sub-arid ecosystem	12 – 36 months	Yes	Yes	Yes*		
Doannio et al. 2006	Côte d'Ivoire	1994	Rural	*An. gambiae* s.l.	-	Cohort	4 houses (10-22 captures per village)	N/A	Selection based on group of dwellings / HLC (inside and outside)	Humid wooded savannah	10 months	Yes*				
Dolo et al. 2004	Mali	1995	Rural	*An. gambiae* s.l.	-	Cohort	2 houses per (6) village	N/A	HLC (inside and outside)	Savannah (10 -15 km)	30 months	Yes*	Yes	Yes*		
Sissoko et al. 2004						Cross-sectional	9134	0 – 14	All children of appropriate age interviewed						Yes	Yes*

Study	Country	Year	Setting (irrigation scheme)	Primary vectors	Transmission / LLIN coverage / IRS coverage	Study design	Study size	Age group	Recruitment of participants / Method of mosquito collection	Control group(s) (distance from rice)	Follow-up	Outcomes included				
												Entomological			Epidemiological	
												HBR[a]	SR[a]	EIR[a]	PR[a]	MI[a]
Briet et al. 2003	Côte d'Ivoire	1996	Rural	*An. gambiae* s.l.	-	Cohort	Every 6 weeks per (13) village	N/A	HLC (inside and outside)	Inland valley without rice cultivation	12 months	Yes*				
Henry et al. 2003	Côte d'Ivoire	1997	Rural	*An. gambiae* s.l.	4% used mosquito nets	Repeated cross-sectional	36217	All ages	Random selection of compounds within randomly selected villages	Lowlands with dense vegetation	N/A				Yes	
						Cohort	42818				10 months					Yes
Betsi et al. 2003	Côte d'Ivoire	1998	Rural	Study concerns *An. funestus*	-	Cohort	3 houses per (3) village	N/A	HLC (inside and outside)	Lowlands with dense vegetation	12 months	Yes* (AF only)	Yes (AF only)	Yes* (AF, AG)		
Betsi et al. 2012	Côte d'Ivoire	1998	Rural	*An. gambiae* s.l.	-	Cohort	3 houses every 6 weeks per (6) village	N/A	HLC (inside and outside)	Lowlands with dense vegetation	13 months	Yes*				
Assi et al. 2013	Côte d'Ivoire	1998	Rural	*An. gambiae* s.l.	-	Repeated cross-sectional	29330	All ages	Random selection of villages	Inland valley without rice cultivation	N/A				Yes	
						Cohort	33678	All ages			12 months					Yes
Baldet et al. 2003	Burkina Faso	1999	Rural (Vallee du Kou)	*An. gambiae* s.l.	-	Cohort	4 houses monthly per (3) village	N/A	HLC (inside)	Savannah	12 months	Yes*		Yes*		
Dabire et al. 2007	Burkina Faso	2000	Rural	Study concerns *An. funestus*	-	Cohort	4 houses weekly per (3) village	N/A	HLC (inside and outside)	Savannah (50 km)	5 months	Yes*	Yes	Yes*		
Mutero et al. 2004	Kenya	2001	Rural (Mwea)	*An. arabiensis*	-	Cohort	12 houses monthly per (4) village	N/A	HLC (inside and outside)	Non-irrigated (16 km)	12 months	Yes*				

Study	Country	Year	Setting (irrigation scheme)	Primary vectors	Transmission / LLIN coverage / IRS coverage	Study design	Study size	Age group	Recruitment of participants / Method of mosquito collection	Control group(s) (distance from rice)	Follow-up	Outcomes included				
												Entomological			Epidemiological	
												HBR[a]	SR[a]	EIR[a]	PR[a]	MI[a]
						Cross-sectional	206	0 – 9	All households with children <10 years of age identified & proportionately sampled		N/A				Yes	
Amusan et al. 2005	Nigeria	2001	Rural	*An. gambiae* s.l.	-	Cohort	4 houses weekly per (2) village	N/A	CDCLT	Rubber & oil plantation within lowland forest	12 months	Yes*				
Okoye 2003	Ghana	2002	Rural	*An. gambiae* s.l.	-	Cohort	4 houses monthly per (2) village	N/A	HLC (inside and outside) and PSC	Non-irrigated (10 km)	6 months	Yes*	Yes	Yes*		
Koudou et al. 2009	Côte d'Ivoire	2002	Rural	*An. gambiae* s.l.	12% slept under a bednet	Repeated cross-sectional	3212	0 – 15	All children randomly selected from primary schools	Subsistence agriculture / intensive vegetable farming	36 months years				Yes	
Koudou et al. 2010						Cohort	4 houses every 2 months per (2) village	N/A	HLC (inside and outside)			Yes	Yes	Yes		
Manoukis et al. 2006	Mali	2004	Rural	*An. gambiae* s.l.	-	Cross-sectional	2 houses per (3) village	N/A	HLC (inside and outside)	Non-irrigated area (10 km)	N/A	Yes				
Muturi et al. 2006	Kenya	2004	Rural (Mwea)	*An. arabiensis*	-	Cohort	30 houses fortnightly	N/A	Equal numbers of houses were selected from centre and periphery / HLC (inside and outside)	Other crops e.g., maize, beans, bananas (15 km)	12 months	Yes*				
Muturi et al. 2008												Yes	Yes	Yes		
Atangana et al. 2012	Cameroon	2004	Rural	*An. arabiensis* & *An. gambiae* s.s.	-	Cohort	40 trap-nights per village	N/A	HLC (inside and outside) and PSC	Market gardening (200 km)	24 months	Yes*	Yes	Yes*		

Study	Country	Year	Setting (irrigation scheme)	Primary vectors	Transmission / LLIN coverage / IRS coverage	Study design	Study size	Age group	Recruitment of participants / Method of mosquito collection	Control group(s) (distance from rice)	Follow-up	Outcomes included				
												Entomological			Epidemiological	
												HBR[a]	SR[a]	EIR[a]	PR[a]	MI[a]
Rumisha et al. 2019	Tanzania	2004	Rural	*An. gambiae* s.l.	50%[b] / 75 – 85% slept under a mosquito net	Cross-sectional	7888	6 – 15	Primary schools within selected villages	Sugar (5 km) and savannah (15 km)	N/A				Yes	
Mboera et al. 2010	Tanzania	2004	Rural	*An. gambiae* s.l.	-	Cohort	3 houses monthly per (5) village	N/A	House selection based on settlement patterns (and similar construction) / CDCLT	Sugar (5 km) and savannah (15 km)	12 months	Yes*	Yes	Yes*		
Mboera et al. 2011	Tanzania	2005	Rural	*An. gambiae* s.l.	-	Cross-sectional	578	0 – 15	Schoolchildren (lower classes 1-4) from 6 primary schools	Sugar (5 km) and savannah (15 km)	N/A				Yes	
Antonio-Nkondjio et al. 2008	Cameroon	2006	Rural	*An. arabiensis, An. gambiae* s.s. & *An. funestus*	-	Cohort	20-30 houses fortnightly per (3) village	N/A	HLC (inside and outside) and PSC	Other crops e.g. maize, millet, groundnut (20 km)	5 months	Yes*	Yes	Yes*		
Ntonga et al. 2010	Cameroon	2006	Rural	*An. gambiae* s.l.	-	Cohort	3 houses monthly per (2) village	N/A	HLC (inside)	Rich in fish species	12 months	Yes*	Yes	Yes*		
Diakite et al. 2015	Côte d'Ivoire	2007	Rural	*An. gambiae* s.l.	-	Repeated cross-sectional	4 sites monthly per (5) village	N/A	HLC (inside and outside)	Non-irrigated / not developed rice cultivation yet	33 months	Yes*	Yes	Yes*		
Toure et al. 2016	Mali	2010	Rural	*An. gambiae* s.l.	40% [b] / 82% children below 10 slept under LLIN night prior survey	Cross-sectional	1145	0.5 – 9	Random selection of households. All children aged 6 months to 9 years enrolled to cohort study	Dry area where ground water pools depend on rainfall	N/A				Yes	
						Cohort	549				12 months					Yes

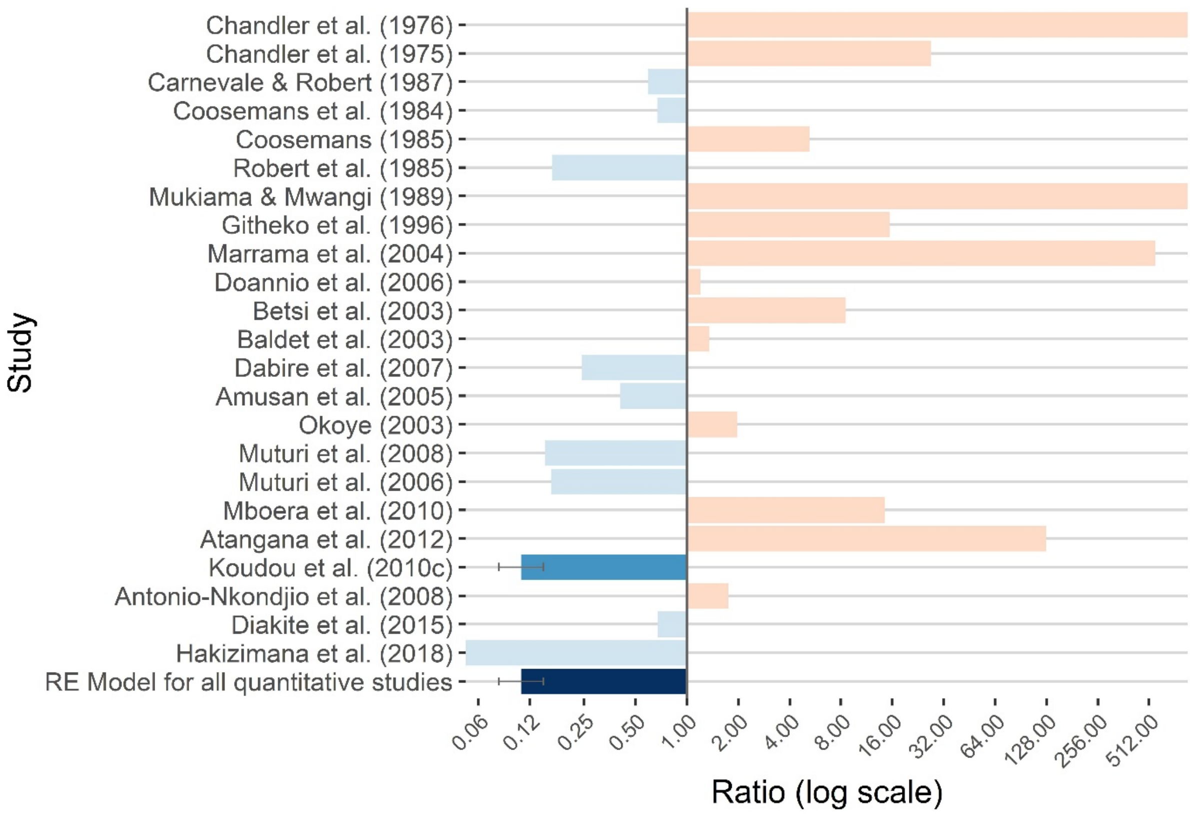
Study	Country	Year	Setting (irrigation scheme)	Primary vectors	Transmission / LLIN coverage / IRS coverage	Study design	Study size	Age group	Recruitment of participants / Method of mosquito collection	Control group(s) (distance from rice)	Follow-up	Outcomes included				
												Entomological			Epidemiological	
												HBR[a]	SR[a]	EIR[a]	PR[a]	MI[a]
Hakizimana et al. 2018	Rwanda	2010	Rural	*An. gambiae* s.l.	-	Cohort	3 houses monthly per (21) village	N/A	HLC (inside and outside)	No rice cultivation	24 months	Yes*	Yes	Yes*		
Mboera et al. 2015a	Tanzania	2012	Rural	*An. gambiae* s.l.	Over 83% of households had ITN	Cross-sectional	3 houses per (5) village	N/A	CDCLT	Dry / wet savannah (5-10 km)	N/A	Yes*	Yes*			
Mboera et al. 2015b							1019	0 – 15	Schoolchildren were recruited		N/A				Yes	
Hien et al. 2017	Burkina Faso	2014	Rural (Vallée du Kou)	*An. gambiae* s.l.	15-30%	Cross-sectional	614	0 – 15	Random sampling on individuals	Subsistence agriculture (15 km)	N/A				Yes	
Babamale et al. 2020	Nigeria	2016	Rural	-	-	Cross-sectional	230	All ages	Voluntary participation based on study criteria	Sugar and yam	N/A				Yes	
Total (quantitative) =												**4**	**17**	**2**	**22**	**4**
Total (semi-quantitative) =												**31**		**16**		
Total (qualitative) =													**2**	**1**	**1**	**1**
Total (qualitative, semi-quantitative & quantitative) =												**36**	**19**	**19**	**23**	**5**
Total =												**36**			**23**	
Total =												**53**				

- = not reported
* = analysed qualitative / semi-quantitative
[a] HBR = human biting rate; SR = sporozoite rate; EIR = entomological inoculation rate; PR = parasite rate; MI = malaria incidence
[b] Prevalence considered for sample size estimation
HLC = human landing catch
CDCLT = CDC light trap
PSC = Pyrethrum spray catch

Appendix 3.3. Sensitivity analysis on the year 2003 as a cut-off point

Year	Risk ratio pre-scale-up	Number of studies pre-scale-up	Risk ratio post-scale-up	Number of studies post-scale-up	Wald-type test p-value
2001	0.81 (0.61 – 1.08)	17	1.61 (0.99 – 2.60)	8	0.016
2002	0.82 (0.63 – 1.06)	18	1.73 (1.01 – 2.96)	7	0.014
2003	0.82 (0.63 – 1.06)	18	1.73 (1.01 – 2.96)	7	0.014
2004	0.87 (0.66 – 1.16)	19	1.62 (0.87 – 3.01)	6	0.075
2005	0.93 (0.71 – 1.22)	21	1.66 (0.64 – 4.29)	4	0.247

Appendix 3.4. Meta-analysis of the association between rice cultivation and *An. funestus* human biting rate



Ratio of human biting rate means (in rice areas compared to non-rice areas) and their 95% confidence intervals (only in quantitative studies, n=1, presented as error bars) are plotted according to year of study. Whilst light-coloured bars indicate semi-quantitative studies, solid-coloured bars indicate quantitative studies. Pooled effect estimates of quantitative studies are presented as dark-coloured bars at the bottom.

Appendix 3.5A. Risk of bias assessment for studies with human biting rate included in the quantitative analysis (cohort studies, n=4).

Study		Selection				Comparability	Outcome			Overall quality assessment (max = 8)
		Representativeness of the exposed group	**Selection of the non-exposed group**	**Ascertainment of exposure (risk factor)**	**Demonstration that outcome of interest was not present at start of study**	**Comparability of groups on basis of the design or analysis**	**Assessment of outcome**	**Was follow-up long enough for outcomes to occur?**	**Adequacy of follow up of cohorts**	
	*	*a) Truly representative of the average individual or household in the community* *b) Somewhat representative of the average individual or household in the community*	*a) Drawn from the same community as the exposed group*	*a) Validated measurement tool* *b) Structured interview*	*a) Yes*	*a) Study controls for age* *b) Study controls for socioeconomic status, bednet use or any additional factor*	*a) Validated measurement tool* *b) Record linkage*	*a) Yes*	*a) Complete follow-up – all subjects accounted for* *b) Subjects lost to follow-up unlikely to introduce bias*	
		c) Selected group of users e.g., nurses, volunteers *d) No description of the derivation of the sample*	*b) Drawn from a different source* *c) No description of the derivation of the non-exposed group*	*c) Written self-report* *d) No description*	*b) No*	*c) Study does not control for other factors*	*c) Self report* *d) No description*	*b) No*	*c) Follow up rate greater than 80% and no description of those lost* *d) No statement*	
Marrama et al. 2004		b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5
Koudou et al. 2010		b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5
Manoukis et al. (2006)		b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5
Muturi et al. 2008		b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5

Appendix 3.5B. Risk of bias assessment for studies with sporozoite rate included in the quantitative analysis (cohort studies, n=17).

Study		Selection				Comparability	Outcome			Overall quality assessment (max = 8)
		Representativeness of the exposed group	**Selection of the non-exposed group**	**Ascertainment of exposure (risk factor)**	**Demonstration that outcome of interest was not present at start of study**	**Comparability of groups on basis of the design or analysis**	**Assessment of outcome**	**Was follow-up long enough for outcomes to occur?**	**Adequacy of follow up of cohorts**	
	*	*a) Truly representative of the average individual or household in the community* *b) Somewhat representative of the average individual or household in the community*	*a) Drawn from the same community as the exposed group*	*a) Validated measurement tool* *b) Structured interview*	*a) Yes*	*a) Study controls for age* *b) Study controls for socioeconomic status, bednet use or any additional factor*	*a) Validated measurement tool* *b) Record linkage*	*a) Yes*	*a) Complete follow-up – all subjects accounted for* *b) Subjects lost to follow-up unlikely to introduce bias*	
		c) Selected group of users e.g. nurses, volunteers *d) No description of the derivation of the sample*	*b) Drawn from a different source* *c) No description of the derivation of the non-exposed group*	*c) Written self-report* *d) No description*	*b) No*	*c) Study does not control for other factors*	*c) Self report* *d) No description*	*b) No*	*c) Follow up rate greater than 80% and no description of those lost* *d) No statement*	
Chandler et al. 1975		b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5
Chandler et al. 1976		b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5
Carnevale & Robert 1987		b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5
Coosemans 1985		b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5
Robert et al. 1985		b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5
Mukiama & Mwangi 1989		b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5
Githeko et al. 1993		b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5
Githeko et al. 1996		b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5
Faye et al. 1993a		b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5

Study		Selection				Comparability	Outcome			Overall quality assessment (max = 8)
		Representativeness of the exposed group	**Selection of the non-exposed group**	**Ascertainment of exposure (risk factor)**	**Demonstration that outcome of interest was not present at start of study**	**Comparability of groups on basis of the design or analysis**	**Assessment of outcome**	**Was follow-up long enough for outcomes to occur?**	**Adequacy of follow up of cohorts**	
	*	*a) Truly representative of the average individual or household in the community*	*a) Drawn from the same community as the exposed group*	*a) Validated measurement tool*	*a) Yes*	*a) Study controls for age*	*a) Validated measurement tool*	*a) Yes*	*a) Complete follow-up – all subjects accounted for*	
		b) Somewhat representative of the average individual or household in the community		*b) Structured interview*		*b) Study controls for socioeconomic status, bednet use or any additional factor*	*b) Record linkage*		*b) Subjects lost to follow-up unlikely to introduce bias*	
		c) Selected group of users e.g. nurses, volunteers	*b) Drawn from a different source*	*c) Written self-report*	*b) No*	*c) Study does not control for other factors*	*c) Self report*	*b) No*	*c) Follow up rate greater than 80% and no description of those lost*	
		d) No description of the derivation of the sample	*c) No description of the derivation of the non-exposed group*	*d) No description*			*d) No description*		*d) No statement*	
Thomson et al. 1994		b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5
Faye et al. 1995		b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5
Githeko et al. 1994		b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5
Ijumba et al. 2002a		b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5
Doannio et al. 2006		b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5
Dolo et al. 2004		b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5
Briet et al. 2003		b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5
Betsi et al. 2003		b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5
Betsi et al. 2012		b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5
Baldet et al. 2003		b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5
Dabire et al. 2007		b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5
Mutero et al. 2004		b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5
Amusan et al. 2005		b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5

Study	Selection				Comparability	Outcome			Overall quality assessment (max = 8)
	Representativeness of the exposed group	Selection of the non-exposed group	Ascertainment of exposure (risk factor)	Demonstration that outcome of interest was not present at start of study	Comparability of groups on basis of the design or analysis	Assessment of outcome	Was follow-up long enough for outcomes to occur?	Adequacy of follow up of cohorts	
*	*a) Truly representative of the average individual or household in the community*	*a) Drawn from the same community as the exposed group*	*a) Validated measurement tool*	*a) Yes*	*a) Study controls for age*	*a) Validated measurement tool*	*a) Yes*	*a) Complete follow-up – all subjects accounted for*	
*	*b) Somewhat representative of the average individual or household in the community*		*b) Structured interview*		*b) Study controls for socioeconomic status, bednet use or any additional factor*	*b) Record linkage*		*b) Subjects lost to follow-up unlikely to introduce bias*	
	c) Selected group of users e.g. nurses, volunteers	*b) Drawn from a different source*	*c) Written self-report*	*b) No*	*c) Study does not control for other factors*	*c) Self report*	*b) No*	*c) Follow up rate greater than 80% and no description of those lost*	
	d) No description of the derivation of the sample	*c) No description of the derivation of the non-exposed group*	*d) No description*			*d) No description*		*d) No statement*	
Okoye 2003	b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5
Koudou et al. 2010	b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5
Muturi et al. 2008	b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5
Muturi et al. 2006	b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5
Mboera et al. 2010	b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5
Manoukis et al. 2006	b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5
Atangana et al. 2012	b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5
Ntonga et al. 2010	b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5
Antonio-Nkondjio et al. 2008	b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5
Diakite et al. 2015	b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5
Hakizimana et al. 2018	b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5
Mboera et al. 2015	b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5

Appendix 3.5C. Risk of bias assessment for studies with entomological inoculation rate included in the quantitative analysis (cohort studies, n=2).

Study		**Selection**				**Comparability**	**Outcome**			**Overall quality assessment (max = 8)**
		Representativeness of the exposed group	**Selection of the non-exposed group**	**Ascertainment of exposure (risk factor)**	**Demonstration that outcome of interest was not present at start of study**	**Comparability of groups on basis of the design or analysis**	**Assessment of outcome**	**Was follow-up long enough for outcomes to occur?**	**Adequacy of follow up of cohorts**	
	*	*a) Truly representative of the average individual or household in the community*	*a) Drawn from the same community as the exposed group*	*a) Validated measurement tool*	*a) Yes*	*a) Study controls for age*	*a) Validated measurement tool*	*a) Yes*	*a) Complete follow-up – all subjects accounted for*	
		b) Somewhat representative of the average individual or household in the community		*b) Structured interview*		*b) Study controls for socioeconomic status, bednet use or any additional factor*	*b) Record linkage*		*b) Subjects lost to follow-up unlikely to introduce bias*	
		c) Selected group of users e.g. nurses, volunteers	*b) Drawn from a different source*	*b) Written self-report*	*b) No*	*c) Study does not control for other factors*	*b) Self report*	*b) No*	*c) Follow up rate greater than 80% and no description of those lost*	
		d) No description of the derivation of the sample	*c) No description of the derivation of the non-exposed group*	*d) No description*			*d) No description*		*d) No statement*	
Koudou et al. 2010		b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5
Muturi et al. 2008		b) *	b)	a) *	b)	c)	a) *	a) *	b) *	5

Appendix 3.5D. Risk of bias assessment for studies with parasite prevalence included in the quantitative analysis (cross-sectional studies, n=22).

Study		**Selection**				**Comparability**	**Outcome**		**Overall quality assessment (max = 9)**
		Representativeness of the sample	**Sample size**	**Non-respondents**	**Ascertainment of exposure (risk factor)**	**Comparability of groups on basis of the design or analysis**	**Ascertainment of outcome**	**Statistical test**	
	**				*a) Validated measurement tool*		*a) Validated measurement tool*		
	*	*a) Truly representative of the average individual or household in the community* *b) Somewhat representative of the average individual or household in the community*	*a) Justified and satisfactory (power calculation included)*	*a) Comparability between respondents and non-respondents characteristics is established, and the response rate is satisfactory*	*b) Non-validated measurement tool, but the tool is available or described*	*a) Study controls for age* *b) Study controls for socioeconomic status, bednet use or any additional factor*	*b) Non-validated measurement method, but the method is available or described*	*a) The statistical test used to analyse the data is clearly described and appropriate, and the measurement of the association is presented, including confidence intervals and probability level*	
		c) Selected group of users e.g. nurses, volunteers *d) No description of the derivation of the sample*	*b) Not justified*	*b) The response rate is unsatisfactory, or the comparability between respondents and non-respondents is unsatisfactory* *c) No description of response rate or the characteristics or the responders and non-responders*	*c) No description of the measurement tool*	*c) Study does not control for other factors*	*c) No description of the measurement tool*	*b) The statistical test is not appropriate, not described or incomplete*	
Audibert et al. 1990		b) *	b)	c)	b) *	a) *	a) **	a) *	6
Carnevale & Robert 1987		b) *	b)	c)	b) *	c)	a) **	a) *	5
Coosemans et al. 1984		b) *	b)	c)	b) *	a) *	a) **	a) *	6
Couprie et al. 1985		b) *	b)	c)	b) *	c)	a) **	a) *	5
Josse et al. 1987		a) *	b)	c)	b) *	a) *	a) **	a) *	6
Boudin et al. 1992		b) *	b)	c)	b) *	a) *	a) **	a) *	6
Faye et al. 1993b		b) *	b)	c)	b) *	a) *	a) **	a) *	6

Study		Selection				Comparability	Outcome		Overall quality assessment (max = 9)
		Representativeness of the sample	Sample size	Non-respondents	Ascertainment of exposure (risk factor)	Comparability of groups on basis of the design or analysis	Ascertainment of outcome	Statistical test	
	**				*a) Validated measurement tool*		*a) Validated measurement tool*		
	*	*a) Truly representative of the average individual or household in the community*	*a) Justified and satisfactory (power calculation included)*	*a) Comparability between respondents and non-respondents characteristics is established, and the response rate is satisfactory*	*b) Non-validated measurement tool, but the tool is available or described*	*a) Study controls for age*	*b) Non-validated measurement method, but the method is available or described*	*a) The statistical test used to analyse the data is clearly described and appropriate, and the measurement of the association is presented, including confidence intervals and probability level*	
		b) Somewhat representative of the average individual or household in the community				*b) Study controls for socioeconomic status, bednet use or any additional factor*			
		c) Selected group of users e.g. nurses, volunteers	*b) Not justified*	*b) The response rate is unsatisfactory, or the comparability between respondents and non-respondents is unsatisfactory*	*c) No description of the measurement tool*	*c) Study does not control for other factors*	*c) No description of the measurement tool*	*b) The statistical test is not appropriate, not described or incomplete*	
		d) No description of the derivation of the sample		*c) No description of response rate or the characteristics or the responders and non-responders*					
Gbakima 1994		b) *	b)	c)	b) *	c)	a) **	a) *	5
Thomson et al. 1994		b) *	b)	c)	b) *	c)	a) **	a) *	5
Faye et al. 1995		b) *	b)	c)	b) *	a) *	a) **	a) *	6
Ijumba et al. 2002b		b) *	b)	c)	a) **	a) *	a) **	a) *	7
Sissoko et al. 2004		b) *	b)	c)	b) *	a) *	a) **	a) *	6
Henry et al. 2003		a) *	a) *	c)	a) **	a) *	a) **	a) *	8
Assi et al. 2013		a) *	a) *	c)	a) **	a) *	a) **	a) *	8
Mutero et al. 2004		a) *	a) *	c)	b) *	a) *	a) **	a) *	7
Koudou et al. 2009		b) *	b)	c)	a) **	a) *	a) **	a) *	7
Rumisha et al. 2019		b) *	a) *	a) *	a) **	a) *	a) **	a) *	9
Mboera et al. 2011		c)	b)	c)	a) **	c)	a) **	a) *	5
Toure et al. 2016		b) *	a) *	c)	a) **	a) and b) *	a) **	a) *	8
Mboera et al. 2015b		c)	b)	c)	a) **	a) *	a) **	a) *	6

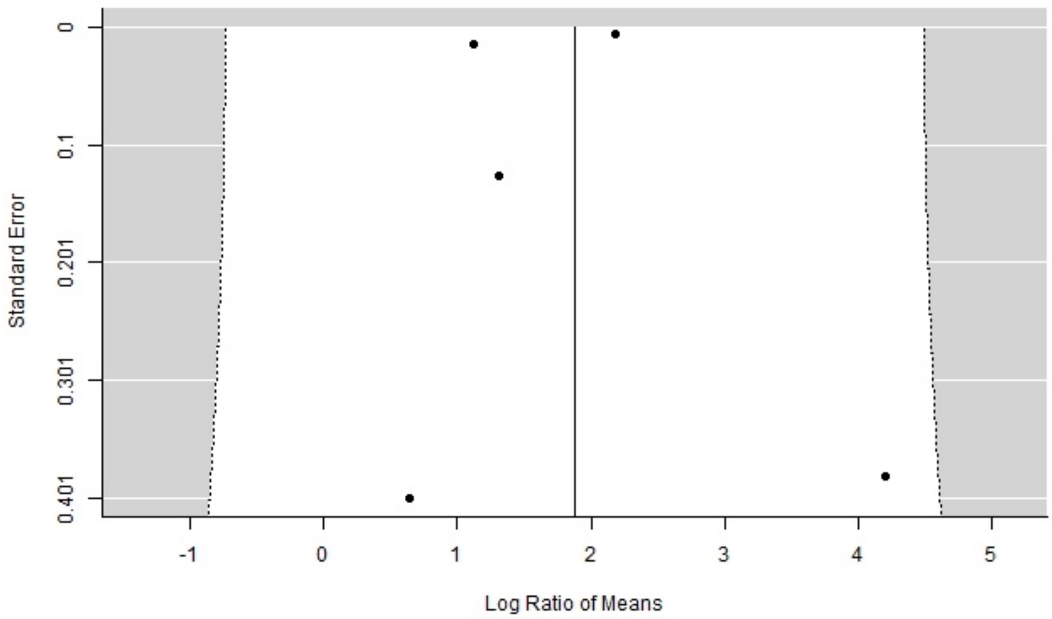
Study		Selection				Comparability	Outcome		Overall quality assessment (max = 9)
		Representativeness of the sample	**Sample size**	**Non-respondents**	**Ascertainment of exposure (risk factor)**	**Comparability of groups on basis of the design or analysis**	**Ascertainment of outcome**	**Statistical test**	
	**				*a) Validated measurement tool*		*a) Validated measurement tool*		
	*	*a) Truly representative of the average individual or household in the community* *b) Somewhat representative of the average individual or household in the community*	*a) Justified and satisfactory (power calculation included)*	*a) Comparability between respondents and non-respondents characteristics is established, and the response rate is satisfactory*	*b) Non-validated measurement tool, but the tool is available or described*	*a) Study controls for age* *b) Study controls for socioeconomic status, bednet use or any additional factor*	*b) Non-validated measurement method, but the method is available or described*	*a) The statistical test used to analyse the data is clearly described and appropriate, and the measurement of the association is presented, including confidence intervals and probability level*	
		c) Selected group of users e.g. nurses, volunteers *d) No description of the derivation of the sample*	*b) Not justified*	*b) The response rate is unsatisfactory, or the comparability between respondents and non-respondents is unsatisfactory* *c) No description of response rate or the characteristics or the responders and non-responders*	*c) No description of the measurement tool*	*c) Study does not control for other factors*	*c) No description of the measurement tool*	*b) The statistical test is not appropriate, not described or incomplete*	
Hien et al. 2017		b) *	a) *	c)	a) **	a) *	a) **	a) *	8
Babamale et al. 2020		b) *	b)	c)	b) *	c)	a) **	a) *	5

Appendix 3.5E. Risk of bias assessment for studies with clinical malaria included in the quantitative analysis (cohort studies, n=4).

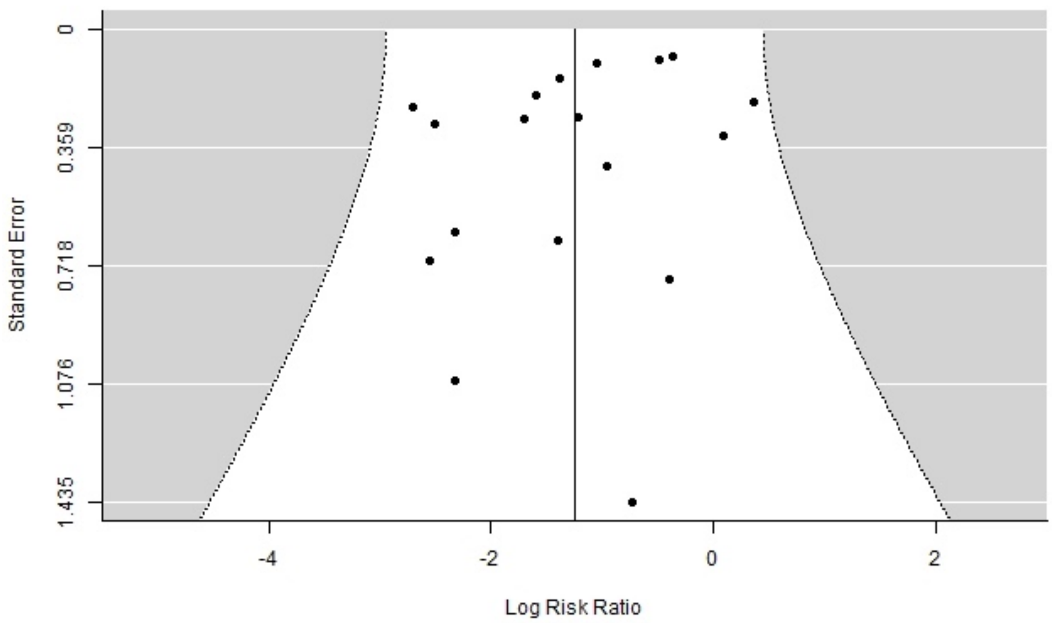
Study		Selection				Comparability	Outcome			Overall quality assessment (max = 8)
		Representativeness of the exposed group	**Selection of the non-exposed group**	**Ascertainment of exposure (risk factor)**	**Demonstration that outcome of interest was not present at start of study**	**Comparability of groups on basis of the design or analysis**	**Assessment of outcome**	**Was follow-up long enough for outcomes to occur?**	**Adequacy of follow up of cohorts**	
	*	*a) Truly representative of the average individual or household in the community* *b) Somewhat representative of the average individual or household in the community*	*a) Drawn from the same community as the exposed group*	*a) Validated measurement tool*	*a) Yes*	*a) Study controls for age* *b) Study controls for socioeconomic status, bednet use or any additional factor*	*a) Validated measurement tool*	*a) Yes*	*a) Complete follow-up – all subjects accounted for* *b) Subjects lost to follow-up unlikely to introduce bias*	
		c) Selected group of users e.g. nurses, volunteers *d) No description of the derivation of the sample*	*b) Drawn from a different source* *c) No description of the derivation of the non-exposed group*	*b) Written self-report* *d) No description*	*b) No*	*c) Study does not control for other factors*	*b) Self report* *d) No description*	*b) No*	*c) Follow up rate greater than 80% and no description of those lost* *d) No statement*	
Ijumba et al. 2002b		b) *	b)	a) *	b)	a) *	a) *	a) *	d)	5
Henry et al. 2003		a) *	b)	a) *	b)	a) *	a) *	a) *	d)	5
Assi et al. 2013		a) *	b)	a) *	b)	a) *	a) *	a) *	b) *	6
Toure et al. 2016		b) *	b)	a) *	b)	a) and b) *	a) *	a) *	b) *	6

Appendix 3.6. Funnel plots assessing publication bias in the meta-analysis of malaria indicators in areas of rice vs. non-rice cultivation.

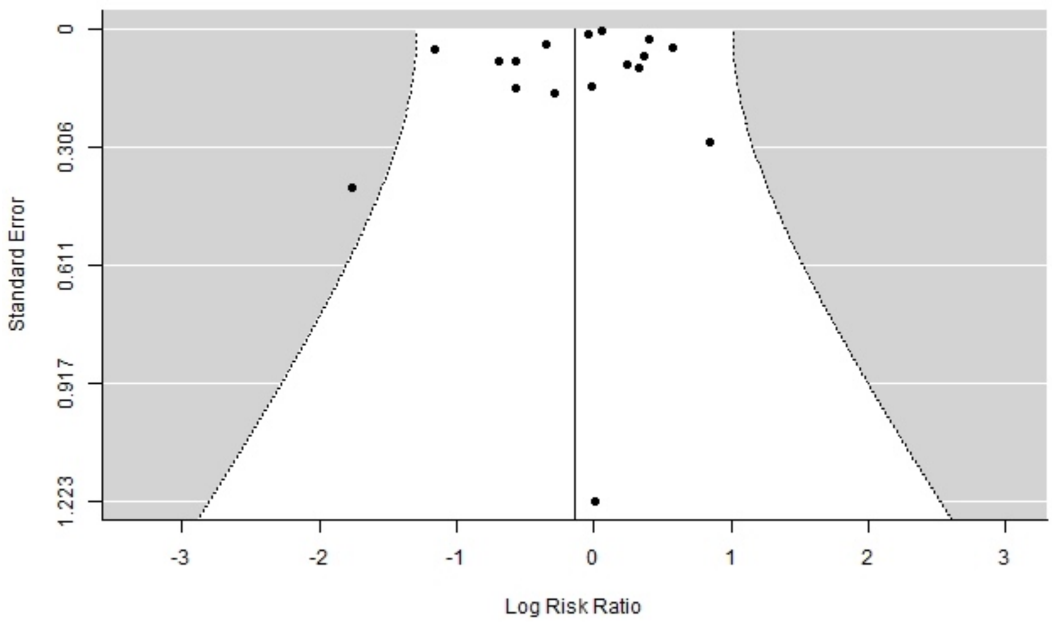
A)



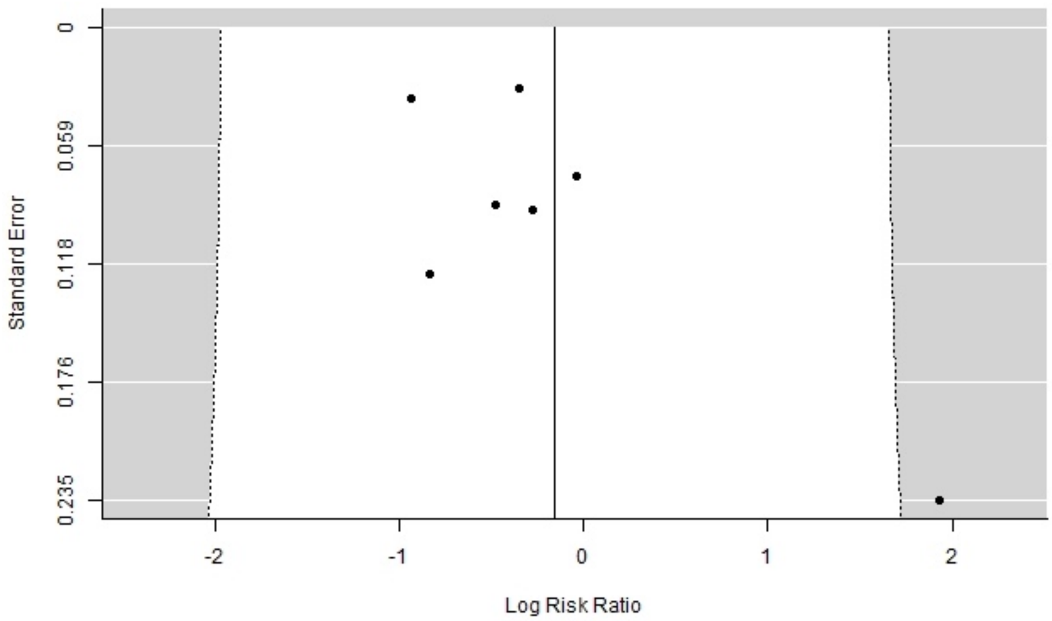
B)



C)



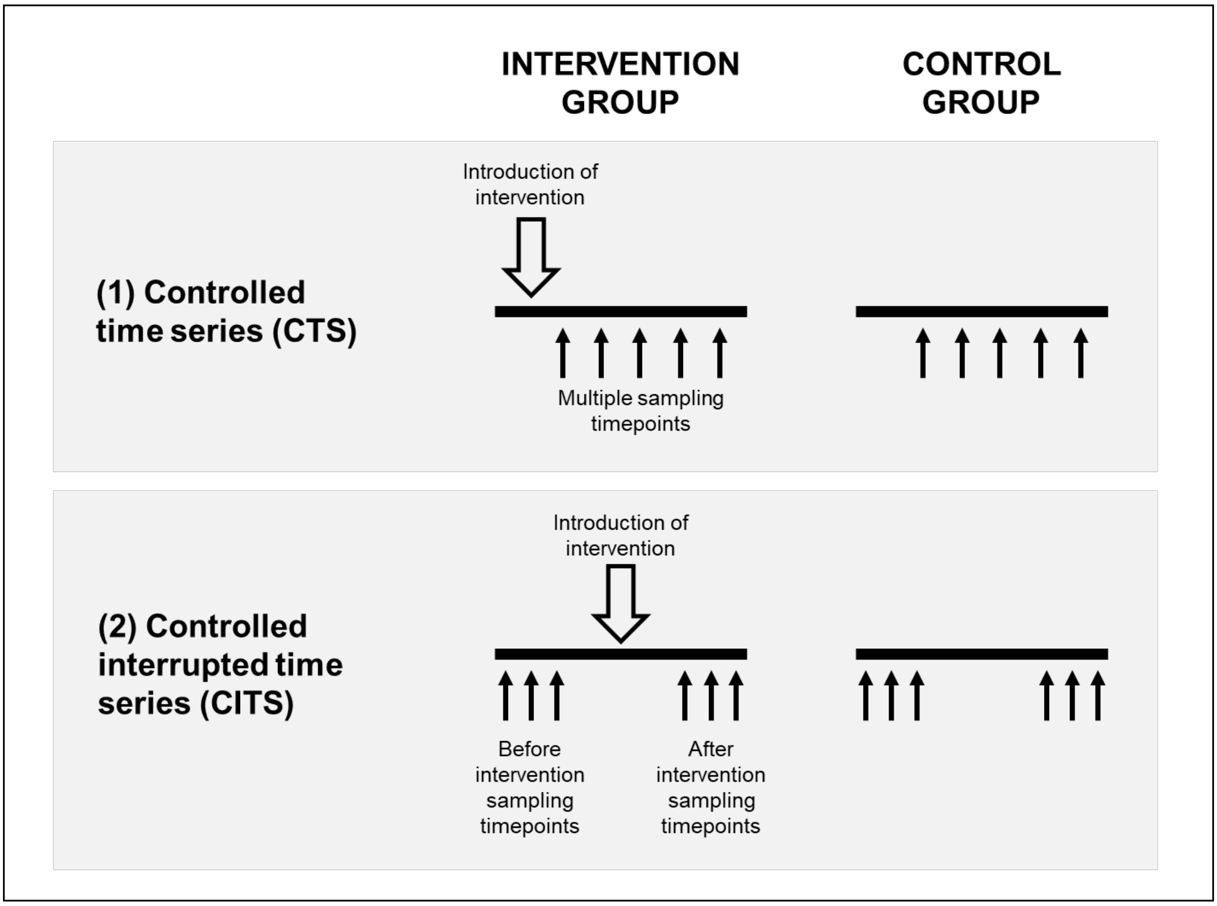
D)



The funnel plots illustrate the estimates of effect sizes against study size, and are used to detect publication bias. In the absence of publication bias, the plot creates a roughly funnel-shaped distribution. An asymmetric funnel indicates the possibility of publication bias, small study effects or selective outcome reporting. Plots show studies reporting (A) *An. gambiae* s.l. human biting rate (test for funnel plot asymmetry: z = 0.51, p = 0.61), (B) *An. gambiae* s.l. sporozoite rates (z = -0.90, p = 0.37), (C) parasite prevalence pre-2003 (z = -0.63, p = 0.53) and (D) parasite prevalence post-2003 (z = 3.19, p = 0.0014).

Appendix 4. Chapter 4 Supplementary Material

Appendix 4.1. Illustration of experimental study designs considered.



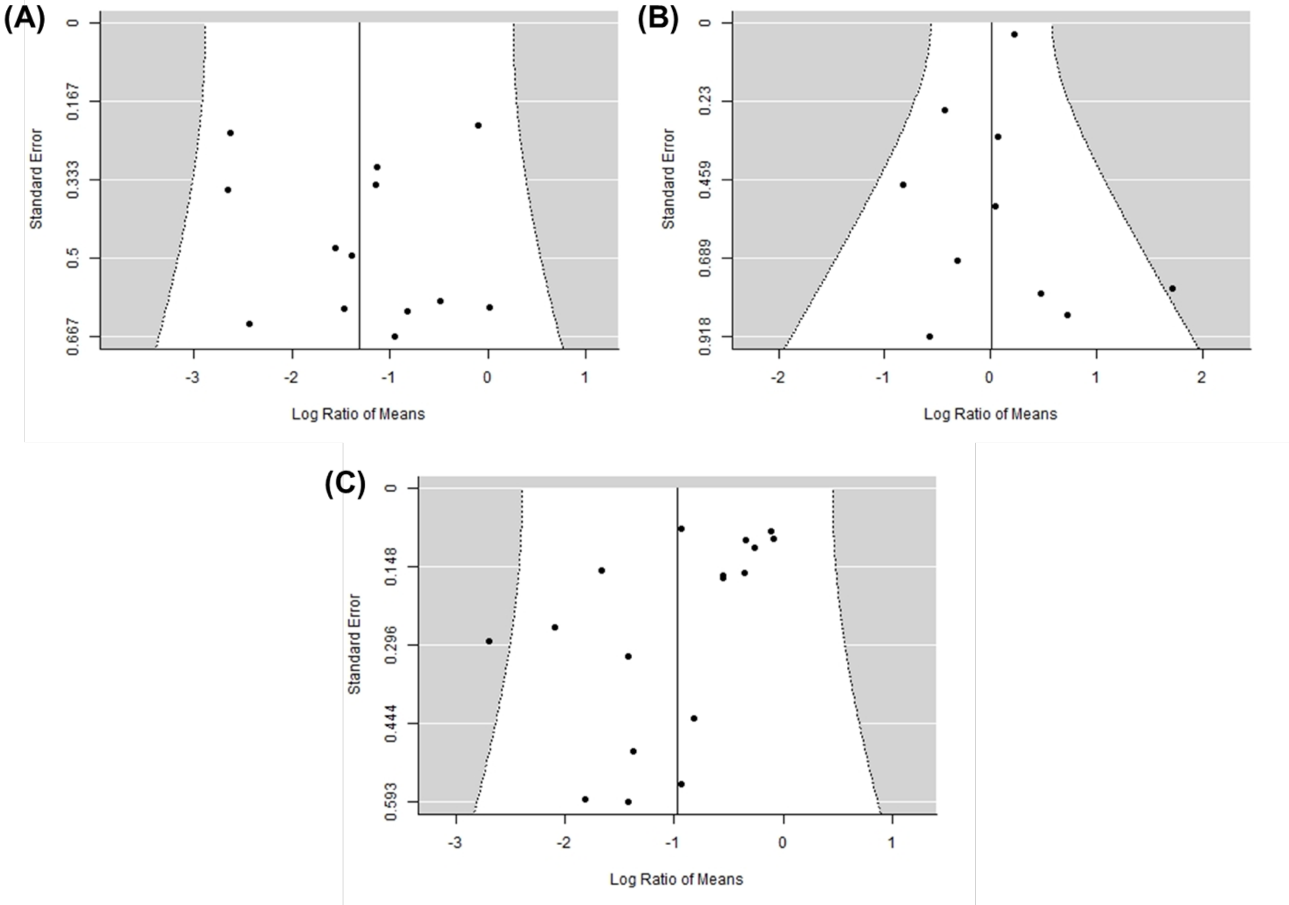
Appendix 4.2. Risk of bias assessment for studies included in the quantitative analysis (controlled time series and controlled before-after studies, n=33).

Reference		**Allocation sequence generation**	**Allocation concealment**	**Baseline outcome measurements**	**Baseline features**	**Incomplete outcome data**	**Blinding (performance)**	**Blinding (detection)**	**Contamination**	**Selective outcome reporting**	**Overall assessment** (Low / some concerns / high)
	Low	Intervention randomly allocated	Allocation conducted by investigators on all units at the start of the study	Outcomes were measured prior to the intervention and no important differences were present across study groups, or if imbalanced but appropriate adjusted analysis was performed	Features of the study and control areas are reported and similar	Missing outcome measures are unlikely to bias the results	Performance bias unlikely due to lack of knowledge of the allocated interventions	Outcomes were assessed blindly or outcomes are objective	It is unlikely that the control group received the intervention	No evidence that outcomes were selectively reported (all pre-specified outcomes are reported)	
	High	When a non-random method is used, and for non-randomised trials and controlled before-after studies	Technicians and investigators could foresee assignment, and for controlled before-after studies	Important differences were present and not adjusted for analysis	No report of characteristics in text or tables or if there are differences between control and intervention areas	Missing outcome data likely to bias the results	Performance bias likely due to knowledge of the allocated interventions	Outcomes were not assessed blindly	It is likely that the control group received the intervention	Some important outcomes are subsequently omitted from the results	
	Unclear	Not specified in the paper	Not specified in the paper	If randomised trials have no baseline measure of outcome	Not specified in the paper	Not specified in the paper (do not assume 100% follow-up unless stated explicitly)	Not specified in the paper	Not specified in the paper	-	Not specified in the paper	
Allen et al. 2008		Unclear	High	Low	Low	Low	High	High	Low	Low	Some concerns
Balaraman et al. 1983		Unclear	High	Unclear	Unclear	Low	High	High	Low	Low	High
Bolay & Trpis 1989		High	High	Low	Low	Low	High	High	Low	Low	Some concerns
Bukhari et al. 2011		High	High	Low	Low	Low	High	High	Low	Low	Some concerns
Dennett et al. 2001		Unclear	High	Low	Unclear	Low	High	High	Low	Low	High
Djegbe et al. 2020		Unclear	High	Unclear	Unclear	Low	High	High	Low	Low	High
Hill & Cambournac 1941		Unclear	High	Low	Low	Low	High	High	High	High	High
Kamel et al. 1972		High	High	High	High	Low	High	High	High	Low	High
Karanja et al. 1994		High	High	Low	Low	Low	High	High	Low	Low	Some concerns
Kim et al. 2002		High	High	Low	Low	Low	High	High	Low	Low	Some concerns
Kramer et al. 1988		Low	High	Low	High	Low	High	Low	Low	Low	Some concerns
Krishnasamy et al. 2003		Unclear	High	High	Unclear	Low	High	High	Low	High	High

Reference		Allocation sequence generation	Allocation concealment	Baseline outcome measurements	Baseline features	Incomplete outcome data	Blinding (performance)	Blinding (detection)	Contamination	Selective outcome reporting	Overall assessment (Low / some concerns / high)
	Low	Intervention randomly allocated	Allocation conducted by investigators on all units at the start of the study	Outcomes were measured prior to the intervention and no important differences were present across study groups, or if imbalanced but appropriate adjusted analysis was performed	Features of the study and control areas are reported and similar	Missing outcome measures are unlikely to bias the results	Performance bias unlikely due to lack of knowledge of the allocated interventions	Outcomes were assessed blindly or outcomes are objective	It is unlikely that the control group received the intervention	No evidence that outcomes were selectively reported (all pre-specified outcomes are reported)	
	High	When a non-random method is used, and for non-randomised trials and controlled before-after studies	Technicians and investigators could foresee assignment, and for controlled before-after studies	Important differences were present and not adjusted for analysis	No report of characteristics in text or tables or if there are differences between control and intervention areas	Missing outcome data likely to bias the results	Performance bias likely due to knowledge of the allocated interventions	Outcomes were not assessed blindly	It is likely that the control group received the intervention	Some important outcomes are subsequently omitted from the results	
	Unclear	Not specified in the paper	Not specified in the paper	If randomised trials have no baseline measure of outcome	Not specified in the paper	Not specified in the paper (do not assume 100% follow-up unless stated explicitly)	Not specified in the paper	Not specified in the paper	-	Not specified in the paper	
Marten et al. 2000		Unclear	High	Low	Low	Low	High	High	Low	Low	Some concerns
Martono 1988		High	High	Low	Low	Low	High	High	Low	Low	Some concerns
McLaughlin et al. 1982		Unclear	High	Low	Low	Low	High	High	Low	Low	Some concerns
Mutero et al. 2000		Low	High	Low	Low	Low	High	High	Low	Low	Some concerns
Palchick & Washino 1986		Unclear	High	Unclear	Unclear	Low	High	High	Low	Low	High
Rajendran & Reuben 1991		Unclear	Unclear	Low	Low	Low	High	High	High	Low	High
Rajendran et al. 1995		Unclear	High	Low	Low	Low	High	High	Low	Low	Some concerns
Rao et al. 1995		Low	High	Low	Low	Low	High	High	Low	Low	Some concerns
Ravoahangimalala et al. 1994		Unclear	Unclear	Low	Low	Low	High	High	Low	Low	Some concerns
Reiter 1980		Unclear	High	Low	Low	Low	High	High	Low	Low	Some concerns
Sogoba et al. 2007		High	High	High	Unclear	Low	High	High	High	Low	High
Sundaraj & Reuben 1991		Unclear	High	Low	Low	Low	High	High	Low	Low	Some concerns
Takagi et al. 1996		Unclear	High	Low	Unclear	Low	High	High	Low	Low	Some concerns
Teng et al. 2005		Low	High	Low	Low	Low	High	High	Low	Low	Some concerns
Victor et al. 1994		Unclear	High	Low	Low	Low	High	High	Low	Low	Some concerns
Victor & Reuben 2000		Low	High	Unclear	Low	Low	High	High	Low	Low	Some concerns
Yap et al. 1982		Unclear	Unclear	High	Low	Low	High	High	Low	Low	Some concerns
Yu et al. 1981		Unclear	Unclear	Unclear	High	Low	High	High	High	Low	High

Reference		**Allocation sequence generation**	**Allocation concealment**	**Baseline outcome measurements**	**Baseline features**	**Incomplete outcome data**	**Blinding (performance)**	**Blinding (detection)**	**Contamination**	**Selective outcome reporting**	**Overall assessment** (Low / some concerns / high)
	Low	Intervention randomly allocated	Allocation conducted by investigators on all units at the start of the study	Outcomes were measured prior to the intervention and no important differences were present across study groups, or if imbalanced but appropriate adjusted analysis was performed	Features of the study and control areas are reported and similar	Missing outcome measures are unlikely to bias the results	Performance bias unlikely due to lack of knowledge of the allocated interventions	Outcomes were assessed blindly or outcomes are objective	It is unlikely that the control group received the intervention	No evidence that outcomes were selectively reported (all pre-specified outcomes are reported)	
	High	When a non-random method is used, and for non-randomised trials and controlled before-after studies	Technicians and investigators could foresee assignment, and for controlled before-after studies	Important differences were present and not adjusted for analysis	No report of characteristics in text or tables or if there are differences between control and intervention areas	Missing outcome data likely to bias the results	Performance bias likely due to knowledge of the allocated interventions	Outcomes were not assessed blindly	It is likely that the control group received the intervention	Some important outcomes are subsequently omitted from the results	
	Unclear	Not specified in the paper	Not specified in the paper	If randomised trials have no baseline measure of outcome	Not specified in the paper	Not specified in the paper (do not assume 100% follow-up unless stated explicitly)	Not specified in the paper	Not specified in the paper	-	Not specified in the paper	
Yu & Lee 1989		High	High	Low	Low	Low	Low	High	Low	Low	Some concerns
Yu et al. 1993		High	High	Low	Low	Low	High	High	Low	Low	Some concerns

Appendix 4.3. Funnel plots assessing publication bias in the meta-analyses of the effect of riceland mosquito control.



(A) synthetic organic chemicals (test for funnel plot asymmetry: z = 0.54, p = 0.59), **(B)** water management techniques (z = 0.44, p = 0.66) and **(C)** bacterial larvicides (z = -2.26, p = 0.024), on *Anopheles* larval density.

Appendix 4.4. Summary of findings of meta-analyses of the effect of riceland mosquito control on *Anopheles* larval density, arranged by the type of control, larval stage, study design and geographical region.

Study	Country	Vector	Details of intervention (application method, rate, dose, fish species)	Study design	Plot size (no. of replications)	Larval stage	Percent difference (95% CI)
Larviciding							
Oils and surface agents							
Bukhari et al. 2011	Kenya	*An. gambiae* s.l.	Monomolecular surface film (Aquatain, silicone-based) at 1 ml/m^2 (1st app.) and at 2 ml/m^2 (2nd app.)	CTS	2000 m^2 (6)	Early	-24.3 (-89.0, +422.8)
Reiter 1980	Kenya	*An. gambiae* s.l.	Monomolecular surface film (lecithin solution) at rate of 2.47 L/ha	CTS	600 m^2 (9)	Early	-52.1 (-74.5, -9.9)
							-49.9 (-72.5, -8.8)
Karanja et al. 1994	Kenya	*An. arabiensis*	Monomolecular surface film (Arosurf MSF) at 4 L/ha every 14 days	CITS	100 m^2 (4)	Early	**-93.5 (-99.8, 114.2)**
Bukhari et al. 2011	Kenya	*An. gambiae* s.l.	Monomolecular surface film (Aquatain, silicone-based) at 1 ml/m^2 (1st app.) and at 2 ml/m^2 (2nd app.)	CTS	2000 m^2 (6)	Late	-32.5 (-71.6, +60.6)
Reiter 1980	Kenya	*An. gambiae* s.l.	Monomolecular surface film (lecithin solution) at rate of 2.47 L/ha	CTS	600 m^2 (9)	Late	-59.5 (-73.0, -39.2)
							-54.9 (-70.0, -32.4)
Bacterial larvicide							
Balaraman et al. 1983	India	*An. subpictus*	*Bti* serotype H-14 (VCRC B-17), with dose 27 x 10^5 spores/ml	CTS	1000 m^2 (5)	Early	-75.8 (-88.8, -47.9)
Dennett et al. 2001	USA	*An. quadrimaculatus*	*Bacillus sphaericus* (*Bs*), VectoLex WDG, aerial application at 1.68 kg/ha	CTS	2000 m^2 (2)	Early	-7.3 (-31.7, +25.8)
Dennett et al. 2001	USA	*An. quadrimaculatus*	*Bs,* VectoLex WDG, aerial application at 0.56 kg/ha	CTS	2000 m^2 (2)	Early	-17.3 (-35.9, +6.8)
Sundaraj & Reuben 1991	India	*An. subpictus*	*Bs,* Biocide-S 1593M, at 2.2 kg/ha	CTS	440 m^2 (3)	Early	-65.5 (-87.3, -6.0)
Sundaraj & Reuben 1991	India	*An. subpictus*	*Bs,* Biocide-S 1593M, at 4.3 kg/ha	CTS	440 m^2 (3)	Early	-65.2 (-89.7, +17.3)
							-46.7 (-67.4, -6.3)
Balaraman et al. 1983	India	*An. subpictus*	*Bti* serotype H-14 (VCRC B-17), with dose 27 x 10^5 spores/ml	CTS	1000 m^2 (5)	Late	-66.5 (-88.3, -3.7)
Dennett et al. 2001	USA	*An. quadrimaculatus*	*Bs*, VectoLex WDG, aerial application at 1.68 kg/ha	CTS	2000 m^2 (2)	Late	-14.5 (-26.4, -0.8)
Dennett et al. 2001	USA	*An. quadrimaculatus*	*Bs*, VectoLex WDG, aerial application at 0.56 kg/ha	CTS	2000 m^2 (2)	Late	-11.0 (-25.5, +6.3)
Sundaraj & Reuben 1991	India	*An. subpictus*	*Bs,* Biocide-S 1593M, at 2.2 kg/ha	CTS	440 m^2 (3)	Late	-89.1 (-96.9, -62.0)
Sundaraj & Reuben 1991	India	*An. subpictus*	*Bs,* Biocide-S 1593M, at 4.3 kg/ha	CTS	440 m^2 (3)	Late	-92.3 (-97.3, -77.9)
							-67.2 (-88.0, -10.7)
Balaraman 1983	India	*An. subpictus*	*Bti* serotype H-14 (VCRC B-17), with dose 27 x 10^5 spores/ml	CTS	1000 m^2 (5)	Pupae	-95.7 (-99.1, -78.2)
Dennett et al. 2001	USA	*An. quadrimaculatus*	*Bs*, VectoLex WDG, aerial application at 1.68 kg/ha	CTS	2000 m^2 (2)	Pupae	-3.1 (-6.3, +0.3)
Dennett et al. 2001	USA	*An. quadrimaculatus*	*Bs*, VectoLex WDG, aerial application at 0.56 kg/ha	CTS	2000 m^2 (2)	Pupae	-1.7 (-5.0, +1.7)
Sundaraj & Reuben 1991	India	*An. subpictus*	*Bs,* Biocide-S 1593M, at 2.2 kg/ha	CTS	440 m^2 (3)	Pupae	-99.0 (-99.8, -93.9)
Sundaraj & Reuben 1991	India	*An. subpictus*	*Bs,* Biocide-S 1593M, at 4.3 kg/ha	CTS	440 m^2 (3)	Pupae	-99.4 (-99.9, -94.8)
							-91.1 (-99.0, -22.0)

Appendix 4.5. Summary of findings of meta-analyses of the effect of riceland mosquito control and rice-growing techniques on *Anopheles* human biting rate (HBR), arranged by the type of control or intervention.

Study	Country	Predominant vector	Details of intervention (application method, rate, dose, fish species)	Study design	Plot size (no. of replications)	Outcome	Relative percent difference (95% CI)
Larviciding							
Synthetic organic chemicals							
Kamel et al. 1972	Egypt	*An. pharoensis*	Iodofenphos (NUVANOL N20U), aerial application at 1.5-3 L/ha	CITS	500,000 m^2 - 1,200,000 m^2	HBR indoors (HLC) HBR outdoors (HLC)	-73.4 (-94.1, +5.4) -52.0 (-80.2, +15.9)
Water management system							
Sogoba et al. 2007	Mali	*An. gambiae* s.l.	*Hors-casier* plot sector (no technical assistance in irrigation system and therefore lack efficient drainage systems) vs. *casier* plot sector (renovated irrigation systems)	CTS	1000 m^2 (4)	HBR	+44.2 (-50.7, +321.9)

Appendix 4.6. The association between riceland mosquito control and larval density in studies included in the qualitative analysis (n=13).

Study	Country	Vector	Details of intervention (application method, rate, dose, fish species)	Study design	Plot size (no. of replications)	Outcome	Relative percent difference (%)
Synthetic organic chemicals							
Gahan & Nob 1955	USA	*An. quadrimaculatus*	Organophosphate (Parathion)	CTS	4000 m^2	Larval density	+57.9
Gahan & Nob 1955	USA	*An. quadrimaculatus*	Organophosphate (Bayer L 13/59)	CTS	4000 m^2	Larval density	+80.0
Gahan & Nob 1955	USA	*An. quadrimaculatus*	Organophosphate (Shell OS 2046)	CTS	4000 m^2	Larval density	+85.0
Magy 1949	USA	*An. freeborni*	Organochlorine (DDT), varying concentrations, aerial application	CITS	7284 ha	Larval density	-55.0 - -100.0
Washino et al. 1972	USA	*An. freeborni*	Organophosphate (Chlorpyrifos, Dursban)	CITS	1855 ha	Adult density	-11.1
Weathersbee et al. 1986	USA	*An. quadrimaculatus*	Mixture of malathion, heavy aromatic naphtha and resmethrin/PBO at 221.8 ml/min, aerial application and *Bti* ground application	CITS	1758 ha	Adult density	-59.9 12 hr post, -6.8 36 hr post
Bacterial larvicides							
Bassi et al. 1989	USA	*An. quadrimaculatus*	*Bti* (Vectobac) at 78 ml AI/ha	CITS	61,000 m^2	Larval density	-72.3
Lacey & Inman 1985	USA	*An. crucians*	*Bti* (Vectobac) at 5.6 kg/ha, aerial application	CITS	4000 m^2	Larval density	-91.9
Lacey & Inman 1985	USA	*An. crucians*	*Bti* (Vectobac) at 11.2 kg/ha, aerial application	CITS	4000 m^2	Larval density	-94.2
Lacey & Inman 1985	USA	*An. crucians*	*Bti* (Vectobac) at 22.5 kg/ha, aerial application	CITS	4000 m^2	Larval density	-96.0
Lacey & Inman 1985	USA	*An. crucians*	*Bti* (Bactimos) at 2.8 kg/ha, hand application	CITS	4000 m^2	Larval density	-100.0

Study	Country	Vector	Details of intervention (application method, rate, dose, fish species)	Study design	Plot size (no. of replications)	Outcome	Relative percent difference (%)
Lacey & Inman 1985	USA	*An. crucians*	*Bti* (Bactimos) at 5.6 5 kg/ha, aerial application	CITS	4000 m^2	Larval density	-100.0
Lacey & Inman 1985	USA	*An. crucians*	*Bti* (Bactimos) at 11.2 5 kg/ha, aerial application	CITS	4000 m^2	Larval density	-100.0
Lacey & Inman 1985	USA	*An. crucians*	*Bti* (Teknar) at 1.7 kg/ha, hand application	CITS	4000 m^2	Larval density	-100.0
Lacey & Inman 1985	USA	*An. crucians*	*Bti* (Teknar) at 3.0 kg/ha, aerial application	CITS	4000 m^2	Larval density	-100.0
Lacey & Inman 1985	USA	*An. crucians*	*Bti* (Teknar) at 7.5 kg/ha, aerial application	CITS	4000 m^2	Larval density	-100.0
Lacey & Inman 1985	USA	*An. quadrimaculatus* & *An. crucians*	*Bs* (isolate 2362), granular formulation	CTS	400 m^2 (3)	Larval density	-68.0
Romi et al. 1993	Madagascar	*An. arabiensis*	*Bti* (Vectobac 12AS) at 1 kg/ha	CITS	180 m^2	Larval density	-89.0
Romi et al. 1993	Madagascar	*An. arabiensis*	*Bti* (Vectobac 12AS) at 0.6 kg/ha	CITS	260 m^2	Larval density	-100.0
Romi et al. 1993	Madagascar	*An. arabiensis*	*Bti* (Vectobac GR) at 10 kg/ha	CITS	220 m^2	Larval density	-100.0
Romi et al. 1993	Madagascar	*An. arabiensis*	*Bti* (Vectobac GR) at 5 kg/ha	CITS	410 m^2	Larval density	-100.0
Romi et al. 1993	Madagascar	*An. arabiensis*	*Bti* (ABG 6185) at 10 kg/ha	CITS	160 m^2	Larval density	-84.0
Romi et al. 1993	Madagascar	*An. arabiensis*	*Bti* (ABG 6185) at 5 kg/ha	CITS	160 m^2	Larval density	-57.0
Sandoski et al. 1985	USA	*An. quadrimaculatus*	*Bti* (Teknar) at 0.54 l/ha	CITS	17,000 m^2	Larval density	-97.9
Sandoski et al. 1985	USA	*An. quadrimaculatus*	*Bti* (Teknar) at 0.27 l/ha	CITS	17,000 m^2	Larval density	-94.4
Sandoski et al. 1985	USA	*An. quadrimaculatus*	*Bti* (Teknar) at 0.11 l/ha	CITS	17,000 m^2	Larval density	-93.0
Sandoski et al. 1985	USA	*An. quadrimaculatus*	*Bti* (Teknar) at 0.07 l/ha	CITS	17,000 m^2	Larval density	-71.1
Sandoski et al. 1985	USA	*An. quadrimaculatus*	*Bti* (Teknar) at 0.04 l/ha	CITS	17,000 m^2	Larval density	-21.8
Stark et al. 1983	USA	*An. quadrimaculatus*	*Bti* (serotype H-14 ABG-6108)	CTS	36 m^2	Larval density	-97.0
Insect growth regulator							
Kottkamp & Meisch 1985	USA	*An. quadrimaculatus*	Insect growth regulator (Bay Sir 8514) at 49 g ai/ha	CTS	36 m^2	Larval density	-73.7
Insect growth regulator and bacterial larvicide							
Bassi et al. 1989	USA	*An. quadrimaculatus*	Methoprene (insect growth regulator) and *Bti* (Duplex) at 78 ml AI/ha	CITS	61,000 m^2	Larval density	-95.1
Biological control							
Blaustein 1992	USA	*An. freeborni*	*G. affinis*: 10 gravid female, 10 male adults	CTS	83.6 m^2 (6)	Larval density	-7.3
Blaustein 1992	USA	*An. freeborni*	*L. cyanellus*: 20 adults	CTS	83.6 m^2 (6)	Larval density	+28.2
Blaustein 1992	USA	*An. freeborni*	*G. affinis*: 10 gravid female, 10 male adults and *L. cyanellus*: 20 adults	CTS	83.6 m^2 (6)	Larval density	-92.2
Hoy et al. 1971	USA	*An. freeborni*	*G. affinis* at stocking rate of 50 per acre	CTS	(3)	Larval density	-83.0
Hoy et al. 1971	USA	*An. freeborni*	*G. affinis* at stocking rate of 100 per acre	CTS	(3)	Larval density	-48.5
Hoy et al. 1971	USA	*An. freeborni*	*G. affinis* at stocking rate of 150 per acre	CTS	(3)	Larval density	-59.2
Hoy et al. 1971	USA	*An. freeborni*	*G. affinis* at stocking rate of 200 per acre	CTS	(3)	Larval density	-87.0
Hoy et al. 1971	USA	*An. freeborni*	*G. affinis* at stocking rate of 250 per acre	CTS	(3)	Larval density	-82.4
Hoy et al. 1971	USA	*An. freeborni*	*G. affinis* at stocking rate of 300 per acre	CTS	(3)	Larval density	-59.2
Intermittent irrigation							

Study	Country	Vector	Details of intervention (application method, rate, dose, fish species)	Study design	Plot size (no. of replications)	Outcome	Relative percent difference (%)
Luh 1984	China	*An. sinensis*	Wet irrigation (a type of intermittent irrigation where fields are filled with a shallow layer of water that may disappear between 24-48 hours) vs. conventional irrigation	CTS		Larval density	-84.0 - -86.0

Appendix 4.7. Summary of findings of meta-analyses of the effect of rice-growing techniques on *Anopheles* larval density, arranged by larval stage, study design and geographical region.

Study	Country	Vector	Comparison	Plot size (no. of replications)	Larval stage	Relative percent difference (95% CI)
Intermittent irrigation						
Hill & Cambournac 1941	Portugal	*Anopheles*	10d wet, 7d dry cycle	2000 m^2 (4)	Early	-36.9 (-64.7, +12.8)
Mutero et al. 2000	Kenya	*An. arabiensis*	Flooded before transplanting, drained during transplanting, flooded after transplanting	750 m^2 (4)	Early	+32.6 (+11.9, +57.2)
Mutero et al. 2000	Kenya	*An. arabiensis*	Flooded before transplanting, drained during transplanting, alternately flooded and drained after transplanting	750 m^2 (4)	Early	+770.6 (+113.4, +3450.9)
						+69.9 (-57.3, +575.3)
Hill & Cambournac 1941	Portugal	*Anopheles*	10d wet, 7d dry cycle	2000 m^2 (4)	Late	-32.6 (-64.5, +28.1)
Mutero et al. 2000	Kenya	*An. arabiensis*	Flooded before transplanting, drained during transplanting, flooded after transplanting	750 m^2 (4)	Late	-34.7 (-45.4, -21.7)
Mutero et al. 2000	Kenya	*An. arabiensis*	Flooded before transplanting, drained during transplanting, alternately flooded and drained after transplanting	750 m^2 (4)	Late	-35.0 (-51.9, -12.1)
Rajendran et al. 1995	India	*An. subpictus*	2.5 cm depth maintained for the first 10-14 DAT. Fields subsequently dried out and re-irrigated to 5 cm depth immediately after all standing water had disappeared (3-5d after irrigation stopped)	16.2 ha - 22.3 ha	Late	-28.8 (-71.0, +74.9)
						-34.5 (-43.5, -24.0)

Appendix 4.8. The adoptability of each intervention according to rice farmers.

	Reference	Intervention or rice-growing method	Description
Interventions and rice-growing techniques that were accepted amongst local farmers	Karanja et al. 1994	Monomolecular surface film	Appropriate for small scale rice cultivation with no more than 1.5 hectares of paddy fields
	Victor & Reuben 1994	Fish	Net profit 2.5 times larger for rice cum fish than rice alone – can provide more income to farmers if successfully promoted
	Bolay & Trpis 1989	Fish	An additional source of protein in tropical countries with malnutrition
	Rajendran & Reuben 1991	Azolla	Grain yields not only increased by 9-14% in treated fields, but weed production halved, reducing need and cost of labour for weeding. Azolla also kept field moist when fields dried out. Farmers were thoroughly impressed.
	Hill & Cambournac 1941	Intermittent irrigation	Economic advantages outweigh disadvantages: increased cost in rebuilding field, relaying irrigation and drainage ditches, increased care necessary in preparation of fields, careful supervision of interruptions to irrigation but once fields are set up for practice, economic advantages resulting from increased yield and better conditions for labour stimulate interest on growers and practice spreading slowly
	Luh 1984	Intermittent irrigation	Rice yield increased by 13% and water consumption considerably lower. Method was therefore well-accepted and used on more than 100,000 ha in 1980
Interventions and rice-growing techniques that needed improvement for increased acceptability	Washino et al. 1972	Synthetic organic chemical	This cannot be done economically on an individual field basis. Low volume technique and synchronisation of large areas required to increase success
	Sundaraj & Reuben 1991	Bacterial larvicide	Application costs were too much; took one person 2.5 hours to spray 0.5 hectares. A less labour-intensive system must be developed (perhaps through multiple point source introduction into irrigation water)
	Rao et al. 1995	Neem	Strong incentive to adopt strategy for economic reasons – but commercial availability at an affordable price will promote large scale use
	Rajendran et al. 1995	Intermittent irrigation	Farmers were convinced of utility based on water conservation but doubted ability to organise equitable distribution during years of water scarcity, preferring neutral supervision of a government agency
	Mutero et al. 2000	Intermittent irrigation	Required more labour, which was already scarce since irrigated rice in the area was labour intensive. Provision of labour only expected if there was a real direct benefit in relation to rice yield, which there was not. Water saving would have benefited farmers during times of water scarcity but no apparent advantage in terms of water saving. The method could not be recommended for use by farmers unless rice yield increased significantly.
	Krishnasamy et al. 2003	Intermittent irrigation	Efficacy heavily dependent on farmer practices and considerable effort would be needed to change practices on a large scale
	Djegbe et al. 2020	Intermittent irrigation and land preparation	Need to assess on a wider scale the feasibility of implementing intermittent flooding with respect to farmer acceptance and required changes in irrigation system design and management

Appendix 4.9. Search strategy in multiple databases (last search date = 10 October 2020).

Search set	Medline	EMBASE	Global Health	Web of Science
1	Exp malaria/	Exp malaria/	Exp malaria/	TS = malari*
2	Exp Anopheles/	Exp malaria control/	Exp anopheles/	TS = anophel*
3	Exp Culicidae/	Exp Anopheles/	Exp Culicidae/	TS = disease vector$
4	Exp mosquito control/	Exp mosquito control/	Exp disease vectors/	TS = mosquito*
5	Exp disease vectors/	Exp insect vector/	Exp entomology/	TS = mosquito control
6	Exp entomology/	Exp mosquito/	Exp medical entomology/	TS = malaria control
7	Malaria.tw.	Exp mosquito vector/	Malaria.ab.	1 OR 2 OR 3 OR 4 OR 5 OR 6
8	Malari*.tw.	Malaria.tw.	Malari*.ab.	TS = rice
9	Anophel*.tw.	Malari*.tw.	Anophel*.ab.	TS = “rice field$”
10	Mosquito*.tw.	Anophel*.tw.	Mosquito*.ab.	TS = “ricefield$”
11	Entomolog*.tw.	Mosquito*.tw.	Entomolg*.ab.	TS = “rice cultivat*”
12	Vector control.tw.	Entomolog*.tw.	1 or 2 or 3 or 4 or 5 or 6	TS = “rice grow*”
13	1 or 2 or 3 or 4 or 5 or 6	1 or 2 or 3 or 4 or 5 or 6 or 7	7 or 8 or 9 or 10 or 11	TS = “rice padd*”
14	7 or 8 or 9 or 10 or 11 or 12	8 or 9 or 10 or 11 or 12	12 or 13	TS = “rice irrigat*”
15	13 or 14	13 or 14	Exp rice/	TS = “water management”
16	Exp oryza/	Exp rice/	Exp flooded rice/	TS = weed*
17	Exp agriculture/	Exp agriculture/	Exp rice fields/	TS = fertili*
18	Herbicides/	Exp “irrigation (agriculture)”	Exp agriculture/	TS = “plant variet*”
19	Rice.tw.	Exp tillage/	Exp irrigation/	8 OR 9 OR 10 OR 11 OR 12 OR 13 OR 14 OR 15 OR 16 OR 17 OR 18
20	Rice field$.tw.	Rice.tw.	Rice.ab.	19 AND 7
21	Ricefield$.tw.	Rice field$.tw.	Rice field$.ab.	
22	Rice adj4 cultivat*.tw.	Ricefield$.tw.	Ricefield$	
23	Rice adj4 grow*.tw.	Rice adj4 cultivat*.tw.	Rice adj4 cultivat*.ab.	
24	Rice adj4 practice$.tw.	Rice adj4 grow*.tw.	Rice adj4 grow*.tw.	
25	Rice adj4 technique$.tw.	Rice adj4 practice$.tw.	Rice adj4 practice$.ab.	
26	Rice adj2 padd*.tw.	Rice adj4 technique$.tw.	Rice adj4 technique$.ab.	
27	Rice adj2 irrigat*.tw.	Rice adj2 padd*.tw.	Rice adj2 padd*.ab.	
28	Rice adj4 method*.tw.	Rice adj2 irrigat*.tw.	Rice adj2 irrigat*.ab.	
29	Monolayer.tw.	Rice adj4 method*.tw.	Rice adj4 method*.ab.	
30	Fertili*.tw.	Monolayer.tw.	Monolayer.ab.	
31	Weed*.tw.	Fertili*.tw.	Fertili*.ab.	
32	Plant adj3 variet*.tw.	Weed*.tw.	Weed*.ab.	
33	Water adj3 management.tw.	Plant adj3 variet*.tw.	Plant adj3 variet*.ab.	
34	16 or 17 or 18	Water adj3 management.tw.	Water adj3 management.ab.	
35	19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33	16 or 17 or 18 or 19	15 or 16 or 17 or 18 or 19	
36	34 or 35	20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34	20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34	
37	15 and 36	35 or 36	35 or 36	
38		15 and 37	14 and 37	

Appendix 5. Chapter 5 Supplementary Material

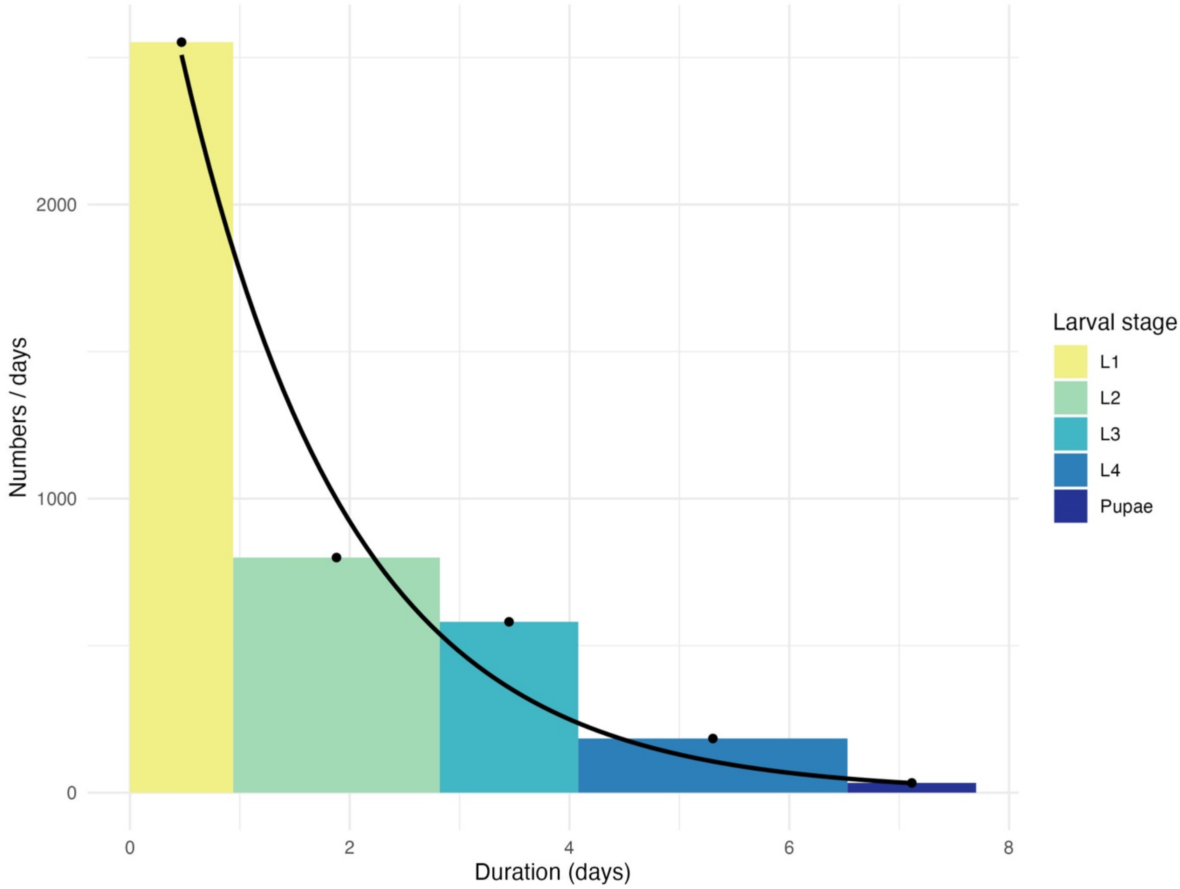
Appendix 5.1. Locally made metal area sampler in M'bé rice field

Appendix 5.2. Mosquito and rice field characteristics sampling survey

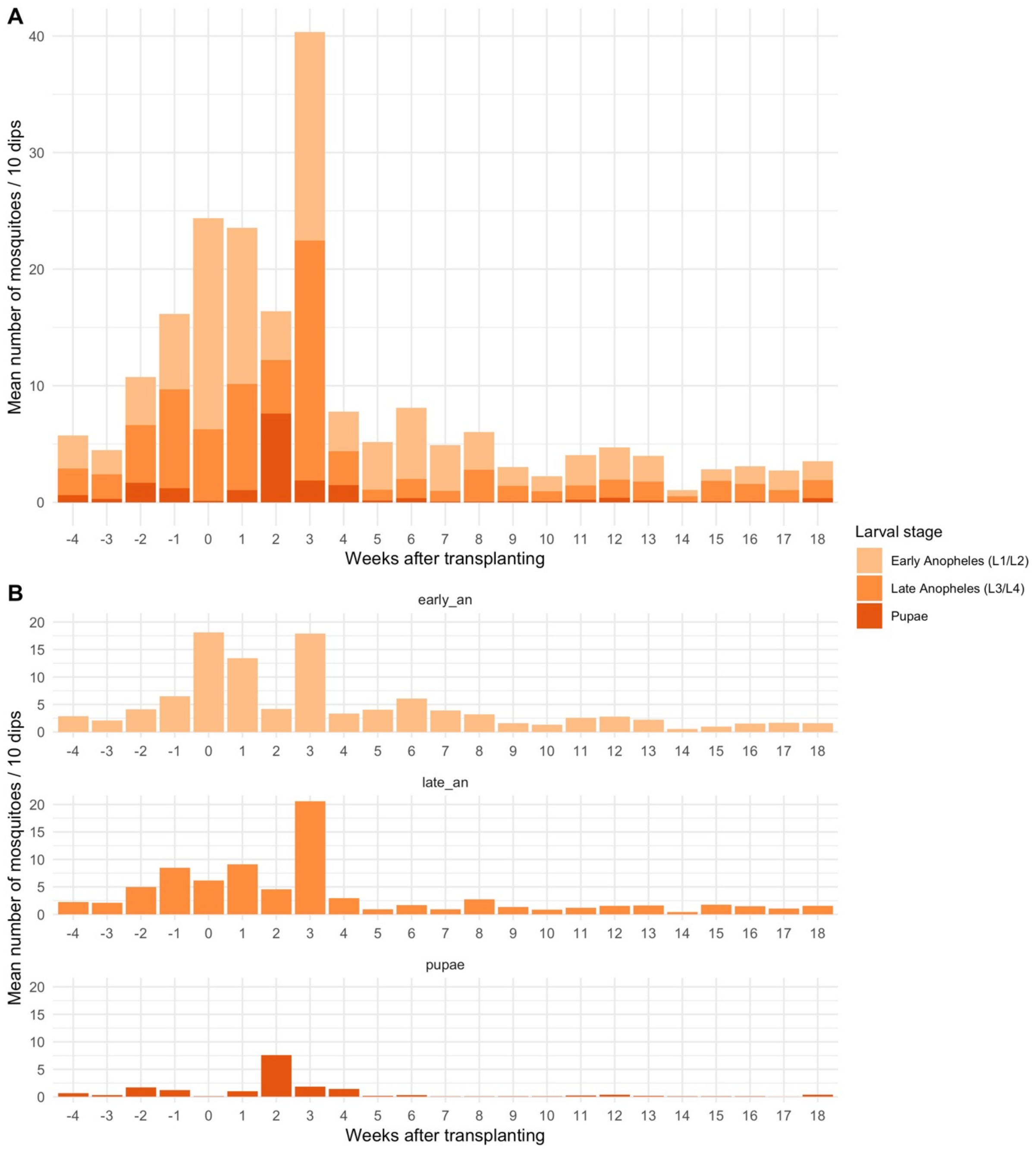
Date	Name of technician collecting	Plot identification (Rep .1 / 2 / 3)	Sampling Point	Dip No.	**Anopheles L1/L2**	**Anopheles L3/L4**	**Anopheles Pupae**	**Culex (0** : Absent / **1** : Present)	**Percentage of plot covered with water (1 m around sampling point) (%)** (**0**: 0% / **1**: 1-25% / **2**: 26-50% / **3**: 51-75% / **4**: 76-99% / **5**: 100%)	**Water transparency** (**1**: Transparent / **2**: A little / **3**: Cloudy / **4**: Very cloudy / **5**: Opaque)	**Rice Height (cm)** (from ground to the highest leaf)	**Water depth (cm)** (from soil surface to water surface)	**Iron soil toxicity (red / brown)** (**0**: Absent / **1**: A little / **2**: Some / **3**: A lot / **4**: Comp-letely covered)	**Other vegetation** (**0**: Absent / **1**: Submerging / **2**: On surface / **3**: Filamentous algae / **4**: Weeds / **5**: Other (please comment) (Select as many as you observe)	**Fauna** (**0**: None / **1**: Fish / **2**: Tadpoles / **3:** Large insects / **4**: Molluscs / **5**: Earthworms / **6**: Other (please comment) (Select as many as you observe)
			1	1											
				2											
			2	1											
				2											
			3	1											
				2											
			4	1											
				2											
			5	1											
				2											
			6	1											
				2											
			7	1											
				2											
			8	1											
				2											

Appendix 5.3 Iron (III) oxide residues in the rice field

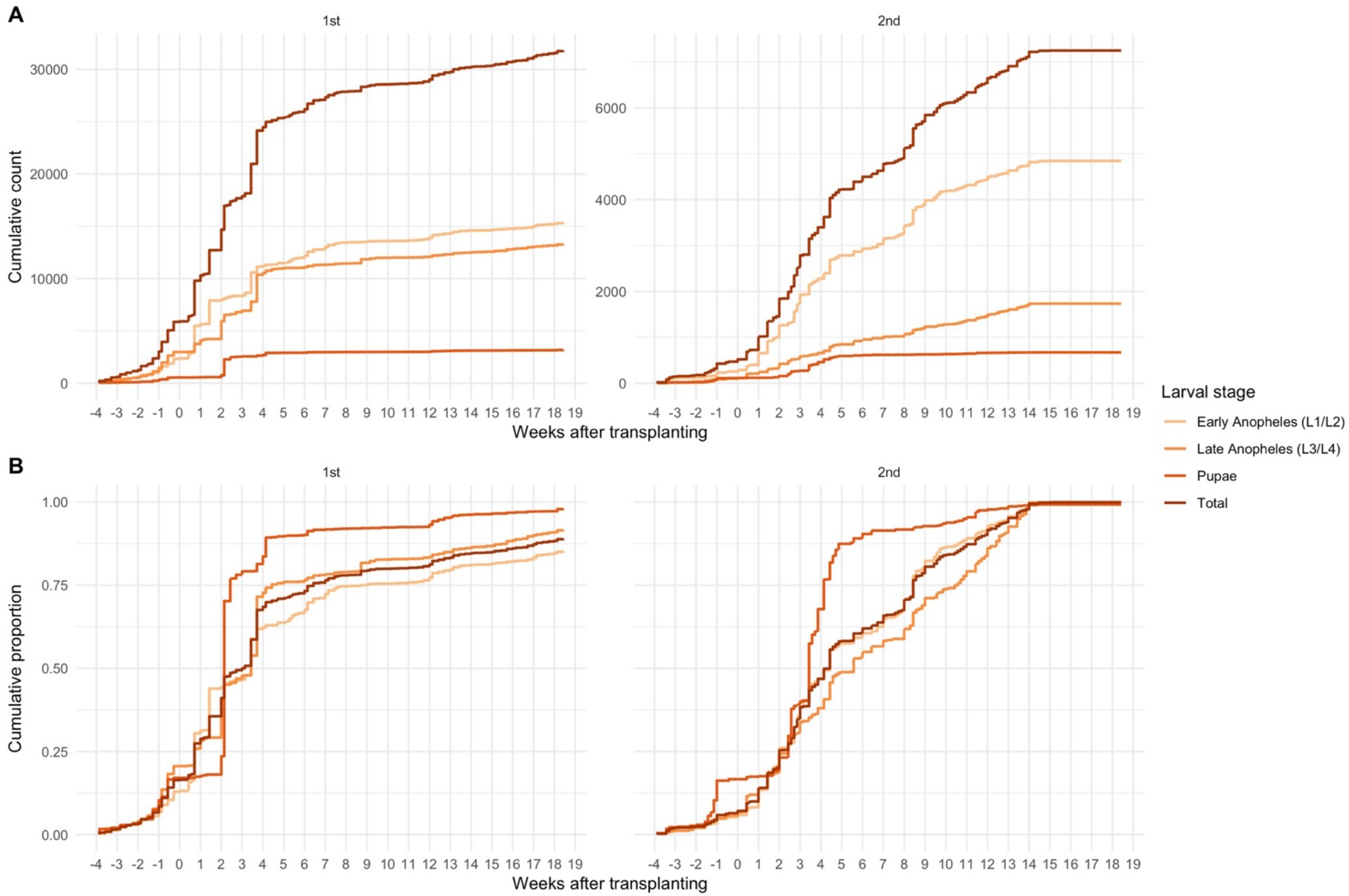
Appendix 5.4. Age distribution and survivorship curve of the immature stages of *An. gambiae* collected from rice fields in Cote d'Ivoire.



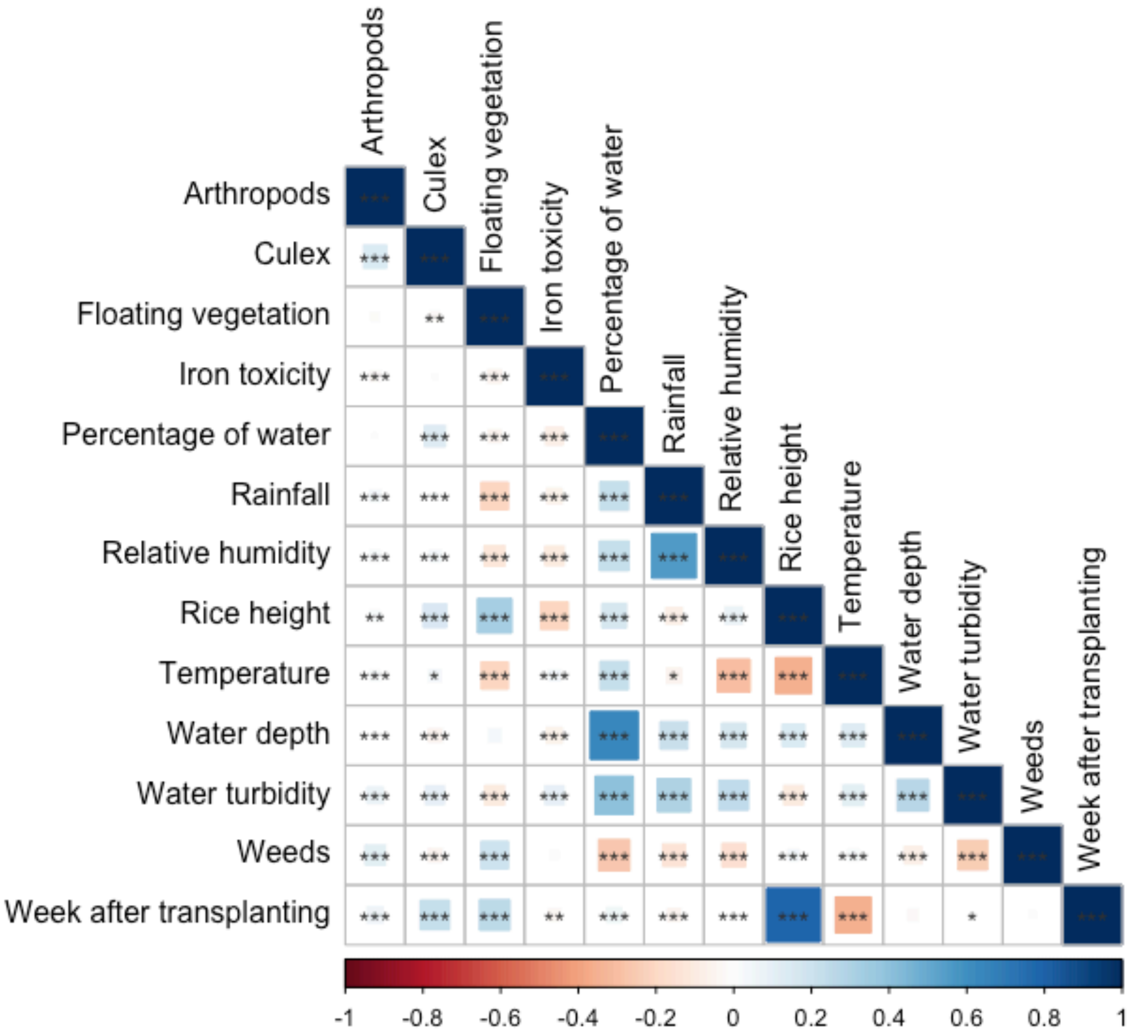
Appendix 5.5. Weekly mean *Anopheles* abundance throughout a rice growing season, (A) accumulated with all developmental stages and (B) separated by developmental stage.



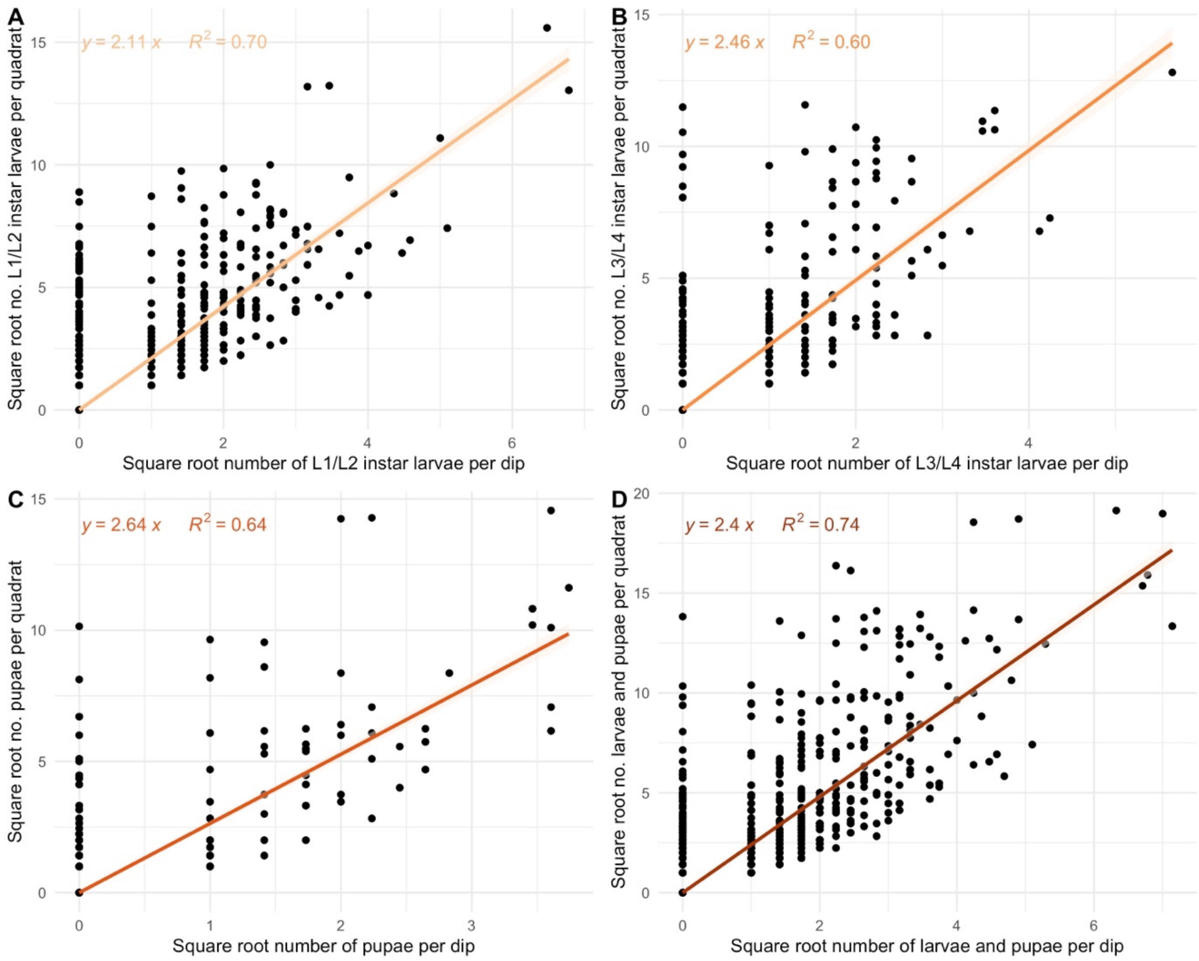
Appendix 5.6. Cumulative (A) count and (B) proportion of weekly mean *Anopheles* abundance throughout a rice season, separated by 1st season (wet season from March to August) and 2nd season (wet-dry cusp from August-December)



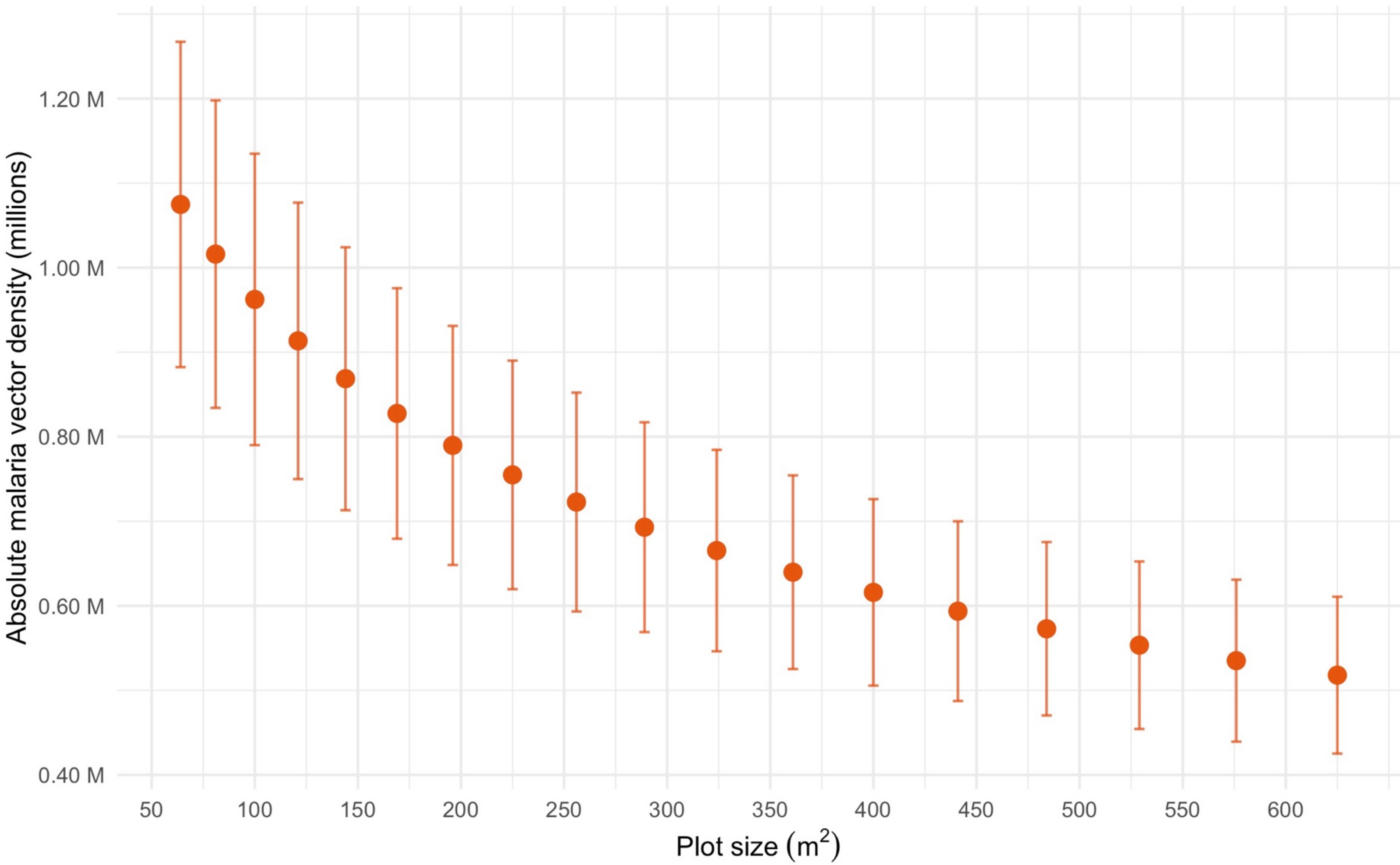
Appendix 5.7. Correlation matrix between biophysical and environmental variables



Appendix 5.8. Calibration factors between two dips and area sampler counts obtained through no-intercept linear regressions for (A) early instars, (B) late instars, (C) pupae, and (D) all immatures



Appendix 5.9. Vector productivity according to plot size



Appendix 6. Chapter 6 Supplementary Material

Appendix 6.1. Images of rice fields in Côte d'Ivoire under various cultivation practices: (a) nursery beds, (b) drum-seeding, (c) stagnant pools in drum-seeded fields, (d) wet direct-seeded fields using broadcasting, (e) line dry direct-seeded fields.

(A) 


(B)

(C)

(D) 

(E) 

Appendix 6.2. Mosquito species composition in Côte d'Ivoire and Tanzania by rice growth phase.

Country and trials	Mosquito species	Rice growth phase					Total
		Land preparation	Vegetative	Reproductive	Ripening	Post-harvest	
Côte d'Ivoire (Trials 1-6)	*Aedes*						
	Ae. cumminsii	22	51	33	21	1	128 (1.0)
	Ae. vittatus	4	0	0	0	0	4 (0.03)
	Anopheles						
	An. broheri	0	1	3	2	0	7 (0.05)
	An. coustani	0	0	1	2	0	3 (0.02)
	An. funestus sl	0	0	0	1	0	1 (0.01)
	An. gambiae sl	565	4656	1034	81	0	6336 (49.0)
	An. pharoensis	4	15	4	18	0	41 (0.3)
	An. ziemanni	45	53	139	816	15	1068 (8.3)
	Culex						
	Cx. annulioris	19	59	139	179	5	401 (3.1)
	Cx. cinereus	570	1460	677	551	13	3271 (25.3)
	Cx. nebulosus	53	127	15	25	2	222 (1.7)
	Cx. pipiens	0	2	0	0	0	2 (0.02)
	Cx. quinquefasciatus	70	815	447	42	0	1374 (10.6)
	Cx. tigripes	27	29	9	10	1	76 (0.6)
	Total	**1379 (10.7)**	**7268 (56.2)**	**2362 (18.3)**	**1748 (13.5)**	**37 (0.3)**	**12934 (100.0)**
Tanzania (Trial 7)	*Aedes*						
	Ae. aegypti	0	2	7	3	0	12 (1.5)
	Ae. hirsutus	0	0	3	3	0	6 (0.8)
	Ae. vittatus	0	0	1	0	0	1 (0.1)
	Anopheles						
	An. coustani	0	0	0	20	0	20 (2.6)
	An. gambiae sl	5	331	79	2	5	411 (54.2)
	Culex						
	Cx. antennatus	0	6	5	103	0	114 (14.6)
	Cx. bitaenorhynchus	0	0	0	2	0	2 (0.3)
	Cx. nebulosus	0	2	0	0	0	2 (0.3)
	Cx. quinquefasciatus	0	0	1	7	0	8 (1.0)
	Cx. univittatus	0	0	1	190	1	192 (24.6)
	Total	**5 (0.6)**	**341 (43.8)**	**97 (12.5)**	**330 (42.4)**	**6 (0.8)**	**779 (100.0)**

Appendix 6.3. Estimated marginal means and 95% confidence intervals of mosquito immatures (per 10 dips) under different rice cultivation practices, under separate trials in Côte d'Ivoire and Tanzania (n=7). Significant differences between treatments are highlighted in green.

		Immature stage															
Rice growth phase	Treatment	All				Early instar				Late instar				Pupae			
		Mean	LCI	UCI		Mean	LCI	UCI		Mean	LCI	UCI		Mean	LCI	UCI	
Trial 1: Land preparation																	
All	21d	17.436	10.675	28.479	a	9.131	5.399	15.442	a	2.499	1.528	4.087	a	1.389	0.574	3.358	a
	14d	14.458	8.707	24.008	a	8.248	4.746	14.332	a	3.412	2.077	5.606	a	1.095	0.436	2.750	a
	7d	14.225	8.951	22.605	a	8.189	4.995	13.423	a	2.780	1.716	4.502	a	0.981	0.442	2.175	a
	4d	11.546	5.444	24.486	a	5.351	2.399	11.937	a	1.980	0.911	4.306	a	1.251	0.310	5.051	a
	2d	14.523	8.685	24.285	a	8.087	4.703	13.906	a	3.287	1.937	5.580	a	0.583	0.240	1.412	a
		trend: 0.011, p=0.530				trend: 0.010, p=0.588				trend: -0.004, p=0.826				trend: 0.033, p=0.293			
Land preparation	21d	6.834	2.314	20.186	a	1.734	0.584	5.148	a	0.599	0.184	1.943	a	2.776	0.171	45.150	a
	14d	3.249	1.201	8.791	a	1.682	0.555	5.103	a	1.665	0.517	5.366	a	0.050	0.002	1.192	a
	7d	4.827	2.079	11.207	a	2.512	0.984	6.412	a	0.941	0.357	2.484	a	1.046	0.131	8.379	a
	4d	2.516	0.500	12.671	a	0.489	0.095	2.525	a	0.554	0.094	3.256	a	0.683	0.017	28.203	a
	2d	4.775	1.794	12.706	a	1.753	0.640	4.797	a	1.291	0.461	3.610	a	0.401	0.038	4.268	a
		trend: 0.005, p=0.886				trend: 0.002, p=0.959				trend: -0.017, p=0.671				trend: 0.010, p=0.927			
Vegetative	21d	42.422	24.134	74.568	a	36.290	19.514	67.490	a	5.066	2.972	8.636	a	0.987	0.446	2.183	a
	14d	43.282	24.464	76.574	a	33.992	18.117	63.775	a	6.986	4.042	12.075	a	1.307	0.564	3.030	a
	7d	37.935	21.858	65.839	a	30.450	16.719	55.458	a	5.744	3.323	9.930	a	0.791	0.341	1.837	a
	4d	36.016	15.193	85.377	a	28.749	11.233	73.581	a	5.204	2.209	12.264	a	1.542	0.395	6.017	a
	2d	38.628	21.245	70.235	a	30.587	15.921	58.764	a	6.573	3.673	11.763	a	0.468	0.187	1.172	a
		trend: 0.008, p=0.699				trend: 0.011, p=0.618				trend: -0.006, p=0.769				trend: 0.029, p=0.324			

		Immature stage															
Rice growth phase	**Treatment**	**All**				**Early instar**				**Late instar**				**Pupae**			
		Mean	LCI	UCI		Mean	LCI	UCI		Mean	LCI	UCI		Mean	LCI	UCI	
Trial 2: Crop establishment & water management																	
All	TP-CF	3.671	2.894	4.655	a	2.836	2.173	3.701	a	0.644	0.486	0.853	a	0.069	0.041	0.116	a
	TP-AWD	3.670	2.896	4.649	a	2.544	1.952	3.315	a	0.791	0.604	1.037	a	0.149	0.096	0.231	ab
	DS-AWD	3.317	2.401	4.582	a	2.508	1.748	3.597	a	0.546	0.371	0.803	a	0.216	0.125	0.374	b
	DS-WET-CF	4.482	3.494	5.749	a	3.231	2.442	4.274	a	0.834	0.625	1.112	a	0.162	0.104	0.254	ab
	DS-DS-CF	4.753	3.764	6.000	a	3.318	2.557	4.306	a	1.055	0.812	1.370	a	0.175	0.116	0.264	b
	DS-DRY-CF	3.865	3.022	4.943	a	2.801	2.126	3.690	a	0.845	0.641	1.113	a	0.117	0.073	0.188	ab
Land preparation	TP-CF	4.060	2.316	7.117	ab	3.036	1.633	5.647	a	0.454	0.240	0.860	a	0.124	0.047	0.327	ab
	TP-AWD	4.722	2.849	7.829	ab	3.872	2.228	6.731	a	0.452	0.249	0.820	a	0.081	0.027	0.246	a
	DS-AWD	1.125	0.322	3.925	a	0.811	0.202	3.248	a	0.000	0.000	Inf	a	0.279	0.060	1.307	ab
	DS-WET-CF	6.622	2.849	15.387	ab	5.068	1.973	13.018	a	0.559	0.228	1.370	a	0.209	0.067	0.654	ab
	DS-DS-CF	9.164	4.497	18.674	b	6.811	3.115	14.890	a	1.217	0.619	2.389	a	0.665	0.266	1.662	b
	DS-DRY-CF	4.309	2.100	8.840	ab	3.248	1.490	7.078	a	0.558	0.258	1.208	a	0.134	0.040	0.453	ab
Vegetative	TP-CF	8.119	5.338	12.350	a	6.271	3.901	10.080	a	1.610	0.986	2.629	a	0.174	0.080	0.379	a
	TP-AWD	10.129	6.698	15.317	a	7.021	4.368	11.286	a	2.281	1.423	3.658	a	0.578	0.317	1.054	ab
	DS-AWD	8.987	5.726	14.104	a	6.471	3.903	10.730	a	1.476	0.858	2.538	a	1.103	0.571	2.131	b
	DS-WET-CF	9.022	6.297	12.927	a	6.864	4.555	10.346	a	1.719	1.131	2.612	a	0.383	0.216	0.678	ab
	DS-DS-CF	10.392	7.419	14.557	a	7.108	4.874	10.366	a	2.370	1.594	3.523	a	0.468	0.272	0.804	ab
	DS-DRY-CF	10.038	7.070	14.253	a	6.685	4.488	9.959	a	2.376	1.596	3.537	a	0.435	0.248	0.763	ab
Reproductive	TP-CF	2.249	1.368	3.699	a	1.641	0.906	2.972	a	0.403	0.229	0.712	a	0.011	0.001	0.170	a
	TP-AWD	2.567	1.602	4.111	a	2.090	1.192	3.664	a	0.526	0.318	0.873	a	0.014	0.001	0.179	a
	DS-AWD	1.133	0.545	2.356	a	0.970	0.418	2.253	a	0.032	0.003	0.290	a	0.019	0.001	0.298	a
	DS-WET-CF	1.553	0.892	2.702	a	0.996	0.513	1.935	a	0.356	0.181	0.702	a	0.045	0.009	0.218	a
	DS-DS-CF	2.964	1.766	4.973	a	2.095	1.125	3.900	a	0.641	0.375	1.095	a	0.004	0.000	0.132	a
	DS-DRY-CF	1.527	0.879	2.655	a	1.194	0.628	2.271	a	0.214	0.092	0.499	a	0.008	0.000	0.145	a
Ripening & Post-harvest	TP-CF	1.007	0.677	1.499	a	0.636	0.412	0.981	a	0.316	0.190	0.524	a	0.024	0.005	0.106	a
	TP-AWD	1.360	0.925	2.000	a	0.606	0.389	0.945	a	0.515	0.335	0.790	a	0.091	0.039	0.210	a
	DS-AWD	1.048	0.677	1.625	a	0.645	0.406	1.025	a	0.421	0.257	0.690	a	0.035	0.009	0.146	a
	DS-WET-CF	1.595	1.140	2.232	a	0.900	0.623	1.302	a	0.562	0.384	0.822	a	0.113	0.053	0.239	a
	DS-DS-CF	1.037	0.721	1.492	a	0.568	0.383	0.845	a	0.378	0.246	0.581	a	0.032	0.009	0.114	a
	DS-DRY-CF	0.993	0.692	1.426	a	0.612	0.412	0.908	a	0.348	0.227	0.534	a	0.050	0.019	0.134	a

		Immature stage															
Rice growth phase	Treatment	All				Early instar				Late instar				Pupae			
		Mean	LCI	UCI		Mean	LCI	UCI		Mean	LCI	UCI		Mean	LCI	UCI	
Trial 3: Water management																	
All	CF	2.785	2.396	3.236	a	1.663	1.395	1.982	a	0.638	0.543	0.750	a	0.138	0.091	0.207	a
	AWD15	2.900	2.492	3.376	a	1.728	1.451	2.058	a	0.664	0.564	0.783	a	0.200	0.135	0.296	a
Land preparation	CF	1.549	1.033	2.324	a	0.789	0.508	1.225	a	0.318	0.203	0.498	a	0.262	0.135	0.509	a
	AWD15	1.339	0.889	2.018	a	0.766	0.493	1.192	a	0.141	0.077	0.260	a	0.183	0.090	0.372	a
Vegetative	CF	4.643	3.493	6.173	a	3.147	2.275	4.353	a	0.680	0.504	0.917	a	0.507	0.268	0.961	a
	AWD15	5.427	4.105	7.174	a	3.326	2.406	4.597	a	0.831	0.622	1.110	a	0.999	0.547	1.825	a
Reproductive	CF	3.741	3.108	4.503	a	2.874	2.270	3.638	a	0.761	0.601	0.963	a	NA	NA	NA	-
	AWD15	3.313	2.748	3.995	a	2.244	1.761	2.859	a	0.860	0.686	1.078	a	NA	NA	NA	-
Ripening & Post-harvest	CF	1.873	1.493	2.352	a	0.853	0.638	1.140	a	0.848	0.624	1.153	a	NA	NA	NA	-
	AWD15	2.195	1.752	2.748	a	1.272	0.968	1.672	a	0.780	0.568	1.072	a	NA	NA	NA	-
		Immature stage															
Rice growth phase	Treatment	All				Early instar				Late instar				Pupae			
		Mean	LCI	UCI		Mean	LCI	UCI		Mean	LCI	UCI		Mean	LCI	UCI	
Trial 4: Water & nutrient management																	
All	CF	2.721	1.914	3.869	a	1.897	1.277	2.816	a	0.605	0.393	0.933	a	0.160	0.076	0.337	a
	AWD15	2.333	1.626	3.349	a	1.624	1.082	2.437	a	0.504	0.322	0.790	a	0.143	0.063	0.323	a
	AWD15-2DAT	2.219	1.558	3.161	a	1.674	1.127	2.486	a	0.405	0.258	0.637	a	0.099	0.044	0.223	a
	FD-II	2.074	1.458	2.949	a	1.398	0.939	2.081	a	0.417	0.266	0.652	a	0.175	0.086	0.358	a
	FD-II2	1.558	1.081	2.244	a	0.818	0.536	1.249	a	0.465	0.297	0.728	a	0.163	0.078	0.342	a
	II3	2.076	1.447	2.979	a	1.583	1.058	2.370	a	0.339	0.208	0.552	a	0.087	0.038	0.201	a
	Rainfed	1.235	0.846	1.803	a	0.764	0.495	1.179	a	0.266	0.156	0.454	a	0.149	0.071	0.312	a
	CF-FD	1.742	1.217	2.495	a	1.242	0.828	1.863	a	0.372	0.225	0.613	a	0.083	0.037	0.190	a
	CF-NONE	1.507	1.045	2.172	a	1.189	0.789	1.791	a	0.246	0.147	0.409	a	0.048	0.019	0.125	a
	No rice	1.642	1.135	2.377	a	1.373	0.909	2.074	a	0.209	0.123	0.357	a	0.033	0.011	0.097	a
Land preparation	CF	3.316	1.542	7.134	a	1.695	0.667	4.306	a	1.138	0.508	2.548	a	0.294	0.091	0.954	a
	AWD15	1.183	0.487	2.871	a	0.724	0.244	2.150	a	0.237	0.068	0.818	a	0.241	0.068	0.859	a
	AWD15-2DAT	1.675	0.727	3.860	a	1.277	0.492	3.318	a	0.279	0.095	0.815	a	0.062	0.009	0.441	a
	FD-II	2.770	1.314	5.839	a	1.285	0.526	3.135	a	0.766	0.344	1.704	a	0.547	0.184	1.630	a
	FD-II2	4.686	1.844	11.908	a	3.135	1.261	7.795	a	0.938	0.395	2.226	a	0.225	0.059	0.857	a
	II3	1.002	0.422	2.378	a	0.747	0.274	2.040	a	0.110	0.022	0.544	a	0.067	0.009	0.490	a
	Rainfed	1.838	0.810	4.169	a	1.253	0.485	3.236	a	0.151	0.041	0.565	a	0.339	0.096	1.196	a
	CF-FD	2.776	1.289	5.981	a	2.483	1.004	6.144	a	0.316	0.112	0.886	a	0.030	0.002	0.418	a
	CF-NONE	4.648	2.079	10.395	a	3.886	1.526	9.895	a	0.692	0.288	1.663	a	0.128	0.028	0.598	a

		Immature stage															
Rice growth phase	Treatment	All				Early instar				Late instar				Pupae			
		Mean	LCI	UCI		Mean	LCI	UCI		Mean	LCI	UCI		Mean	LCI	UCI	
	No rice	2.112	0.662	6.740	a	1.706	0.554	5.251	a	0.105	0.021	0.522	a	0.032	0.002	0.448	a
Vegetative	CF	3.652	1.983	6.725	b	2.601	1.339	5.053	b	0.707	0.303	1.647	a	0.315	0.106	0.941	a
	AWD15	2.624	1.396	4.932	ab	1.857	0.937	3.679	ab	0.704	0.326	1.522	a	0.101	0.026	0.402	a
	AWD15-2DAT	2.448	1.308	4.580	ab	1.554	0.790	3.060	ab	0.525	0.236	1.169	a	0.116	0.034	0.393	a
	FD-II	2.283	1.215	4.291	ab	1.527	0.766	3.047	ab	0.388	0.168	0.899	a	0.219	0.075	0.636	a
	FD-II2	1.237	0.626	2.444	ab	0.468	0.213	1.028	a	0.168	0.059	0.476	a	0.372	0.132	1.050	a
	II3	2.060	1.084	3.913	ab	1.756	0.879	3.508	ab	0.131	0.044	0.393	a	0.084	0.021	0.329	a
	Rainfed	1.087	0.530	2.230	ab	0.570	0.244	1.335	ab	0.253	0.100	0.641	a	0.215	0.070	0.663	a
	CF-FD	1.798	0.936	3.456	ab	0.957	0.461	1.988	ab	0.463	0.180	1.189	a	0.146	0.045	0.477	a
	CF-NONE	1.206	0.605	2.405	ab	0.824	0.381	1.781	ab	0.164	0.052	0.513	a	0.071	0.018	0.283	a
	No rice	0.778	0.389	1.556	a	0.613	0.285	1.316	ab	0.096	0.029	0.320	a	0.050	0.010	0.236	a
Reproductive	CF	2.867	1.364	6.028	a	2.492	1.135	5.470	b	0.240	0.082	0.703	a	0.042	0.007	0.259	a
	AWD15	2.190	1.024	4.681	a	1.699	0.757	3.816	ab	0.327	0.110	0.976	a	0.202	0.073	0.557	a
	AWD15-2DAT	2.088	0.970	4.495	a	1.860	0.827	4.185	ab	0.157	0.048	0.516	a	0.075	0.018	0.318	a
	FD-II	1.729	0.808	3.701	a	1.591	0.714	3.548	ab	0.115	0.032	0.408	a	0.038	0.006	0.253	a
	FD-II2	0.570	0.247	1.315	a	0.270	0.100	0.731	a	0.206	0.069	0.616	a	0.072	0.017	0.301	a
	II3	2.907	1.371	6.166	a	2.495	1.124	5.540	b	0.308	0.109	0.870	a	0.057	0.011	0.286	a
	Rainfed	0.935	0.419	2.088	a	0.677	0.286	1.602	ab	0.160	0.044	0.580	a	0.040	0.006	0.244	a
	CF-FD	1.411	0.636	3.127	a	1.034	0.443	2.413	ab	0.200	0.053	0.754	a	0.062	0.014	0.279	a
	CF-NONE	0.980	0.437	2.199	a	0.905	0.390	2.099	ab	0.034	0.004	0.272	a	0.000	0.000	Inf	a
	No rice	1.252	0.565	2.776	a	1.170	0.507	2.701	ab	0.069	0.016	0.292	a	0.019	0.001	0.251	a
Ripening & Post-harvest	CF	0.793	0.469	1.343	a	0.534	0.290	0.983	a	0.175	0.076	0.407	a	0.040	0.011	0.151	a
	AWD15	0.909	0.541	1.528	a	0.558	0.300	1.039	a	0.186	0.080	0.430	a	0.103	0.041	0.259	a
	AWD15-2DAT	1.325	0.833	2.108	a	0.792	0.460	1.363	a	0.373	0.196	0.711	a	0.077	0.028	0.208	a
	FD-II	0.925	0.557	1.536	a	0.422	0.221	0.806	a	0.358	0.183	0.698	a	0.060	0.019	0.184	a
	FD-II2	1.169	0.712	1.920	a	0.451	0.234	0.869	a	0.614	0.338	1.115	a	0.032	0.007	0.146	a
	II3	1.156	0.710	1.884	a	0.655	0.365	1.173	a	0.338	0.171	0.667	a	0.080	0.029	0.217	a
	Rainfed	1.071	0.645	1.778	a	0.610	0.330	1.126	a	0.324	0.159	0.658	a	0.086	0.032	0.233	a
	CF-FD	1.517	0.939	2.453	a	1.054	0.610	1.820	a	0.383	0.195	0.750	a	0.052	0.015	0.175	a
	CF-NONE	1.217	0.742	1.998	a	0.936	0.537	1.631	a	0.263	0.124	0.559	a	0.021	0.003	0.132	a
	No rice	1.575	0.998	2.487	a	1.135	0.681	1.890	a	0.380	0.197	0.734	a	0.020	0.003	0.123	a

		Immature stage															
Rice growth phase	Treatment	All				Early instar				Late instar				Pupae			
		Mean	LCI	UCI		Mean	LCI	UCI		Mean	LCI	UCI		Mean	LCI	UCI	
Trial 5: Water & nutrient management																	
All	CF	6.454	5.010	8.314	a	4.639	3.415	6.301	a	1.402	1.060	1.856	a	0.199	0.131	0.302	ab
	AWD15	6.410	4.994	8.229	a	4.251	3.132	5.771	a	1.448	1.102	1.901	a	0.457	0.323	0.647	c
	AWD30	5.706	4.423	7.362	a	3.880	2.856	5.270	a	1.277	0.962	1.695	a	0.279	0.189	0.412	abc
	DF	7.211	5.626	9.241	a	5.281	3.919	7.116	a	1.416	1.075	1.865	a	0.376	0.261	0.544	bc
	F-BF	7.839	6.117	10.046	a	6.150	4.555	8.301	a	1.174	0.891	1.547	a	0.274	0.185	0.405	abc
	F-DA	6.195	4.789	8.014	a	4.853	3.559	6.619	a	1.012	0.757	1.353	a	0.156	0.101	0.241	a
Land preparation	CF	4.249	2.176	8.295	a	3.577	1.593	8.030	a	NA	NA	NA	-	NA	NA	NA	-
	AWD15	3.270	1.713	6.241	a	2.591	1.207	5.561	a	NA	NA	NA	-	NA	NA	NA	-
	AWD30	3.224	1.648	6.306	a	2.491	1.100	5.638	a	NA	NA	NA	-	NA	NA	NA	-
	DF	3.302	1.721	6.337	a	2.331	1.083	5.020	a	NA	NA	NA	-	NA	NA	NA	-
	F-BF	2.555	1.315	4.965	a	2.140	0.955	4.794	a	NA	NA	NA	-	NA	NA	NA	-
	F-DA	3.539	1.748	7.164	a	2.844	1.192	6.786	a	NA	NA	NA	-	NA	NA	NA	-
Vegetative	CF	12.761	7.955	20.471	a	7.815	4.520	13.511	a	2.884	1.552	5.361	a	0.434	0.192	0.980	a
	AWD15	13.092	8.323	20.593	a	8.863	5.227	15.030	a	3.646	2.022	6.574	a	0.589	0.286	1.214	a
	AWD30	13.086	8.158	20.991	a	9.235	5.267	16.193	a	3.175	1.747	5.771	a	0.525	0.243	1.133	a
	DF	18.203	11.558	28.668	a	12.947	7.598	22.064	a	3.411	1.900	6.125	a	0.811	0.387	1.701	a
	F-BF	13.146	8.366	20.657	a	9.001	5.354	15.131	a	2.742	1.492	5.041	a	0.194	0.079	0.475	a
	F-DA	9.620	6.017	15.381	a	6.886	3.989	11.887	a	1.615	0.858	3.038	a	0.254	0.103	0.628	a
Reproductive	CF	7.104	4.474	11.280	a	5.381	3.011	9.617	a	1.559	0.997	2.437	a	0.196	0.091	0.422	a
	AWD15	9.229	5.887	14.468	a	6.291	3.578	11.061	a	1.974	1.286	3.030	a	0.383	0.201	0.729	a
	AWD30	5.981	3.757	9.521	a	4.390	2.467	7.813	a	1.009	0.622	1.638	a	0.349	0.175	0.697	a
	DF	9.111	5.688	14.594	a	7.346	4.037	13.367	a	1.457	0.930	2.283	a	0.386	0.199	0.748	a
	F-BF	10.443	6.671	16.349	a	8.448	4.795	14.885	a	1.461	0.939	2.273	a	0.313	0.161	0.608	a
	F-DA	8.913	5.595	14.200	a	7.246	4.045	12.981	a	1.468	0.927	2.326	a	0.298	0.144	0.618	a
Ripening & Post-harvest	CF	2.831	1.899	4.220	a	1.792	1.107	2.901	a	0.909	0.605	1.363	a	0.082	0.035	0.191	a
	AWD15	2.214	1.489	3.293	a	1.195	0.728	1.960	a	0.765	0.511	1.145	a	0.161	0.088	0.296	a
	AWD30	2.666	1.793	3.965	a	1.530	0.944	2.480	a	0.997	0.670	1.482	a	0.060	0.023	0.159	a
	DF	2.829	1.925	4.158	a	1.868	1.177	2.966	a	0.751	0.499	1.130	a	0.129	0.066	0.251	a
	F-BF	3.796	2.598	5.546	a	2.591	1.634	4.107	a	0.997	0.680	1.463	a	0.203	0.118	0.352	a
	F-DA	2.857	1.923	4.245	a	1.905	1.185	3.062	a	0.833	0.552	1.256	a	0.030	0.008	0.109	a

		Immature stage															
Rice growth phase	Treatment	All				Early instar				Late instar				Pupae			
		Mean	LCI	UCI		Mean	LCI	UCI		Mean	LCI	UCI		Mean	LCI	UCI	
Trial 6: Water management																	
All	CF	2.665	1.530	4.642	a	1.691	0.805	3.555	a	0.667	0.373	1.194	a	0.168	0.071	0.396	a
	AWD15	2.423	1.461	4.018	a	1.319	0.668	2.603	a	0.791	0.468	1.338	a	0.186	0.087	0.397	a
	FD-II	4.908	2.978	8.088	a	3.143	1.629	6.064	a	1.118	0.662	1.887	a	0.179	0.078	0.411	a
	FD-II2	5.204	3.144	8.616	a	3.411	1.745	6.669	a	1.331	0.796	2.227	a	0.316	0.159	0.629	a
	Supplemental	4.481	2.587	7.761	a	3.098	1.497	6.411	a	0.821	0.459	1.470	a	0.327	0.153	0.697	a
Land preparation	CF	NA	NA	NA	-	NA	NA	NA	-	NA	NA	NA	-	NA	NA	NA	-
	AWD15	NA	NA	NA	-	NA	NA	NA	-	NA	NA	NA	-	NA	NA	NA	-
	FD-II	NA	NA	NA	-	NA	NA	NA	-	NA	NA	NA	-	NA	NA	NA	-
	FD-II2	NA	NA	NA	-	NA	NA	NA	-	NA	NA	NA	-	NA	NA	NA	-
	Supplemental	NA	NA	NA	-	NA	NA	NA	-	NA	NA	NA	-	NA	NA	NA	-
Vegetative	CF	5.311	2.777	10.156	a	3.225	1.167	8.914	a	1.159	0.571	2.354	a	0.439	0.184	1.047	a
	AWD15	5.905	3.264	10.685	a	3.424	1.343	8.731	a	1.888	1.042	3.419	ab	0.609	0.291	1.275	a
	FD-II	6.309	3.430	11.604	a	3.300	1.251	8.709	a	2.152	1.178	3.931	ab	0.605	0.282	1.296	a
	FD-II2	13.123	7.288	23.631	a	6.866	2.697	17.481	a	4.778	2.735	8.346	b	1.235	0.656	2.327	a
	Supplemental	6.220	3.263	11.858	a	3.106	1.120	8.609	a	2.037	1.065	3.896	ab	0.694	0.327	1.473	a
Reproductive	CF	1.548	0.212	11.322	a	0.458	0.110	1.912	a	NA	NA	NA	-	NA	NA	NA	-
	AWD15	0.532	0.101	2.816	a	0.166	0.027	1.008	ab	NA	NA	NA	-	NA	NA	NA	-
	FD-II	4.750	1.087	20.764	a	2.660	0.949	7.458	b	NA	NA	NA	-	NA	NA	NA	-
	FD-II2	1.170	0.210	6.538	a	0.551	0.142	2.145	ab	NA	NA	NA	-	NA	NA	NA	-
	Supplemental	2.826	0.560	14.254	a	2.575	0.769	8.622	ab	NA	NA	NA	-	NA	NA	NA	-
Ripening & Post-harvest	CF	1.586	0.567	4.435	a	0.646	0.185	2.252	a	0.733	0.256	2.095	a	0.040	0.002	0.971	a
	AWD15	1.897	0.718	5.010	a	0.754	0.222	2.563	a	0.867	0.326	2.306	a	0.081	0.011	0.590	a
	FD-II	2.277	0.868	5.974	a	0.998	0.319	3.125	a	1.141	0.442	2.945	a	0.173	0.023	1.294	a
	FD-II2	2.468	0.985	6.185	a	1.762	0.605	5.129	a	0.704	0.267	1.857	a	0.000	0.000	Inf	a
	Supplemental	3.616	1.358	9.633	a	2.207	0.696	6.997	a	1.147	0.429	3.064	a	0.258	0.024	2.745	a

		Immature stage															
Rice growth phase	Treatment	All				Early instar				Late instar				Pupae			
		Mean	LCI	UCI		Mean	LCI	UCI		Mean	LCI	UCI		Mean	LCI	UCI	
Trial 7: Water management (Tanzania)																	
All	CF	9.689	5.340	17.58	b	7.798	4.247	14.32	b	1.900	0.900	4.009	a	0.720	0.395	1.312	b
	AWD15	2.942	1.637	5.286	a	2.296	1.243	4.240	a	0.960	0.450	2.047	a	0.280	0.146	0.538	ab
	AWD30	6.624	3.639	12.06	ab	5.327	2.875	9.871	ab	1.089	0.510	2.323	a	0.282	0.147	0.544	ab
	DF	2.951	1.641	5.306	a	2.064	1.128	3.778	a	1.006	0.469	2.156	a	0.437	0.233	0.819	ab
	II3	3.653	2.068	6.453	ab	2.913	1.627	5.214	ab	1.330	0.627	2.819	a	0.180	0.090	0.362	a
Land preparation	CF	1.281	0.225	7.276	a	1.270	0.225	7.158	a	NA	NA	NA	-	NA	NA	NA	-
	AWD15	0.581	0.096	3.534	a	0.583	0.096	3.528	a	NA	NA	NA	-	NA	NA	NA	-
	AWD30	1.960	0.380	10.12	a	1.961	0.381	10.08	a	NA	NA	NA	-	NA	NA	NA	-
	DF	0.354	0.058	2.172	a	0.355	0.058	2.162	a	NA	NA	NA	-	NA	NA	NA	-
	II3	0.359	0.058	2.234	a	0.360	0.058	2.227	a	NA	NA	NA	-	NA	NA	NA	-
Vegetative	CF	143.7	59.13	349.2	a	131.2	54.01	322.5	a	9.404	3.378	26.18	a	1.123	0.448	2.817	a
	AWD15	57.21	23.62	138.6	a	54.14	22.26	131.7	a	1.401	0.462	4.248	a	0.546	0.197	1.516	a
	AWD30	94.15	38.18	232.2	a	87.58	35.29	217.4	a	1.934	0.684	5.466	a	0.644	0.263	1.581	a
	DF	83.31	34.14	203.3	a	79.16	32.39	193.9	a	2.599	0.909	7.433	a	0.596	0.237	1.498	a
	II3	89.79	36.77	219.2	a	84.82	34.63	207.8	a	2.041	0.688	6.052	a	0.463	0.169	1.267	a
Reproductive	CF	13.99	2.247	87.17	a	15.21	2.378	97.23	a	NA	NA	NA	-	NA	NA	NA	-
	AWD15	6.296	1.287	30.81	a	4.452	0.899	22.05	a	NA	NA	NA	-	NA	NA	NA	-
	AWD30	0.823	0.095	7.169	a	0.882	0.100	7.743	a	NA	NA	NA	-	NA	NA	NA	-
	DF	1.773	0.257	12.25	a	1.586	0.229	11.00	a	NA	NA	NA	-	NA	NA	NA	-
	II3	8.265	1.449	47.13	a	6.698	1.176	38.14	a	NA	NA	NA	-	NA	NA	NA	-
Ripening & Post-harvest	CF	0.610	0.211	1.767	a	0.484	0.152	1.538	a	NA	NA	NA	-	0.092	0.014	0.613	a
	AWD15	0.639	0.217	1.881	a	0.506	0.153	1.675	a	NA	NA	NA	-	0.105	0.017	0.655	a
	AWD30	0.628	0.217	1.816	a	0.480	0.153	1.507	a	NA	NA	NA	-	0.052	0.008	0.338	a
	DF	0.753	0.268	2.120	a	0.466	0.148	1.472	a	NA	NA	NA	-	0.262	0.052	1.333	a
	II3	0.649	0.189	2.220	a	0.424	0.107	1.683	a	NA	NA	NA	-	0.066	0.007	0.607	a

Appendix 6.4. Biophysical characteristics of rice fields under different rice cultivation practices, separated by trial in Côte d'Ivoire and Tanzania (n=7).

Field characteristic	Treatments				
Trial 1: Land preparation					
	21d	**14d**	**7d**	**4d**	**2d**
Anopheles larvae					
Absent	65.8	64.9	72.3	79.1	70.2
Present	34.2	35.1	27.7	20.9	29.8
Culex larvae					
Absent	77.4	75.4	84.3	83.1	77.2
Present	22.6	24.6	15.7	16.9	22.8
Aquatic arthropods					
Absent	43.7	34.5	40.2	18.5	37.1
Present	56.3	65.5	59.8	81.5	62.9
Floating vegetation					
Absent	43.2	28.1	39.0	20.5	36.8
Present	56.8	71.9	61.0	79.5	63.2
Weeds					
Absent	83.5	90.7	87.3	61.0	86.0
Present	16.5	9.3	12.7	39.0	14.0
Water transparency					
None	5.0	0.0	0.9	8.1	0.9
Clear	41.6	38.6	40.5	45.9	42.1
A little cloudy	47.5	50.5	43.2	29.7	40.4
Cloudy	5.0	8.9	14.4	16.2	16.7
Opaque	1.0	2.0	0.9	0.0	0.0
Water depth					
0	5.0	0.0	0.9	8.1	0.8
0-5	58.4	54.5	59.5	56.8	51.8
5-10	36.6	45.5	39.6	35.1	47.4
Iron toxicity					
Absent	59.4	65.3	69.4	16.2	74.6
Present	40.6	34.7	30.6	83.8	25.4

Field characteristic	Treatments					
Trial 2: Crop establishment & water management						
	TP-CF	TP-AWD	DS-AWD	DS-WET-CF	DS-DS-CF	DS-DRY-CF
Anopheles larvae						
Absent	82.7	81.4	82.2	79.4	78.2	81.3
Prese	17.3	18.6	17.8	20.6	21.8	18.7
Culex larvae						
Absent	86.8	86.6	83.1	84.9	84.7	85.7
Present	13.2	13.4	16.9	15.1	15.3	14.3
Aquatic arthropods						
Absent	70.8	73.4	78.7	75.2	74.2	71.8
Present	29.2	26.6	21.3	24.8	25.8	28.2
Floating vegetation						
Absent	76.6	78.7	69.0	74.9	76.3	74.4
Present	23.4	21.3	31.0	25.1	23.7	25.6
Weeds						
Absent	59.4	59.7	74.4	61.3	59.2	59.8
Present	40.6	40.3	25.6	38.7	40.8	40.2
Water transparency						
None	5.9	5.7	9.5	5.8	6.1	7.3
Clear	37.4	35.6	50.4	38.0	41.3	36.1
A little cloudy	52.6	56.1	35.8	54.9	51.3	54.3
Cloudy	3.6	1.8	3.7	1.3	0.4	0.9
Opaque	0.5	0.8	0.7	0.0	0.9	1.4
Water depth						
0	5.8	5.7	9.5	5.8	6.1	7.3
0-5	45.9	42.5	43.1	44.2	50.9	40.2
5-10	44.2	47.4	46.0	45.1	36.9	16.2
10+	4.1	4.4	1.5	4.9	6.1	6.3
Iron toxicity						
Absent	60.8	53.6	57.7	62.1	59.6	57.5
Present	39.2	46.4	42.3	37.9	40.4	42.5

Field characteristic	Treatments	
Trial 3: Water management		
	CF	**AWD15**
Anopheles larvae		
Absent	77.6	76.0
Present	22.4	24.0
Culex larvae		
Absent	80.8	79.3
Present	19.2	20.7
Aquatic arthropods		
Absent	74.7	73.4
Present	25.3	26.6
Floating vegetation		
Absent	67.4	64.2
Present	32.6	35.8
Weeds		
Absent	65.0	63.2
Present	35.0	36.8
Water transparency		
None	12.7	10.5
Clear	63.9	66.0
A little cloudy	17.2	17.9
Cloudy	3.7	2.6
Opaque	2.4	3.0
Water depth		
0	11.9	10.7
0-5	53.1	53.5
5-10	27.4	30.8
10+	7.5	5.0
Iron toxicity		
Absent	13.5	10.0
Present	86.5	90.0

Field characteristic	Treatments									
Trial 4: Water & nutrient management										
	CF	**AWD15**	**AWD15-2DAT**	**FD-II**	**FD-II2**	**II3**	**Rainfed**	**F-FD**	**F-NONE**	**No rice**
Anopheles larvae										
Absent	82.7	86.0	86.8	84.5	89.8	88.2	86.3	84.8	87.9	88.2
Present	17.3	14.0	13.2	15.5	10.2	11.8	13.7	15.2	12.1	11.8
Culex larvae										
Absent	86.0	84.8	83.8	86.0	89.2	85.0	87.8	85.6	89.7	89.2
Present	14.0	15.2	16.2	14.0	10.8	15.0	12.1	14.4	10.3	10.8
Aquatic arthropods										
Absent	81.8	79.6	81.2	82.5	89.4	83.0	83.2	83.9	85.0	81.3
Present	18.2	20.4	18.8	17.5	10.6	17.0	16.8	16.1	15.0	18.7
Floating vegetation										
Absent	99.1	96.4	88.4	99.9	82.6	83.1	92.4	84.8	92.5	84.5
Present	0.9	3.6	11.6	0.1	17.4	16.9	7.6	15.2	7.5	15.5
Weeds										
Absent	98.9	97.4	96.7	95.8	96.2	95.2	96.9	96.0	97.1	64.3
Present	1.1	2.6	3.3	4.2	3.8	4.8	3.1	4.0	2.9	35.7
Water transparency										
None	24.4	17.6	23.1	23.9	35.2	14.1	25.8	14.6	26.9	21.1
Clear	39.7	46.4	38.5	40.3	36.7	44.5	48.4	40.8	36.2	29.7
A little cloudy	33.6	33.6	33.8	34.3	21.9	38.3	23.4	41.5	33.8	42.2
Cloudy	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8
Opaque	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8
Water depth										
0	24.4	17.6	22.7	23.9	34.9	14.1	25.5	14.6	26.7	22.7
0-5	64.9	68.0	66.9	67.9	53.1	69.5	62.5	71.5	60.0	57.0
5-10	10.7	14.4	9.2	6.7	10.9	15.6	10.9	12.3	13.1	15.6
10+	0.0	0.0	1.2	1.5	1.1	0.8	1.1	1.5	0.2	4.7
Iron toxicity										
Absent	11.5	18.4	15.4	14.2	15.6	18.0	14.8	16.9	15.4	16.4
Present	88.5	81.6	84.6	85.8	84.4	82.0	85.2	83.1	84.6	83.6

Field characteristic	Treatments					
Trial 5: Water & nutrient management						
	CF	**AWD15**	**AWD30**	**DF**	**F-BF**	**F-DA**
Anopheles larvae						
Absent	70.9	72.1	73.0	70.4	70.8	72.1
Present	29.1	27.9	27.0	29.6	29.2	27.9
Culex larvae						
Absent	75.6	77.1	80.3	74.4	74.8	77.2
Present	24.4	22.9	19.7	25.6	25.2	22.8
Aquatic arthropods						
Absent	44.6	43.6	40.5	39.6	44.5	42.8
Present	55.4	56.4	59.5	60.4	55.5	57.2
Floating vegetation						
Absent	46.1	43.1	41.6	38.3	43.1	45.2
Present	53.9	56.9	58.4	61.7	56.9	54.8
Weeds						
Absent	58.4	59.9	57.9	54.7	57.9	59.4
Present	41.6	40.1	42.1	45.3	42.1	40.6
Water transparency						
None	19.3	19.6	15.9	14.5	19.8	17.3
Clear	47.4	47.4	48.9	51.5	50.2	49.8
A little cloudy	23.7	26.1	26.0	28.2	21.1	23.8
Cloudy	5.7	4.4	5.7	4.0	7.1	6.9
Opaque	4.0	2.6	3.5	1.8	1.8	2.2
Water depth						
0	18.6	19.5	16.1	14.4	18.9	16.9
0-5	62.3	60.0	62.6	61.7	59.9	61.0
5+	17.5	19.6	19.4	22.5	19.4	20.8
10-50	1.5	0.9	1.9	1.4	1.8	1.3
Iron toxicity						
Absent	21.5	23.5	22.5	21.1	24.2	19.9
Present	78.5	76.5	77.5	78.9	75.8	80.1

Field characteristic	Treatments				
Trial 6: Water management					
	CF	**AWD15**	**FD-II**	**FD-II2**	**Rainfed**
Anopheles larvae					
Absent	86.5	84.6	84.2	78.1	80.8
Present	13.5	15.4	15.8	21.9	19.2
Culex larvae					
Absent	89.9	90.2	90.3	91.5	86.2
Present	10.1	9.8	9.7	8.5	13.8
Aquatic arthropods					
Absent	35.4	36.6	33.2	45.1	31.7
Present	64.6	63.4	66.8	54.9	68.3
Floating vegetation					
Absent	28.1	31.6	28.1	40.0	21.7
Present	71.9	68.4	71.9	60.0	78.3
Weeds					
Absent	80.5	81.6	79.0	81.9	79.0
Present	19.5	18.4	21.0	18.1	21.0
Water transparency					
None	23.0	19.0	18.0	30.5	16.7
Clear	39.3	39.7	49.2	44.1	48.3
A little cloudy	26.2	36.2	24.6	20.3	30
Cloudy	6.6	1.7	3.3	5.1	1.7
Opaque	4.9	3.5	4.9	0.0	3.3
Water depth					
0	23.0	19.0	18.0	30.4	16.2
0-5	55.7	60.3	57.4	49.2	61.7
5-10	18.0	20.7	23.0	16.9	20.0
10+	3.3	0.0	1.6	3.5	2.1
Iron toxicity					
Absent	19.7	19.0	14.8	18.6	23.3
Present	80.3	81.0	85.2	81.4	76.7

Field characteristic	Treatments				
Trial 7: Water management (Tanzania)					
	CF	**AWD15**	**AWD30**	**DF**	**II**
Anopheles larvae					
Absent	80.7	89.3	91.1	86.5	88.2
Present	19.3	10.7	8.9	13.5	11.8
Culex larvae					
Absent	90.2	91.7	91.0	92.2	93.3
Present	9.8	8.3	9.0	7.8	6.8
Aquatic arthropods					
Absent	99.2	99.8	99.9	99.9	99.8
Present	0.8	0.2	0.1	0.1	0.2
Floating vegetation					
Absent	80.5	82.1	84.9	78.4	82.0
Present	19.5	17.9	15.1	21.6	18.0
Weeds					
Absent	97.8	99.2	99.6	99.0	98.7
Present	2.20	0.8	0.4	1.0	1.3
Water transparency					
None	47.8	60.9	66.5	55.3	62.0
Clear	23.9	19.9	19.5	19.3	19.0
A little cloudy	27.0	16.1	9.8	23.6	16.6
Cloudy	1.3	2.5	4.3	1.9	2.5
Opaque	0.0	0.6	0.0	0.0	0.0
Water depth					
0	47.8	61.9	66.3	54.8	61.8
0-5	47.8	34.8	29.9	40.4	35.0
5-10	4.4	3.3	3.8	4.8	3.2
Iron toxicity					
Absent	6.9	8.1	3.7	5.0	4.3
Present	93.1	91.9	96.3	95.0	95.7

Appendix 6.5. Sample size calculations for testing rice-growing practices on *Anopheles* vector abundance

Sample size calculations for the rice trials are estimated using data collected from preliminary trials that were included in Chapter 5. These calculations were conducted using STATA/SE v17.0.

Under continuous flooding (control), the mean density of pupae and larvae per plot per day (the main outcomes of interest) were estimated to be 0.35 and 3.07, respectively (Table 6.1).

Table 6.1. Summary of mosquito abundance collected in the pilot study.

Descriptive statistic	Pupae	Larvae (early & late instars)
Mean	0.35	3.07
Median	0	0
Minimum	0	0
1st quantile	0	0
3rd quantile	0	2
Maximum	78	358
Variance	6.37	121.16
Standard deviation	2.52	10.99
Standard error	0.03	0.11
Within-plot variance	187.90	4459.94
Between-plot variance	58219.80	1105749.70
Intra-class correlation coefficient (ICC)	0.00181	0.00267

Sample size calculations were estimated based on the following:

- The number of replicate plots (per treatment) that agronomists use in a trial, which is typically 3 replicates
- The mean "cluster"[37] size, which is typically 352 samples per plot throughout a cropping season (calculated from 8 samples per plot x 22 weeks in a cropping season x 2 sampling occasions per week)
- The intra-class correlation coefficient (ICC), which indicates the degree of similarity between samples within a plot, which was estimated to be 0.00181 for pupae and 0.00267 for larvae (Table 6.1).

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[37] Samples were grouped by plot to form a "cluster"

For a fixed sample size design, in order to achieve a power of 1 - β = 0.80 to conduct a linear regression at level α = 0.05, **an effect size of 0.40** was required for pupae (i.e., 114.3% reduction / 7-fold reduction) and **1.865** for larvae (i.e. 60.7% reduction / 2.55-fold reduction).

Previous water management trials have shown much smaller reductions than ths: for example, a 1.54-fold reduction in late instar larvae between treatment and control plots [44]. Using these typical observations, of effect sizes of 0.227 for pupae and 1.993 for larvae, a total number of **31 replicates** would be required to detect a significant difference in pupae between treatment and control plots and **9 replicates** to do the same for larvae.

The agronomists that we were collaborating with at Africa Rice Center regarded these levels of plot replication as unreasonable because of additional labour and costs. We were therefore faced with the choice of (a) abandoning the trial, or (b) doing the trial but without AfricaRice, thus abandoning the collaboration, or (c) persist with the underpowered trial in order to build collaboration and shared experience with AfricaRice, in the hope of learning lessons together about replication and experimental power. We decided that for our long-run purposes, option (c) was the best choice. Our hope, in making this choice, was that our rice agronomist colleagues might eventually be persuaded that it is their job to address the problem of mosquitoes, and having done so, then they would elect to use experimental designs that could adequately measure mosquitoes.

Appendix 6.6. The differentiation of developmental stages of mosquito immatures by technician

To compare the ratios of early and late instar larvae captured in a plot between technicians, a generalised linear mixed-effect model with negative binomial distribution was fitted using the "glmer.nb" function from the "lme4" package in R. As dips conducted within a plot may be correlated, plots were accounted for in the model as a random effect. The trial that mosquitoes were collected in was accounted for as a fixed-effect explanatory factor. Estimated marginal means (i.e., mosquito density means that were adjusted for other factors in the model) were computed using the "emmeans" package. *Post-hoc* Tukey's Honest Significant Difference (HSD) tests were run for multiple pair-wise comparisons between treatments.

No significant differences were seen in early:late instar ratios between technicians (Table 6.2). This indicates that there was no detected bias in the subjectivity of technicians when distinguishing between early and late instar larvae.

Table 6.2. The ratio of early and late instar mosquito larvae captured between technicians

Technician	Early:late instar (95% CI)
A	1.87 (1.33 – 2.63) a
B	3.53 (2.29 – 5.45) a
C	2.93 (2.26 – 3.81) a

Appendix 7. Chapter 7 Supplementary Material

Appendix 7.1 In-depth interview topic guide

Participant ID:		**Participant gender:**	Male / Female
Researcher initials:		**Date:**	____ /____/_____
Audio file number:	- start recording -		

Introduction

I am _______________ from __________________, collaborating with the London School of Hygiene & Tropical Medicine.

Aims of the interview	*To explore rice communities' views and perspectives on mosquitoes*
Expected duration	*Approximately one hour*
Why the participant's cooperation is important	*We need to know their experiences to find out if, and how, we can reduce mosquito populations in rice farming communities*
What will happen with the collected information?	*Results will be used to design the next part of the study*
Confidentiality	*- Inform them -*
Any questions?	*- Ask -*
Use of tape recorder	*- Inform them -*
Consent form	*- Signature -*

Demographic and Work History

Can I ask some details about you and your household?

School education level:		**Year of graduation:**	
How long have you lived in this house?		**Are you originally from this area / district?**	Yes / No
How old are you?			

Now, I would like to ask you some questions about your experiences with rice farming and mosquitoes.

Domain	Topic and Probes	
1.Rice farming	a)	Does your household farm any rice? Who works on the farm? *Where is the farmland? How long has your family cultivated this land? And of that time, how long has it been rice?*
	b)	What kind of rice do you grow? *Variety, agrosystem (rain-fed/irrigated)*

	c)	How easy is it to grow rice? *What are the difficulties with growing rice? Is water readily available? What are main choices/decisions to be made at the start of the season? What influences those decisions (why choose to do x not y)?*
	d)	What are the disadvantages of living next to rice fields? *Are you busier? What about your health?*
2.Mosquitoes	a)	Are there mosquitoes here? *Where? When? What factors influence whether there are many or few?*
	b)	Are mosquitoes a problem? What kind of problem? What are the consequences of mosquitoes? *Are mosquitoes a nuisance? How much of a nuisance (compared to flies, bed bugs, …)? Why are mosquitoes a problem? Nuisance? Diseases? Lack of sleep?*
	c)	When are mosquitoes a problem? When do they come? - *What part of the day?* - *Which season(s)?* - *What part of the season?* - *What part of the year?*

	d)	The history of mosquitoes: are there more or less now? *Compared to the last 10 years? Has anything changed? Do you remember a time where it was not a problem? Or a very large problem?*
	e)	Where do mosquitoes come from? *Physically, where?*
3. Mosquito control	a)	Can you control mosquito numbers in the house? *What can you do in the house to protect yourself? Is there anything you can personally do to control the number of mosquitoes you and your family are exposed to? Ask them for their own ideas.*
	b)	If you can control mosquito numbers in the house, do you do them? What do you do? How much does this cost? How effective are they? Is one better than the other? *If not, why don’t you do them? What would have to happen for you to think about doing these things?*
	c)	Can you control mosquito numbers in the community? *What can we do to reduce the number of mosquitoes in the village? What can an individual rice farmer do?*

	d)	If you can control mosquito numbers in the community, do you do them? What do you do? *If not, why don't you do them? What would have to happen for you to think about doing these things?*
	c)	Can mosquitoes grow in water? *Is there any change you can make to the way you grow rice that will reduce the number of mosquitoes in your area?*
Closing		Is there anything else you think is important in rice farming and mosquitoes that we have not talked about? - Summarise - Thank participant

Contact Summary

1	How would you describe the atmosphere and context of the interview?
2	What were the main points made by the respondent during this interview?
3	What non-verbal behaviour (gestures and actions) were made throughout the course of the interview?

4	What new information did you gain through this interview compared to previous interviews?
5	Was there anything surprising to you personally? Or that made you think differently?
6	What main messages did you take from this interview about mosquitoes?
7	Were there any problems with the topic guide (e.g., wording, order of topics, missing topics) you experienced in this interview?

Appendix 7.2 Focus group discussion topic guide

Rice farmers' views on their responsibility for mosquito production	Where do they think mosquitoes come from?
	Does rice generate mosquitoes?
Collective initiatives to control mosquitoes, according to the rice farmers	What has been done to control mosquitoes?
Prospects for collective solutions to control mosquitoes	What can be done to control mosquitoes?
Decision-making process in reducing mosquito production	Why hasn't anything been done to control mosquitoes?

Appendix 7.3 Images of *bas-fonds* in central Côte d'Ivoire

(A) Image of M'bé irrigation scheme taken from a drone

(B) Image of M’bé irrigation scheme taken during the wet season

(C) Image of M’bé irrigation scheme taken during the dry season

Appendix 8. Chapter 8 Supplementary Material

Appendix 8.1. The effect of rice cultivation techniques on malaria vector densities according to studies conducted in this thesis.

Aspect	Operation	Treatment	Control	Effect on malaria vector densities (percent difference)	Evidence (Number of studies, Countries)
Land preparation	Tillage	Minimal tillage	Deep tillage	Decrease (-65%)	Chapter 4: 1 study (Benin)
	Levelling	Normal levelling	Abnormal levelling	Inconsistent	Chapter 4: 1 study (Benin)
	Timing of flooding between primary and second tillage	2 days	21 days	Decrease (-36% early instars)	Chapter 6: Côte d'Ivoire
Crop establishment	Direct seeding	Line wet seeding with drum-seeder / manual wet direct broadcast seeding	Transplanting	Increase (+47% late instars)	Chapter 6: Côte d'Ivoire
	Plant variety	Tall (99 cm)	Short (45 cm)	Inconsistent	Chapter 4: 1 study (Japan)
	Plant spacing	60 hills/m^2	80 hills/m^2	Inconsistent	Chapter 4: 1 study (India)
Water management	Alternate wetting and drying irrigation (AWD)	AWD-15/30	Continuous flooding	Inconsistent (increase in pupae in Côte d'Ivoire, decrease in late instars in Tanzania)	Chapter 6: Côte d'Ivoire, Tanzania
	Forced drainage	Forced drainage at 25 DAT followed by AWD or intermittent irrigation	Continuous flooding	Inconsistent	Chapter 6: Côte d'Ivoire
	Intermittent irrigation	Intermittent irrigation at 3 day-wet and 3 day-dry intervals	Continuous flooding	Inconsistent	Chapter 6: Côte d'Ivoire, Tanzania
		Active or passive drainage (general)	Continuous flooding	Decrease (-35% late instars)	Chapter 4: 7 studies (USA, Portugal, Benin, Kenya, India)
	Water depth	Medium/deep (3-8 inches)	Shallow (1-2 inches)	Increase (+96%)	Chapter 4: 1 study (USA)
	Water management system	Inefficient drainage system in irrigation system	Renovated irrigation system	Inconsistent	Chapter 4: 1 study (Mali)

Weed management	Herbicide application	Herbicide	None	Increase (+77%)	Chapter 4: 1 study (USA)
Nutrient management	Fertiliser application	Fertiliser	None	Increase (-48%)	Chapter 6: Côte d'Ivoire
		Forced drainage before application	Normal timing	Inconsistent	Chapter 6: Côte d'Ivoire
		Fertiliser applied before flooding during land preparation	Normal timing	Inconsistent	Chapter 6: Côte d'Ivoire
		Fertiliser application delayed at 20 DAT	Normal timing	Inconsistent	Chapter 6: Côte d'Ivoire
Pest management	Pesticide application	Pesticide	None	Decrease (-76%)	Chapter 4: 1 study (Indonesia)
Other	Fish	Various species (e.g., carp, tilapia)	None	Decrease (-82 to -87%)	Chapter 4: 6 studies (USA, India, S. Korea, Liberia)
	Azolla	Azolla	None	Inconsistent	Chapter 4: 1 study (India)
	Neem	Neem	None	Inconsistent	Chapter 4: 1 study (India)

Appendix 8.2. Comparison of the riceland mosquito sampling programmes used in studies included in Chapter 4.

- Most studies used dippers (usually ranging between 350 to 500 ml) to sample for mosquito immatures in rice fields (n=23/25).
- Mosquito sampling frequency depended on the type of intervention. Studies that were testing chemical and biological larvicides tended to sample 24 hours before treatment and a select number of days following treatment (n=8). Other studies tended to sample two or three times per week.
- The number of dips in a plot or replicate depended on the study but most studies conducted 20 or more dips within a plot.
- When quantifying mosquito immatures, many studies separated them by developmental stage (n=15), whilst others counted all immatures together (n=9).
- When comparing mosquito densities between treatments, most studies used analysis of variance (ANOVA) on log(x+1) transformed mosquito counts (n=8). It is no longer applicable to transform count data [348]. This is because regression of transformed variables can lead to impossible predictions i.e., negative numbers of individuals. Thus, it has been suggested to fit Poisson or negative binomial models to count data instead.

Reference	Plot size (m^2, per treatment)	No. of repl-icates	Mosquito sampling method	Mosquito sampling frequency and duration throughout rice season	Mosquito sampling frequency within a plot	Differentiation into immature development stages	Statistical analysis method
Hill & Cambournac 1941	100-2000	4	Dip	-	100 dips	Total	-
Reiter 1980	600	9-15	350 ml dipper	-	Not reported	L/P	-
Yu et al. 1981	0.5 acre	2	500 ml dipper	-	4/+	Total	-
McLaughlin et al. 1982	30	3	350 ml dipper	Pre- + 1,2,3 days post-intervention	-	Total	-
Yap et al. 1982	69-365	2	15.5 cm dipper	Pre- + 2,4,7,14,21,28 days post- intervention	Along levees	Total	-
Balaraman et al. 1983	1000	5	10 cm dipper	Pre- + up until 19 days post-intervention	10 dips	L1/L2/L3/L4/P	-
Kramer et al. 1988	1000	3	400 ml dipper	Weekly	40 dips at margin, 20 dips in middle	Total	One-way ANOVA
Martono 1988	250	2	11 cm dipper	-	500 dips	Total	-
Rajendran & Reuben 1991	40	2	350 ml dipper	3 times / week	20 dips at margin	L1/L2/L3/L4/P	ANOVA
Sundaraj & Reuben 1991	440	3	Dip	Pre-intervention + 3 times / week until harvest	10 dips	L1/L2/L3/L4/P	Bespoke formula

Ravoahangimalala et al. 1994	43-110	2	Dip	Pre- + 1,2,3 + every 3 days until 4 weeks post-intervention	10 dips	L3/L4/P	Mulla's equation, then Student t-test
Victor et al. 1994	400	3	350 ml dipper	3 times / week, from TP[b] until 6 weeks	20 dips	L1/L2/L3/L4/P	Log(x+1) transformation, then ANOVA
Rajendran et al. 1995	16.2-22.3 ha	78-85	350 ml dipper	3 times / week, from TP until harvest	1 dip	L1/L2/L3/L4/P	Late instars * proportion of total area under water, log(x+1), then ANOVA
Rao et al. 1995	400	3	350 ml dipper	3 times / week	20 dips at margin	L1/L2/L3/L4/P	Log(x+1) transformation, then factorial ANOVA
Takagi et al. 1996	1500	2	13 cm dipper	Weekly for 6 weeks	10 dips	L1/L2/L3/L4/P	Log(x+1) transformation, then Tukey & Kramer method
Marten et al. 2000	100	2	Dip	-	100 dips	L1/L2/L3/L4/P	-
Mutero et al. 2000	750	4	350 ml dipper	2 times / week from TP until 2 weeks after mature rice formed canopy	20 dips at margin	L1/L2/L3/L4/P	Log(x+1) transformation, then ANOVA
Victor & Reuben 2000	40	4	Quadrat	3 times / week, for 6 weeks after TP	Within 2 quadrats	L3/L4/P	Log(x+1) transformation, then two-way ANOVA
Dennett et al. 2001	2000	2	Dip	Pre- + 2,8,14,23 days post- intervention	160 dips	L1/L2/L3/L4/P	Square-root transformation, then ANOVA
Krishnasamy et al. 2003	Varying sizes	5	350 ml dipper	3 times / week	20 dips at margin	L1/L2/L3/L4/P	ANOVA
Teng et al. 2005	119-194	4	14 cm dipper	Pre- + 2,4,7,14,20,28,35,42 days post-intervention	150 dips	L1/L2/L3/L4/P	-
Sogoba et al. 2007	1000	4	500 ml dipper	Fortnightly collection for a year	20 dips	Total	-
Allen et al. 2008	13-15 hectares	2	350 ml dipper	Pre- + 1,3,5,6 days post- intervention	100 dips along transect	Total	Log(x+1) transformation, then ANOVA
Bukhari et al. 2011	2000	6	$0.085m^2$ area sampler	Every 4-5 days	9 sites along transect	L1/L2/L3/L4/P	Log(x+1) transformation, then Mulla's[a] equation
Djegbe et al. 2020	16.5	5	350 ml dipper	-	20 dips	Total	ANOVA

[a] Mulla's equation 1971 to calculate percent reduction

[b] Transplanting

Appendix 8.3. Variations in irrigated rice cultivation techniques

Intervention	Choices – 1st degree	Choices – 2nd degree	Examples	Choices – 3rd degree
Land preparation	Wet	Timing of pre-irrigation	3-7 days	
		Type of primary tillage	Minimal / reduced tillage	
			Deep tillage	
		Timing of puddling	<1-3 weeks	
		Type of secondary tillage	(As above)	
		Type of levelling	Manual levelling	
			Laser levelling	
		Timing of flooding before crop establishment	2-4 days	
Rice variety	Rice variety			
Crop establishment	Transplanting	Type of seedling preparation	Wet-bed	
			Dry-bed	
		Timing of transplanting		
		Type of transplanting	Manual transplanting	Spacing: random/straight-row
			Mechanical transplanting	Spacing: random/straight-row
	Wet direct seeding	Timing of wet direct seeding		
		Type of wet direct seeding	Broadcasting	
			Dibbling / line seeding	Spacing
	Dry direct seeding	Timing of dry direct seeding		
		Type of dry direct seeding	Broadcasting	
			Drilling / line seeding	Spacing

Water management	Continuous flooding	Changes in water depth throughout season		
	Non-continuous flooding	Type of drainage	Passive drainage (e.g., alternate wetting and drying irrigation)	Timing / water level below soil
			Active drainage (e.g., mid-season drainage)	Timing
Weed management	Weeding (or no weeding)	Timing of weeding		
		Type of weeding	Herbicide	Water conditions
			Manual weeding	Water conditions
			Mechanical weeding	Water conditions
Nutrient management	Fertiliser application (or no application)	Timing of fertiliser application		
		Type of fertiliser	Inorganic	Water conditions
				Application rate
			Organic	Water conditions
			Combination of inorganic and organic	Water conditions