Larval ecology of malaria vectors and the impact of larviciding on malaria transmission in The Gambia

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Abstract

The study reported in this thesis explored the ecology of aquatic stages of mosquitoes in the middle reaches of the Gambia River in order to assess the feasibility and impact of microbial larviciding on malaria transmission in large river ecosystems in sub-Saharan Africa.

All accessible water bodies in four study zones covering 400 km^2 were mapped and sampled for mosquitoes. Microbial larvicides were applied in the four zones in a cross-over design and the impact of larviciding on mosquito densities assessed.

Anopheline and culicine mosquitoes were found in all sampled habitats, apart from those with moving water. Similarly, all habitats, except puddles and water channels, had similar larval and pupal densities. *Anopheles gambiae sensu lato*, the major malaria vector in Africa, exploited a wide range of habitats and despite a decrease in population density during the dry season, could be found in breeding sites throughout the year. Mosquitoes shared habitats with other invertebrates including their predators. A closer look at rice fields revealed that mosquitoes were abundant in rice fields closer to the landward edge of the floodplains where water is fresher and contains high quantities of nutrients.

Mosquitoes of The Gambia were highly susceptible to both *Bacillus thuringiensis* var. *israelensis* (*Bti*) and *B. sphaericus* microbials, however no residual activity against anopheline larvae was observed. The basic training of personnel in identification of habitats, calibration of application equipment and active larviciding proved to be successful.

Routine larviciding was associated with > 91 % reduction ($p < 0.001$) in anophelines late stage larval density and 72 % ($p < 0.001$) in culicines. Overall, larviciding was associated with a 28% ($p = 0.005$) reduction in the number of adult female *Anopheles gambiae s.l.* found indoors, although this rose to 42%, when the study zone with the greatest abundance of breeding sites was excluded from the analysis. No significant reduction in adult culicines was observed.

Ground application of *Bti* in areas with extensive floodplains is unlikely to contribute to a substantial reduction in malaria transmission in The Gambia, therefore vector control in such areas should target adult mosquitoes.

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I dedicate this thesis to my wife Ange-Nadine Munezero for her immeasurable support, love and faithfulness; to my parents and family for their everlasting love and encouragements. I return all the glory and honour to God for His grace upon my life and for seeing me through easy and tough times.

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List of abbreviations

ACT	Artemisinin combination therapies
BHC	Benzene Hexachloride
CI	Confidence interval
DDT	dichlorodiphenyltrichloroethane
EIR	Entomological inoculation rate
ELISA	Enzyme-linked immune-sorbent assay
GDP	Gross domestic product
GEE	Generalised estimating equations
IGR	Insect growth regulator
IPT	Intermittent preventive treatment
IRS	Indoor residual spraying
ITN	Insecticide-treated bed nets
IVM	Integrated vector management
LLIN	Long lasting insecticide-treated bed nets
LSM	Larval source management
MRC	Medical research council
NIBP	National impregnated bed net programme
NIH	National Institutes of Health
NMCP	National malaria control programme
OCP	Onchocerciasis control programme
OR	Odds ratio
P	Probability value (in statistical analysis)
PCR	Polymerase chain reaction
POP	Persistent organic pollutants
RBM	Roll back malaria
SSA	sub-Saharan Africa
USD	United States dollars
UTM	Universal transverse Mercator
WHO	World Health Organisation

Declarations

None of the material contained in this thesis has been previously submitted for a degree in this or any other university. Chapters 2 and 4 appeared as papers in peer-reviewed journals in a modified format. The contributions of other people to each chapter are listed below:

Chapter 1: Background and introduction: The burden of malaria, vector ecology and control measures

Silas Majambere (SM) was responsible for the identification and collation of papers for inclusion in the review and wrote the review. Steve Lindsay (SL) edited the review.

Chapter 2: Spatial distribution of mosquito larvae and the potential for targeted larval control in The Gambia

SM, SL and Ulrike Fillinger (UF) designed the study. SM was responsible for the implementation of field work, Clare Green (CG) was responsible for the molecular work and David Sayer (DS) helped with GIS work. SM, UF and SL contributed to the analyses, SM wrote the first draft and all authors contributed to editing it.

Chapter 3: Productivity of different habitats for *Anopheles* larvae and pupae in rural Gambia

SM, UF and SL designed the study. SM was responsible for the implementation of field work. CG was responsible for the molecular work. SM did the analysis and wrote the chapter. SL edited the chapter.

Chapter 4: Agriculture and the promotion of insect pests: swamp rice cultivation and malaria vectors in The Gambia

SL, SM and UF designed the study. Lamin Jarju (LJ) was responsible for the implementation of field work. SM trained and supervised LJ in the field. CG was responsible for the molecular work. Vasilis Louca (VL) helped with fish sampling and measurement of water parameters. SL did the analysis, wrote the first draft and all authors contributed to editing it.

Chapter 5: Microbial larvicides for malaria control in The Gambia
SM, UF and SL designed the study. SM was responsible for the implementation of field work, CG was responsible for the molecular work and Balla Kandeh (BK) helped in recruiting spraymen from the NMCP. SM, UF and SL contributed to the analyses, SM wrote the first draft and all authors contributed to editing it.

Chapter 6: Impact of larviciding on malaria vectors in The Gambia
SM, UF and SL designed the study. SM was responsible for the implementation of field work, CG was responsible for the molecular work. SM did the analysis and wrote the chapter. UF and SL edited the chapter.

Chapter 7: General conclusions
SM wrote the conclusion for the study, SL edited it.

For the entire study, field work was carried out by field assistants and spraymen, supervised by a senior field assistant and SM. SM, SL and UF participated in the recruitment and training of field staff and SM was responsible for their overall supervision. SM checked all data forms and data were double entered and validated by data entry clerks supervised by a data manager.

Ethical clearance
Ethical approval for the study was given by the Joint Gambian Government and Medical Research Council's Laboratories in The Gambia (SCC 959, L2005.55 & L2006.20.26), Science faculty Institutional Review Board (IRB), Durham University (No number) and the NIH (DMID Protocol number 05-0067/68). Verbal consent for the study was obtained from local leaders and the community at large before collecting baseline data. Prior to the implementation of larval control operations, the community was again briefed on the nature of larviciding and consent obtained to apply microbials.

Chapter 1

Background and introduction

The burden of malaria, vector ecology and control measures

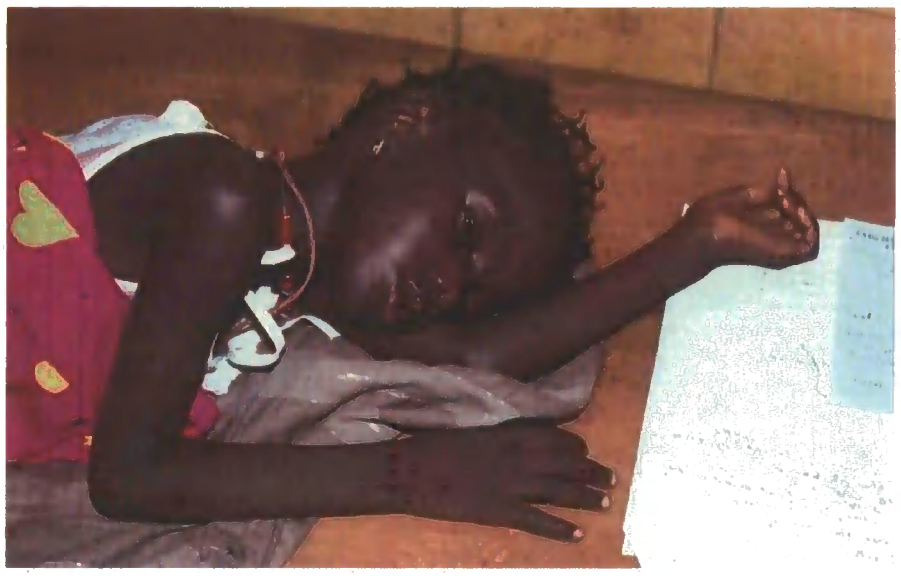

Figure 1.1 Child suffering from malaria

Background and introduction

The burden of malaria, vector ecology and control measures

The burden of malaria

Although malaria is a preventable and curable disease, it remains one of the most prevalent parasitic diseases of humans (Gardiner et al. 2005). Most figures showing the burden of malaria in the World particularly in Africa are a result of best guesses (Hay and Snow 2006) because of the absence of informative maps of malaria risk. However, recent estimates based on epidemiological data linked to the endemicity of malaria in different regions of the World show that in 2002 there were 515 million (range 300-660 million) episodes of clinical *Plasmodium falciparum* malaria (Snow et al. 2005). A staggering 70% of these cases (365 million clinical cases) occurred in Africa, representing close to a 40% increase over only 10 years from the figures reported by same authors in 1995 (Snow et al. 1999). It is worth noting that the 1995 figures were based on epidemiological data while the 2005 were more specific and took into account the endemicity of different regions. Malaria increased in the 1990s compared to the 1980s when measured in terms of proportions of populations at risk, severity of infections and number of deaths (RBM 2005). While mortality resulting from other diseases decreased, child mortality due to malaria increased two-fold between the 1980s and early 1990s (RBM 2005).

The economic burden caused by malaria is enormous and the gross domestic product (GDP) in malarious country is estimated to be more than five times less than in countries without high levels of malaria (Gallup and Sachs 2001). Countries with a high proportion of the population living in malarious areas had an annual economic growth rate 1.3% lower than other countries between 1965 and 1990 (Gallup and Sachs 2001). It is estimated that malaria alone costs African countries USD12 billion each year in lost GDP and consumes up to 25% of household income and 40% of government health spending (RBM 2005). Although the causes of poverty in sub-Saharan Africa (SSA) cannot be explained by malaria alone, it is striking that where malaria prospers most, human societies prosper less (Sachs and Malaney 2002) but the causality in the relationship between poverty and malaria seem to run in both directions. Malaria causes poverty and poverty results in more malaria.

Malaria transmission

Studies on transmission stability show that malaria is transmitted more robustly in SSA than it is elsewhere and this would be due to the intrinsic properties of vectors coupled with favourable climatic conditions than to differences in health systems or malaria interventions (Kiswewski et al. 2004). The annual entomological inoculation rate (EIR expressed as the number of infective mosquito bites per person per year) as a measure of transmission is very heterogeneous in Africa. A review done in 2000 on the EIR across Africa from 1980 onwards show an average of 121 infective bites in 159 spatially distinct sites with a maximum of 884 and a minimum of zero (Hay et al. 2000). This variation is usually due to the heterogeneity in the ecology of the area and human activities (Mabaso et al. 2007) which to a great extent determine vector behaviour. Overall rural areas are more exposed to malaria transmission, followed by irrigated areas (extensive and usually industrial agriculture), and urban areas are less exposed, although this varies in different locations (Hay et al. 2000). Rainfall seasonality, minimum temperature and irrigation in some areas are the major determinants of the intensity of the EIR (Mabaso et al. 2007).

Malaria eradication and control

The Global Malaria Eradication programme initiated by the World Health Organisation (WHO) in 1955 did not include Africa although this is the continent that bore the greatest burden allegedly because Africa was not ready to undergo a major eradication or control programme. Since then malaria control in Africa was neglected until the late 1990s when WHO inaugurated a new programme to Roll Back Malaria (RBM) (Dobson et al. 2000). The ambitious vision of the RBM partnership is that by 2015 malaria would not be a major cause of mortality and a barrier to social and economic development anywhere in the World. Its strategic approach is to support countries to have access to effective treatment and prevention against malaria, to improve management and healthcare, to maintain malaria high on the development agenda and provide a comprehensive research agenda from product development to implementation strategies (RBM 2005).

Although targets and promises are not always met by the donor's community funding malaria research and control (Attaran et al. 2004), there is more awareness today for the problems caused by malaria and expansion of resources and more commitment from endemic country governments (Yukich et al. 2007). In order to

achieve its goals, RBM recommends use of efficient drugs for case management, insecticide-treated bed nets (ITNs) and indoor residual spraying (IRS) for vector control and other environmental and biological measures (RBM 2005). Cheap drugs which were once efficient, such as chloroquine, are no longer advocated due to a spread of resistant strains of parasites against this drug. More recently resistance against sulfadoxine-pyrimethamine has been recorded in East and Southern Africa (EANMAT 2003). Artemisinin has proven to be an effective treatment against *Plasmodium* species and in order to avoid the spread of resistance to the single molecule, artemisinin-based combination therapies (ACT) are being advocated as first line drug policy in many African countries (Mutabingwa 2005). The major challenge with this new policy is the cost of antimalarials and the implementation of the policy in low income countries to make ACTs available for patients at an affordable price (Mutabingwa 2005). WHO is subsidising the ACT in some countries to make it affordable to many but most countries would benefit from a free-drug policy in order to reach all in need.

More than 100 trials in different settings worldwide have shown the protective impact of ITNs in reducing childhood and adult morbidity and mortality (Lengeler 2004b). ITNs reduced malaria incidence by 50% compared to no use of ITNs and by 39% compared to use of untreated bednets in areas of stable malaria. The incidence was reduced by 62% compared to no use of ITNS and by 43% compared to use of treated bednets in areas with unstable malaria. Close to six lives were saved for every 1000 children protected by ITNs (Lengeler 2004a). The use of ITNs is associated with a reduction in malaria infections in pregnant women and a reduction in low birth weight in babies and fewer children dying before birth compared to those not using the ITNs (Gamble et al. 2006). ITNs are viewed as the most efficacious and feasible intervention to prevent malaria morbidity and mortality in Africa (Lengeler et al. 1998, Phillips-Howard et al. 2003, Rozendaal 1997). Long lasting insecticide-treated nets (LLINs) have recently been manufactured that can last for at least three years without need of re-treatment (N'Guessan et al. 2001). In some areas these LLINs are being distributed freely or heavily subsidised for pregnant women and children less than five years old, however there is an ongoing debate on whether to subsidise the nets or simply make them free for all who need them (Roberts 2007). The challenge with ITNs is the spread of resistance against pyrethroids in a number of malaria endemic countries (Awolola et al. 2002, Diabate et al. 2002, Hargreaves et al. 2000). This is of

great concern since the pyrethroids among other insecticides, have many advantages; they are safe, highly active and with a long persistence (Kolaczinski and Curtis 2004). The development of multiple insecticide resistance mechanisms in *An. gambiae* is particularly alarming for the future of malaria control with insecticides (Corbel et al. 2007).

Indoor residual spraying (IRS) has been part of vector control programmes since 1911 when it was used in military barracks in Dar es Salaam and later on in the 1930s in South Africa to control malaria epidemics (Kouznetsov 1977). IRS contributed to the eradication of malaria in countries such as Brazil and Egypt in the 1930s and early 1940s (Soper and Wilson 1943) although both programmes relied heavily on larval control of vectors. However, there are very few countries in Africa that have used IRS in their routine vector control programmes among them South Africa, Zimbabwe, Botswana, Madagascar and Ethiopia (Curtis and Lines 2000). Large-scale malaria control programmes using dichlorodiphenyltrichloroethane (DDT) and Benzene Hexachloride (BHC) started as early as 1948-49 in southern Africa and these projects were extended to other parts of Africa in 1952-53 using DDT, BHC and Dieldrin (Kouznetsov 1977). IRS has seen its rise and fall when DDT was once considered as "the" solution for malaria vector control in the 1960s but after 30 years of tangible success mainly outside Africa, it was withdrawn in most parts of the World for fear of its possible harmful side-effects on the environment and humans. Another reason for its withdrawal was the quick development of resistance that occurred in against *Anopheles sacharovi* in Greece in 1949 (http://timlambert.org/2005/06/ddt10/ accessed 11/09/2008)

The impact of DDT on people and the environment when it is strictly used for indoor spraying remains unclear. The controversy around the use of DDT has been in existence for many decades. However the report of the WHO in Brazzaville in 1972 suggested that DDT was the most effective weapon in areas where time-limited eradication was not practical (Kouznetsov 1977). The Stockholm convention on persistent organic pollutants (POPs), which aims at protecting human health and the environment by eliminating or reducing the release of POPs into the environment, did not exclude totally the use of DDT (http://www.pops.int/ accessed 30.01.2008). With the development of resistance to DDT and its feared side-effects, other organophosphates, carbamates and pyrethroids compounds have been formulated such as fenitrothion, dichlorvos, malathion and propoxur. However their costs and

possibility of development of resistance do not allow use of these compounds at large scale. In 2006 the WHO backed the use of IRS for malaria control in areas with constant and high transmission including throughout Africa (www.who.int/mediacentre/news/releases/2006/pr50/en/ accessed 17.01.2008). The same report stressed that DDT has advantages over the other dozen of insecticides recommended by WHO for IRS. DDT is more effective, presents no health risk when properly used and is cost-effective. However, a study published in 2007 shows that women who were exposed at an early age (below 14 years) to *p, p'* -DDT, the active ingredient of DDT, had a higher risk of breast cancer (Cohn et al. 2007). By 2006, up to 14 countries in SSA were using IRS for malaria control and 10 of these were using DDT. However, upon release of this report, the idea of reintroducing DDT for malaria control at large scale in SSA was challenged mainly because of the lack of infrastructure required to implement large-scale interventions (Ahmad 2006).

The advances in molecular biology and the progress brought forth by the completion of the sequence of genomes of *Anopheles gambiae* (Holt et al. 2002) and *P. falciparum* (Kissinger et al. 2002) was seen as a breakthrough in the malaria research community. It has raised hopes of acquiring effective drugs and vaccine against malaria and genetically engineered mosquitoes that could disrupt the spread of the disease. Research into genetic manipulation of malaria mosquitoes aims on the one hand at replacing naturally malaria-transmitting species with genetically modified ones refractory to *P. falciparum*. On the other hand it aims at releasing sterile males to out-compete natural populations in mating and produce no offspring, which in the long term would eradicate the population (Catteruccia 2007).

The historical slow progress in developing a vaccine against malaria could be explained by (1) the fact that *Plasmodium* has several antigens varying throughout its life cycle therefore requiring sequential consecutive immune response, (2) the immunity to malaria is strain specific, (3) the knowledge on acquired immunity is limited and incomplete and no surrogate markers of immunity have been found so far, and (4) the absence of an appropriate animal model requires costly efficacy trials (Aide et al. 2007). The RTS,S/AS02A trial in 2004 showed 58% efficacy of the vaccine for severe malaria after six months in children 1-4 years old. The vaccine reduced prevalence of *P. falciparum* infection by 37% and its efficacy for extending the time to first infection was 45% (Alonso et al. 2004). Recently there has been more progress supporting the feasibility of a malaria vaccine (Aide et al. 2007). However, the

problem remains that even if an efficient vaccine was found it would take up to 10-12 years before it could be marketed (Bonn 2005).

Successes in malaria control have been registered recently in a number of African countries mainly due to the surge in ITN coverage, the return of IRS control measures, the switch to ACTs as first line drug in most countries and increased and new funding opportunities (Okiro et al. 2007, Wakabi 2007). A study in Kenya showed that after the expansion of ITN use, a 44% reduction in children mortality was achieved (Fegan et al. 2007). There is optimism that Mali, Senegal, Benin and Togo might reach the Abuja target (RBM 2003) of halving the malaria burden by 2010 (Wakabi 2007). WHO reported a dramatic reduction by more than 50% in malaria cases and deaths for Rwanda and Ethiopia and a less dramatic reduction in Zambia and Ghana (http://www.who.int/ accessed 30.04.2008). These successes were attributed to a wide distribution of LLINs and ACTs in the public sector. Amidst these encouraging successes the Bill and Melinda Gates foundation on 17th October 2007 called for the world to launch a malaria eradication campaign (http://www.gatesfoundation.org/ accessed 30.04.2008). A few days later the controversy caused by the call for eradication prompted an editorial in one of the leading journals in medical research, the Lancet, and a publication in the journal Science discussing the consequences of such ambitious plans. For the success of this daunting task new tools would be needed such as an effective vaccine and strong health care systems. The two publications also call for caution because the eradication campaign might divert scarce resources and if it failed might undermine support for global health initiatives in the future (Anonymous 2007a, Roberts and Enserink 2007)

Malaria in The Gambia

Malaria started to be studied systematically in The Gambia in the 1950s. In late 1980s malaria was responsible for 4% infant deaths and 25% of deaths of children between 1 and 4 years (Greenwood et al. 1987). In a study published six years later, it was reported that malaria was responsible for about 40% of deaths in children aged 1-4 years (Alonso et al. 1993).

The EIR in The Gambia is very heterogeneous like in other places in Africa and was estimated to average 42 varying between 0 and 177 (Hay et al. 2000). A recent study done in the central region of the country reported a variation of EIR between 0 and 166 (Bøgh et al. 2007). Malaria prevention relies heavily on the use of

bed nets. Until the 1990s the level of bed net use against mosquitoes in The Gambia was one of the highest on the continent, reaching a 76% coverage in the central division of the country and 58% overall (D'Alessandro et al. 1994). The Government of The Gambia launched a National Impregnated Bed net Programme (NIBP) in 1992 through the primary health care system (Muller et al. 1997). An evaluation of the implementation of the programme suggested that 77% of children under five years old and 78% of women at childbearing age were sleeping under ITNs (Cham et al. 1996). However, in villages where the insecticide for re-treatment of nets was charged, coverage was as low as 14% compared to 77% coverage in areas where free insecticide was distributed (Cham et al. 1997). Despite a long history of bed net use only one in four people were aware that mosquitoes cause malaria (Aikins et al. 1993). However a study done three years later show that 50% of interviewed women said that bed nets prevented malaria (Clarke 2001). Expenditure on bed nets including treatment and repair in The Gambia constitute only 10% of total expenditure towards mosquito prevention, while 81% is spent on coils, indoor sprays, smoke and aerosols (Wiseman et al. 2006).

The strategy for malaria control in The Gambia follows the RBM-WHO guidelines with a main focus on case management (NMCP 2002). Prevention is centred on the use of bed nets and larval control of malaria vectors is done on a very small scale in the Banjul area and occurs only in the rainy season. The policy towards use of intermittent preventive treatments (IPT) in children or pregnant women was introduced late in West Africa (Newman et al. 2006) but is part of the prevention programme in The Gambia for pregnant women.

Ecology of mosquito larvae

The *An. gambiae* complex has been described as including the most efficient vectors of malaria known from anywhere in the world. The complex comprises at least six named species: *An. gambiae sensu stricto, An. arabiensis, An. melas, An. merus, An. bwambae, An. quadriannulatus* and one unnamed species referred to as *An. quadriannulatus* B (Hunt et al. 1998). *An. gambiae* is widely distributed in tropical Africa except at high altitudes and in deserts (Coetzee et al. 2000, Gillies and DeMeillon 1968). Although it has long been considered to breed mostly in shallow open sun-lit pools, its larvae exploit a wide range of water bodies such as borrow-pits, drains, brick-pits, ruts, car-tracks, hoof-prints, pools resulting from overflow of rivers

or left by receding rivers, backwaters, and rainwater in natural depressions (Gillies and DeMeillon 1968). In rural Kenya where malaria is perennial, *An. gambiae* exploits both natural and artificial water bodies and permanent and semi-permanent water bodies are equally productive (Fillinger et al. 2004). The oviposition cues for malaria vectors in Africa are still poorly understood and the preferential behaviour of these mosquitoes to different water habitats varies greatly in different ecological settings (Holstein 1954, Huang et al. 2006).

In the dry savannas of Africa mosquito populations are highly seasonal with high densities occurring in the rainy season. During the rains, anopheline mosquitoes exploit sunny and temporal breeding sites such as rain-fed pools not covered with vegetation (Taylor et al. 1993). In the dry season they exploit pools created by receding rivers, edges of man-made lakes, irrigated gardens, wells and flooded borrow pits (White and Rosen 1973). In some areas, distinct habitat types show a higher production of mosquito immatures than others. Rice fields are very productive for *An. gambiae* when recently flooded and rice is low (Holstein 1954, Ijumba and Lindsay 2001, Snow 1983). Later, when rice is fully grown, breeding may continue at a lower level on the fringes of the rice fields (Gillies and DeMeillon 1968). In some instances, vegetated areas, irrigated channels and permanent wells can be colonised by mosquito larvae and even permanent marshes as observed in the Congo (Gillies and DeMeillon 1968).

All these examples illustrate the plasticity of *An. gambiae* to exploit a variety of breeding sites with different ecological conditions. However, productivity of mosquitoes is not homogeneous in all types of breeding sites. In the western Kenyan highlands, farmlands were more productive for *An. gambiae* than natural forest or swamps (Munga et al. 2006). In The Gambia the landscape is shaped by the Gambia River which bisects the country and creates wide areas of floodplains. These areas are the major source of mosquitoes where most breeding occurs up to 1400 m from the edge of alluvial floodplains (Bøgh et al. 2003). The main vectors of malaria in this area are *An. gambiae s.s.*, *An. melas* and *An. arabiensis* (Bryan 1983, Lindsay et al. 1993). A study done in this area showed a niche partitioning of different members of the complex. *An. melas* developed mainly in *Avicennia* mangroves monthly covered by tides, *An. gambiae s.s.* was associated with *Paspalum* and *Eleocharis* species and *An. arabiensis* was dominant in rice fields fed by rain water (Bøgh et al. 2003, Giglioli 1964).

There is little information available on the importance of chemical and physical factors in breeding sites occupied by *An. gambiae* (Gillies and DeMeillon 1968). The same is true for organic matter content which varies widely in different water bodies occupied by *An. gambiae*. However, algae (Laird 1988) and detritus (Merritt et al. 1992, Wallace and Merritt 1999) are well known as a food source for *An. gambiae* larvae. In western Kenya, a study concluded that the primary food source for *An. gambiae* larvae was derived from algal growth and the second source was bacterial growth (Gimnig et al. 2002). Although water temperature is an important factor for the survival and development of mosquitoes, *An. gambiae* larvae seem to have a wide range of thermo tolerance. Some authors report that minimum temperatures around 7°C and maximum of 42°C are generally accepted but other authors believe no natural collection of water could be too hot for the larvae (Gillies and DeMeillon 1968). In The Gambia under laboratory conditions all larvae died at cold temperatures (10-12°C) and at high temperatures (38-40°C (Bayoh and Lindsay 2004)). The development rate from larvae to adult was highest between 28 and 32°C and adult emergence was highest between 22 and 26°C (Bayoh and Lindsay 2003). *An. gambiae* is usually a pioneer species and develops in high densities in small pools formed after the rains before development of many predators. Apart from predation and cannibalism determining larval survival (Koenraadt and Takken 2003, Koenraadt et al. 2004b), the development rate of *An. gambiae* varies mainly with temperature. In general it takes 1-2 days for eggs to hatch and 6-9 days for larvae to develop and 1-2 days for the pupal stage. However under optimal conditions the cycle might be completed in 6 days (Gillies and DeMeillon 1968). Under laboratory conditions in The Gambia development from larvae to adult took 9 days at 28°C (Bayoh and Lindsay 2003). It has been shown in western Kenya, which is generally cooler than The Gambia, that water temperature in shallow pools can reach 38-39° C (Paaijmans et al. 2008).

Adult mosquito distribution

Population dynamics of *An. gambiae* follow the seasonal pattern of rainfall. In the Sahel region with only one rainy season, mosquito numbers rise at the onset of the rainy season and reach a peak in the middle of the rainy season (Gillies and DeMeillon 1968). In the Gambia, this peak is observed around September (Lindsay et al. 1993) and it is during this time that most larvae are caught when the alluvial soils bordering the river are covered by relatively fresh water (Bøgh et al. 2003). Afterwards mosquito

numbers decrease as vegetation grows high and predators colonise the breeding sites (Gillies and DeMeillon 1968) or oviposition decreases as the breeding sites remain available for a long time and predators increase in abundance (Service 1977a). Moderate rain distributed over the rainy season sustains pools for a long time and therefore contributes to a higher production of mosquitoes. On the contrary heavy down pours over a short time although creating many breeding sites could result in flushing out of pools (Gillies and DeMeillon 1968, Paaijmans et al. 2007) decreasing production of adult mosquitoes. In areas with large river systems affected by tides such as The Gambia, the availability of breeding sites is usually regulated by the tidal movement of water. It was observed that adult mosquitoes in these areas may invade villages 8 to10 days after the spring tides (Mouchet et al. 1994).

The flight range of mosquitoes varies with species and its study is less conclusive because it is usually influenced by external factors difficult to adjust for such as wind speed and vegetation cover prevailing at the time of the study. Another challenge is that testing the flight range of mosquitoes is usually determined by mark-release-recapture techniques. These methods require a huge number of mosquitoes to be released in the wild in order to recapture a significant number. Extensive reviews on these studies show that the recapture rate is generally low (Service 1997, Vlach et al. 2006). These kinds of studies are not only difficult to design in the field but they also pose an ethical problem of releasing potentially harmful vectors in the wild. However, large semi-field systems are being developed where such studies could be successfully done in the future (Ferguson et al. 2008). The flight range of one of the widespread mosquito species in the Americas, the black salt marsh mosquito *Ochlerotatus taeniorynchus* has been reported to reach 48-96 km (Vlach et al. 2006). A study done in Tanzania (Takken et al. 1998) suggests that dispersal of *An. funestus* and *An. gambiae* is not random but influenced by the availability and type of breeding sites, and could also be affected by the distribution of hosts (Gillies 1961). In The Gambia it was shown that vector density and EIR decreased away from breeding sites and for people within 4 km away from breeding habitats there was a 11.5 fold reduction in infective bites per person (Bøgh et al. 2007).

Lessons from the past

Successful programmes for malaria control in the past have relied mainly on controlling the vectors. However the complexity of malaria transmission and vector behaviour implies that no single tool can be sufficient to control the disease successfully. Interventions in the past have relied on integrated vector management tools to achieve success in malaria control. Change in land use, agricultural methods, house design and targeted vector control together contributed to the elimination of malaria from North America and Europe (Greenwood and Mutabingwa 2002).

One of the most efficient tools for vector control is larval source management (LSM). Between the two World wars larval control was almost the only method used for malaria control at large-scale in Africa (Kouznetsov 1977). This could be done either through larval source reduction by manipulation of the environment such as drainage of flooded areas and swamps, modification of river boundaries and vegetation clearance or through larviciding (Utzinger et al. 2001). However, a successful control programme would require that most productive breeding sites are reached and treated, and in places with numerous and scattered breeding sites, larviciding would be logistically demanding (Walker and Lynch 2007). Malaria control across Europe, Asia and the Americas before World War II focused on environmental manipulation and larviciding of breeding sites using chemical or biological agents (Kitron and Spielman 1989, Walker 2002). The advantage of control measures directed at larval stages is that they suppress the vectors before they are capable of transmitting diseases. Because mosquito larvae do not move far (Koenraadt et al. 2003) they are not capable of escaping control measures targeted at their habitats.

The African malaria vector *An. arabiensis* (Parmakelis et al. 2008) was eradicated from an area approximately 54,000 km^2 in Brazil with ecological settings similar to those in many parts of Africa (Killeen et al. 2002a). This is an outstanding example of the success of larval management for malaria control (Soper and Wilson 1943). The main efforts of this programme were larviciding with Paris Green, an arsenic-based insecticide, which was very effective and could be mixed with dust and sand therefore not needing expensive diluents. This unprecedented success was mainly achieved by well defined and rigorous organisation of larval control activities (Killeen et al. 2002a) and was successfully repeated in the River Nile valley of Egypt (Shousha 1948). One of the advantages in these two areas where larval control was successful is that they were relatively confined areas and therefore not subjected to invasion of mosquitoes from other places. However, these were very large areas covering

thousands of kilometres suggesting that this method could be successful in a number of malaria endemic countries in Africa. Another successful programme is the control of malaria in the copper belt in Zambia between 1930 and 1950 (Watson 1953). This integrated programme relied heavily on larval source reduction by clearing vegetation, modification of river boundaries, draining swamps and oil application to water bodies. Houses were screened and part of the population was treated with quinine and the occupants were stimulated to use bed nets. This programme resulted in a reduction of malaria related mortality, morbidity and incidence rates by 70-95% (Utzinger et al. 2001).

A variety of compounds have been used to kill mosquito larvae and pupae such as crude kerosene and distilled petroleum oils, Paris Green, temephos, fenitrothion and chlorpyriphos. The juvenile hormone mimic pyriproxifen has been used for a long time for mosquito control mainly in Asia. It only requires low concentrations (0.01 mg a.i./l) to inhibit the development of larvae and prevent adult emergence (Yapabandara et al. 2001), therefore its impact can only be seen in the adult mosquito density. Pyriproxifen was found to be safe for non-target organisms such as fish, mammals and other invertebrates including mosquito predators (Mulla et al. 1986).

Expandable polystyrene beads (EPB) have also been used mainly for the control of *Culex* mosquitoes breeding in enclosed habitats such as wet pit latrines, cess pits and flooded cellars (Curtis 2005). The EPB suffocate mosquito larvae and by obstruction prevent further oviposition when properly applied (Curtis et al. 1989). This approach is cost-effective because the beads do not rot and would continue to prevent oviposition if they are not moved (Curtis et al. 1989). In Zanzibar the application of EPB in 500 pit latrines in a community of 12000 people achieved ~ 98% reduction in mosquitoes entering bedrooms (Curtis 2005). In Sri Lanka a comparable study of pyriproxifen, polystyrene beads, temephos, used engine oil and filling of pits revealed that pyriproxifen was the most cost-effective approach because it required only two applications per year (Yapabandara and Curtis 2002). In Tanzania an integration of polystyrene beads in enclosed breeding sites and pyriproxifen in open breeding sites reduced the density of *Culex quinquefasciatus* by 46-77% (Chavasse et al. 1995).

Since some of the products used for larval source management such as Paris Green were highly toxic to people, a generation of safer chemical and biological larvicides were developed. Biological larvicides are better than chemical ones because they are often specific to target organisms and do not harm the environment where

they are applied (WHO 1999). The efficacy of these larvicides against malaria vectors is increasingly stimulating research and control communities to integrate larval control as an additional tool for malaria control.

Microbial larvicides

A great number of studies have been conducted in the laboratory to assess the potency of different bacterial agents against mosquito larvae. Among them the mosquitocidal properties of spore-forming bacteria *Bacillus thuringiensis* var. *israelensis* (*Bti*) and *B. sphaericus* (*Bs*) have been proven (Lacey 2007). Both bacteria have a specific mode of action against Culicidae and Simulidae and can be produced inexpensively in artificial media (Regis et al. 2001).

During sporulation *Bti* and *Bs* produce crystals and when released in water and ingested by larvae, the alkaline pH and intestinal proteinases of the larval midgut solubilise the crystals and release the toxins. These toxins bind to the midgut cells and cause lysis of the epithelium of the gut which result in death of the larvae (Charles 1987). *Bti* releases four toxins: Cry 4A, Cry 4B, Cry 11A Cyt1A which act in synergy (Poncet et al. 1995) and on different target molecules. This combination of different toxins explains why no resistance to *Bti* has occurred in the field after 30 years in use. In contrast *Bs* releases only two similar toxins (Bin A and B) which although acting in synergy cannot be considered as individual toxins as those of *Bti*. These toxins use the same receptors and therefore easily display cross-resistance. It has been suggested that *Bs* should be used with caution while monitoring the development of resistance, and preferably in combination with other species such as *Bti*.

Bti larvicidal properties have been known since 1977 (Goldberg and Margalit 1977). Its efficacy and specificity for certain families of Nematocera enabled *Bti* to be quickly registered as a commercial larvicide and to be used in routine large-scale mosquito control programmes in Europe (Becker 1998). It was the first bacterium to be used in a public health programme to control *Simulium damnosum*, the vectors of *Onchocerca volvulus*. This Onchocerciasis Control Programme (OCP) started in 1974 and covered 11 countries in West Africa using organophosphates for control in the first instance. When larvae became resistant to the organophosphates, *Bti* was introduced in 1983 to manage the resistance (Regis et al. 2001). Within 16 years onchocerciasis was contained and was no longer a public health problem in those countries (Hougard et al. 1997). *Bti* was also used on large scale in a *Similium* control programme in Brazil and

in temperate countries for control of nuisance mosquitoes. Since 1981 *Bti* has been widely used in Germany against *Culex pipiens* and floodwater or snow-melt mosquitoes (Becker and Rettich 1994). *Bti* tested in increasing dosages up to 10 kg/ha had no effect on rotifers, *Cyclops* sp, *Daphnia* sp, ostracods, *Baetis* sp, corixids, notonectids, coleoptera, and other non-target invertebrates were not affected (Ali 1981). In general, dosages of *Bti* that kill mosquito larvae are far less than those that could kill non-target organisms. One set back with *Bti* is that it does not persist long in the environment and requires re-application at short intervals, sometimes weekly. In contrast *Bs* has been advocated for larval control mainly because in many environments it persists longer and might be recycled under natural conditions (Hertlein et al. 1979, Mulligan et al. 1980, Skovmand and Bauduin 1997), and in larval cadavers (Correa and Yousten 1995). Therefore the best practice would be to apply *Bs* for its long residual effect together with *Bti* to counter development of resistance (Wirth et al. 2004).

The level of efficacy of these bacteria vary with the environment where they are used and with the susceptibility of indigenous mosquitoes (Becker and Rettich 1994). Therefore it is important to evaluate them locally before they can be used as mosquito control agents. The success of control measures relying on larviciding would require a good understanding of the behaviour and ecology of mosquitoes and their environment. Because larviciding is logistically demanding, the most efficient intervention would be one that targeted the most productive breeding sites. In places with seasonal malaria transmission, it would be cost-effective to target larviciding at the transmission season preferably before the peak in mosquito numbers wherever appropriate.

In summary, today it is widely acknowledged that the best approach to tackle malaria vectors is through integrated vector management programmes. This strategy uses interventions that have shown efficacy in combination or separately instead of relying on a single tool. Such interventions reduce the selection of pressure on insecticides or drugs if they were used alone (WHO 2004). There is a need for as many effective tools as possible to fight the disease and vectors and an informed decision on which tools to use in different settings in order to achieve good control. The complexity and heterogeneity of malaria vector ecology observed in different areas requires a thorough understanding of ecosystems where intervention programmes are planned. One of the best ways to suppress malaria vectors is through targeting them at

the larval stage before they become adults and spread disease. This historical approach has led to eradication of malaria in ecosystems similar to those where malaria is endemic today, therefore raising hope that this approach might be a valid tool to consider in these areas. A successful implementation of anti-larval measures for malaria control requires a comprehensive knowledge of vector breeding sites in order to target larviciding efforts at the most productive habitats wherever possible. It also requires an efficient and specific larviciding agent and one that can persist in the environment without causing adverse effects to non-target organisms. And finally it requires efficient organisation, management and monitoring of the implementation process.

Study Goal

The goal of this study was to determine whether mosquito larval control through the use of *Bti* and *Bs* will reduce malaria transmission in The Gambia.

Objectives

The specific objectives were to:

1. describe immature mosquitoes' spatial distribution and the most productive habitats for mosquitoes in the middle reaches of the Gambia River;
2. test the efficacy of *Bti* and *Bs* on Gambian mosquitoes;
3. implement routine larval control in the area, and measure its impact on immature and adult mosquito densities.

Hypotheses

The hypotheses for this research were as follow:

1. The distribution and abundance of mosquito larvae and pupae is not random in rural Gambia but clustered to some habitats.
2. Microbial larvicides *Bti* and *Bs* are efficient against mosquitoes in The Gambia.
3. Larval control can be used to reduce malaria vector densities and consequently malaria transmission in rural Gambia if applied routinely in the most productive habitats.

Chapter 2

Spatial distribution of mosquito larvae and the potential for targeted larval control in The Gambia*

Figure 2.1 A sample of mosquito breeding habitats found in rural Gambia

*This chapter will appear as a paper, in a modified format with the same title by S. Majambere, U. Fillinger, C. Green, D.R. Sayer, and S.W. Lindsay in the *American Journal of Tropical Medicine and Hygiene*

Spatial distribution of mosquito larvae and the potential for targeted larval control in The Gambia

Abstract

Background There is a growing interest in the scientific community for use of larval control as a tool for integrated vector management. Here the distribution of the aquatic stages of malaria vectors in rural Gambia was examined to assess the practicality of targeting larval control.

Method Every accessible water body in a 400 km^2 area in rural Gambia was mapped and sampled for two consecutive years. Each water body was characterised by its distance to the edge of the alluvial plains, perimeter, habitat type, landcover type and the presence or absence of mosquito larvae assessed by standard dipping. Sampling was continuous in each site through the rainy and dry seasons.

Results During the rainy season, the peak period of malaria transmission, habitats in the floodplain of the Gambia River were 70% more likely to have anopheline larvae than upland habitats ($p < 0.001$). However, mosquitoes were found in all habitats, apart from moving water. Habitats most often colonised by anopheline larvae were the largest water bodies, situated near the landward edge of the floodplain, where culicine larvae were present. In the wet season 49% of all sites had anophelines versus 19% in the dry season ($p < 0.001$).

Interpretation Mosquitoes colonise a wide range of habitats, therefore larval control targeted at specific habitats is unlikely to be successful in this setting. Nonetheless, larval control initiated at the end of the dry season and run throughout the rainy season could help reduce transmission.

Introduction

There is a growing interest in using larval control as a tool for integrated vector management programmes for malaria control in SSA (Fillinger et al. 2003, Fillinger and Lindsay 2006, Gu and Novak 2005, Killeen et al. 2002a, Killeen et al. 2002b, Killeen et al. 2006, Shililu et al. 2007, Utzinger et al. 2002, Utzinger et al. 2001). The first operational larviciding programmes in modern Africa recently commenced in the city of Dar es Salaam, Tanzania (Fillinger et al. 2008). Data needed to inform these programmes are starting to grow. Recently pilot studies in lowland and highland Kenya showed that microbial larvicides could reduce *Anopheles* larval densities by 95% with a concomitant reduction in exposure to mosquito bites of over 90% (Fillinger and Lindsay 2006). Most importantly, in highland sites larviciding was associated with a 50% reduction in malaria parasite infection (Fillinger U and others, unpublished data). Similar reductions in vector productivity have been achieved with larval source management using microbial larvicides in Eritrea (Shililu et al. 2007).

The eradication of *Anopheles arabiensis* (Parmakelis et al. 2008), one of the principal vector of malaria in SSA, from large flooded areas in Brazil in the 1930s (Soper and Wilson 1943) suggested a similar approach could be effective in comparable habitats in SSA, including those in rural Gambia. Before embarking on a larval control campaign it is crucial to understand where and when the aquatic stages of the vectors are found in order to direct control activities at these sites. Since larviciding in large river ecosystems like the Gambia River would be logistically complicated and expensive, identifying the sites where *Anopheles* larvae occur most frequently and/or in highest density for targeting larval control (Gu and Novak 2005, Killeen et al. 2006) would increase the cost-effectiveness of the operation.

In SSA *Anopheles* larval habitats are frequently associated with human activity (Gillies and DeMeillon 1968). These are typically open sunlit pools created when depressions made by people and their animals fill with rain or ground water. Such sites are common close to human habitation and in fields. In addition, regions with large river systems including The Gambia often face seasonal flooding which creates large areas of standing water for extended periods of time and provides potential breeding sites for mosquitoes (Bøgh et al. 2003, Giglioli and Thornton 1965).

Few larval surveys have been conducted in The Gambia (Bertram et al. 1958, Bøgh et al. 2003, Giglioli 1964, Thomas and Lindsay 2000) and most of these studies were small scale or along transects confined largely to the floodplains in the rainy

season making it difficult to generalise the findings from these studies. Originally it was recognised that higher numbers of adult mosquitoes were captured in villages close to the Gambia River compared with those further away (Lindsay et al. 1993). Since the river is too fast flowing to provide mosquito breeding sites an investigation of the riverine habitats was carried out. This investigation revealed that the number of adult mosquitoes found in a village was positively related to the proximity and extent of pooled sediments bordering the river, suggesting that this was the most productive area for anophelines (Thomas and Lindsay 2000). This finding was confirmed several years later when the highest densities of mosquito larvae collected along transects were close to the landward edge of the alluvial plains, although high numbers could also be found more than a kilometer into the alluvial floodplains (Bøgh et al. 2003). *Anopheles gambiae sensu stricto* and *An. melas* were found mainly within the flooded areas, whilst *An. arabiensis* occurred mainly in rain-fed rice fields close to this area.

The present study was carried out to prepare for a large trial of microbial larvicides where all potential breeding habitats within the study area needed to be identified. Larval data collected during this study was used to determine and characterise those water bodies commonly frequented by anopheline larvae, both in the floodplains and the upland sites, during the dry and wet seasons. This information is essential for determining whether targeting interventions at a limited number of specific habitats highly colonised by mosquitoes would be a viable option for malaria control in rural Gambia, and in other areas with major river systems. The study represents a comprehensive longitudinal survey of potential larval breeding sites, surveying every accessible water body in a 400 km^2 area from the river to the Senegalese borders of the country repeatedly over a two year period.

Material and methods

Study area

The study was carried out East of Farafenni town in The Gambia from June 2004 to May 2006. The Gambia is in the southern Sahel and is characterised by a short rainy season from June to October and a long dry season from November to May. The country lies in an area of open flat Sudan savannah that is dominated by the River Gambia, a large, slow moving waterway, characterised by tidal movements and saltwater intrusions as far as 200 km up river. River Gambia is representative of many large river systems in Africa. Its tidal movements flood successive belts of vegetation

from the mangrove forest through flooded *Phragmites*, sedge and grass species, punctuated by large bands of barren floodplain.

The study area was selected to comprise the most common habitats found in large river ecosystems, where many water bodies contained brackish, as well as freshwater. Four zones, each approximately 100 km^2 in area, were selected (Figure 2.2) two on the north bank of River Gambia around Balanghar Ker Nderry (Zone 1 UTM: 1510598N, 456756E) and Bantanto Jawara (Zone 2 UTM: 1513745N, 473014E), and two on the south bank, near Jalangbereh (Zone 3 UTM: 1480043N, 457259E) and Sutukung (Zone 4 UTM: 1489734N, 470794E). Each zone can be divided broadly into (1) the upland area that is predominantly woodland savannah and farmland, where the main crops are millet and groundnuts, and (2) the river floodplains, where large areas of alluvial soils are flooded during the rainy season and rice is grown. The average annual rainfall during the study period was 837 mm.

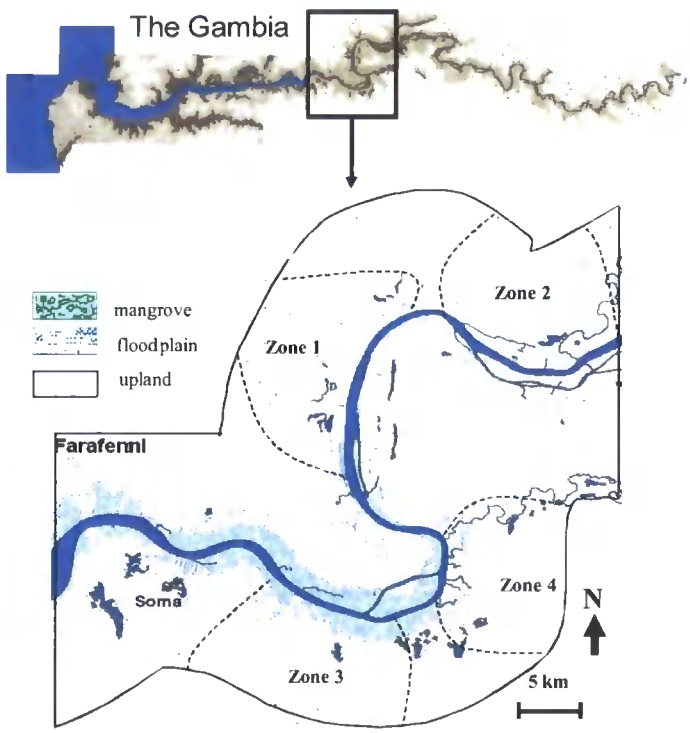

Figure 2.2 Map of the study area. Discontinuous lines show zone limits.

Water body measurements

Each water body encountered while walking the entire breadth of the study area was given a unique identification number and its position recorded using a handheld Global

Positioning System (GPS, Garmin GPS 12 XL, 15 meters accuracy). The depth, size, type of water body (referred to as habitat here forth), and surrounding landcover was recorded for every accessible aquatic habitat found during the study. Surveys were carried out continuously, with each zone being surveyed 6 to 8 times during the study. Water depth was classified as shallow when the water level was below knee-high and deep when it was above the knee. The perimeter of each breeding site was categorised by eye as: (1) < 10 m, (2) 10 - 100 m, or (3) > 100 m. Each aquatic habitat was classified into one of the following categories which are usually found in succession from the village towards the main river: (1) Brick or sand pits: borrow pits (> 2 m diameter) resulting from brick-making or other construction activities, (2) Cattle troughs attached to village pumps, (3) Pools: discrete (< 200 m diameter) and shallow (< 50 cm) standing water bodies, usually drying out towards the end of the dry season, (4) Edges of floodwater: the shallow landward edges of the extensive floodwater in the floodplains of the river or its tributaries, partly barren and partly associated with grass (*Paspalum* and *Sporobolus* sp.) and sedge (*Eleocharis* sp.), (5) Ponds: discrete and permanent water bodies, more than 100 m in circumference fed by groundwater and deeper than pools, (6) Water channels: used for irrigation or drainage, (7) Stream fringes: the shallow edges of permanent streams associated with grass or sedge, and tall reeds in deeper parts, (8) Puddles or tyre tracks: small natural or vehicle-made depressions, (9) Footprints: made by people, cattle or other animals where water collects, often associated with edges of large water bodies (floodwater, streams, pools and ponds), (10) Floodwater: inundated areas in the floodplain further away from the landward edge, towards the river, (11) Rice fields: seasonally flooded areas used to grow rice, and (12) Mangrove: water body characterised by densely growing mangrove trees (*Rhizophora* and *Avicennia* sp.) near the main river.

Additionally, the dominant landcover around each aquatic habitat was recorded as: (1) Upland grassland (Poaceae): vegetation dominated by *Paspalum* and/or *Sporobolus* species: not affected by the river, (2) Upland agriculture: such as fields of groundnuts, maize, pumpkins, sorghum and millet, (3) Shrubs of the West Sudanian savannah ecoregion, (4) Forest: densely growing, tall trees, (5) Barren floodplain: under tidal influence without any vegetation, (6) Sea-purslane (*Sesuvium* sp.): a succulent salt indicator plant forming a low carpet of thick leaves, (7) Grass (Poaceae) on the floodplain: vegetation dominated by *Paspalum* and/or *Sporobolus* species, (8) Sedge (Cyperaceae): vegetation dominated by the spike-rush (*Eleocharis* sp.), (9) Rice

(*Oryza sativa*) plantations, (10) Reeds: *Phragmites karka* and *Cyperus papyrus* form the reed beds, usually found in deep water, and (11) Mangrove forest of *Rhizophora* or *Avicennia* sp. usually next to the main river and large tributaries.

Larval sampling

Purposeful sampling was done to maximise collection of the aquatic stages of mosquitoes using a 350 mL dipper (Clarke Mosquito Control Products, Illinois, USA). At each site 10 dips were made in places likely to harbor mosquito larvae, such as around tufts of submerged vegetation or substrate, edges of water bodies and around floating debris. In extensive water bodies dipping was carried out over a 100 m walk. Larvae were classified either as anophelines or culicines. Anopheline larvae were stored in 100% ethanol which was refreshed upon reaching the laboratory. Random sub-samples of anopheline larvae were selected during the routine mapping of the area and sibling species of the *An. gambiae* complex identified by amplification of ribosomal DNA using the polymerase chain reaction (PCR) (Scott et al. 1993b).

Statistical analysis

Coordinates of each water body were entered into a Geographical Information System (ArcGIS-ArcInfo Version 9.1 software) and plotted on a map of The Gambia using the Geographic Coordinate System: GCS WGS 1984, Datum: D WGS 1984. The map templates were obtained from the Department of Lands and Surveys (The Government of the Republic of The Gambia, 2004). These maps were used to localise and visualise all surveyed aquatic habitats. The distance between a specific breeding site and the nearest human settlement was obtained by measuring the distance between points (breeding sites) and polygons defining the human settlements using the ArcGIS software. A layer was defined along the edges of the alluvial floodplains and the nearest distance between all breeding sites and this edge determined. Habitats close to the alluvial edge, created by floodwater were referred to as 'edge of floodwater'. For the purpose of estimating the area of this habitat type, its width was assumed to be 50 m based on maps from the Department of Lands and Surveys. Further into the floodplain the habitats are usually deeper and semi-permanent and are described as 'floodwater', a further category.

The impact of different water body characteristics on the presence or absence of mosquito larvae was explored individually. Comparisons between proportions were

made using chi-square analysis. All variables were incorporated in a mathematical model and their overall impact on the presence of anopheline larvae tested using Generalised Estimating Equations (GEE). This model was used because it takes account of repeated measures in the analysis since the same water bodies were repeatedly sampled during the study. The habitat ID was used as subject unit for repeated measures assuming an exchangeable correlation matrix. Larval data (presence or absence) was fitted to a binomial distribution with a logit link function. After testing for collinearity of predictors in the model those that were not highly correlated (R > 0.9) were used together in the model. The 'edge of floodwater' was selected as the reference group in the model for comparison of different habitat types, since this habitat had been identified in an earlier study as most likely to be colonised by mosquito larvae (Bøgh et al. 2003). Various landcover types were compared to floodplain areas without any vegetation which is characteristic of many parts of the edge of floodwater. Regression analysis was used to test for relationships between key variables. Logistic regression was used to elucidate any differences between sites with *An. gambiae s.l.* and sites with other anophelines and between anopheline early and late instar larvae. Analyses were performed with SPSS version 15 and EpiInfo version (TM). Missing data were excluded from the analysis.

Results

Characteristics of aquatic habitats

A total of 6038 visits were made to 1076 different water bodies in the four study zones over two consecutive years. 71% of the water bodies in the floodplain contained anopheline larvae on at least one occasion (528/739), compared with 50% in the upland (138/337; $P < 0.001$). 60% (3673/6038) of visits took place in the dry season and 40% (2410/6038) in the rainy season. Most habitats were visited on six to eight occasions over the study period with 35% of habitats occurring in Zone 3 (373/1076), 25% in Zone 4 (269/1076), 21% in Zone 1 (224/1076) and 19% in Zone 2 (210/1076). Although there were more aquatic habitats in Zone 3, the risk of habitats being colonised by anopheline larvae in Zone 3 was less compared to other zones ($P < 0.001$). On occasions when sites were visited, 84% (2031/2410) contained water during the rainy season, while only 45% (1666/3673) had water during the dry season ($P < 0.001$). Sites contained water on 88% of occasions in the floodplains and 67% of occasions in the uplands during the rainy season ($P < 0.001$). In the dry season sites

were wet on 58% of occasions in the river's floodplains and only 15% of occasions in the upland ($P < 0.001$).

Characteristics of larval habitats

Since the presence of late instar anopheline larvae was strongly correlated with early instars ($R^2 = 0.59$, $P < 0.001$) the results for early and late instars were pooled for all further analyses. 42 of 3695 (1%) records for anophelines were missing in the dataset and were not included in the analysis.

GEE modelling for the entire data set adjusting for study zone, the location of the habitat in the upland or in the floodplains, the season of sampling, the habitat type, the habitat size, distance to the landward edge of the alluvial plains and dominant landcover type demonstrated that anopheline larvae were four times more likely to be found during the rainy season than during the dry season (OR = 4.06; 95% CI = 3.31-4.99; $P < 0.001$) and were less common in the upland than in the floodplains, although this was of borderline significance (OR = 0.64; 95% CI = 0.41-1.01; $P = 0.055$). However when only the rainy season was considered the likelihood of finding anophelines was significantly less in the upland sites compared to the floodplain (OR = 0.30; 95% CI = 0.22-0.39; $P < 0.001$).

Given these differences between occurrence of larvae in floodplain and upland sites and between dry and rainy season, data were analysed in subsets to identify potential risk factors for the presence of mosquitoes in the floodplains and upland during rainy (June-October) and dry season (November-May) separately. The distance from a habitat to the nearest village as well as the water depth of habitats were not significantly associated with the presence of anopheline larvae and were therefore not included in any of the final models.

In the floodplains during the rainy season (Table 2.1) habitats farther away than 1 km from the landward edge of the alluvial plains were 58% less frequently colonised by anophelines than those within the first 1km ($P < 0.001$) and larger habitats, with more than 100 m in perimeter, seven times more frequently than smaller ones ($P = 0.006$). Notably, these most colonised habitats represent those most frequently encountered (over 80% of the site visits).

Rice fields (n = 413), open floodwater (n = 439), stream fringes (n = 295) and pools (n = 105) were most frequently flooded in the floodplains during the rainy season and the majority of the *Anopheles* samples were taken from these sites (n = 272,

190, 125 and 105, respectively). Nevertheless, although some habitat types were available more frequently, GEE modelling revealed that adjusting for the location of the habitat and its size, the risk of finding *Anopheles* larvae is the same for the majority of habitat types. There was a positive association between anopheline abundance and habitats in areas dominated by grass, sedge and rice as compared to floodplain areas without vegetation.

The probability of finding *Anopheles* larvae in the floodplains in the dry season (Table 2.2) was the same for the entire width of the floodplain area and independent of the size and type of habitats or the dominant land cover type.

Table 2.1 Factors associated with the presence and absence of anopheline larvae in the floodplain in the rainy season. C.I. is 95% confidence interval.

Factor	Number of visits (n)	Anophelines present		Odds ratio	Lower C.I.	Upper C.I.	P
		Occasions	Proportion (%)				
Zone							
Zone 4	411	234	56.9	0.55	0.37	0.83	0.004
Zone 3	346	122	35.3	0.24	0.16	0.37	<0.001
Zone 2	469	243	51.8	0.26	0.17	0.38	<0.001
Zone 1	304	205	67.4	1.00			
Distance to edge of alluvial plains							
1-3 km	142	45	31.7	0.42	0.27	0.64	<0.001
< 1km	1388	759	54.7	1.00			
Perimeter							
> 100 m	1285	696	54.2	6.81	1.72	26.92	0.006
10-100 m	222	101	45.5	3.20	0.82	12.55	0.095
< 10 m	23	7	30.4	1.00			
Habitat types							
brick or sand pits	10	8	80.0	0.56	0.13	2.49	0.450
cattle troughs	8	4	50.0	0.67	0.14	3.28	0.625
pool	165	105	63.6	0.93	0.46	1.86	0.831
pond	14	10	71.4	1.42	0.19	10.62	0.730
water channel	33	8	24.2	0.56	0.20	1.57	0.266
stream fringe	295	125	42.4	0.49	0.25	0.95	0.036
puddles or tyre tracks	50	23	46.0	1.17	0.42	3.32	0.762
footprints	5	1	20.0	0.13	0.02	0.82	0.030
floodwater	439	190	43.3	0.56	0.31	1.01	0.056
rice fields	413	272	65.9	0.62	0.27	1.43	0.265
edge of floodwater	98	58	59.2	1.00			
Landcover							
Mangrove	39	3	7.7	0.59	0.15	2.42	0.467
Reeds	298	107	35.9	1.78	0.71	4.44	0.220
Sea-purslane	20	4	20.0	0.58	0.12	2.76	0.490
Bush	52	29	55.8	1.24	0.41	3.73	0.706
Sedge	260	138	53.1	3.11	1.26	7.63	0.013
Rice	355	238	67.0	3.33	1.09	10.16	0.035
Grass	476	280	58.8	2.92	1.25	6.84	0.013
Barren floodplain	30	5	16.7	1.00			
Culicines							
present	656	556	84.8	18.35	13.19	25.54	<0.001
absent	859	233	27.1	1.00			

Table 2.2 Factors associated with the presence and absence of anopheline larvae in the floodplain in the dry season. C.I. is 95% confidence interval.

Factor	Number of visits (n)	Anophelines present		Odds ratio	Lower C.I.	Upper C.I.	P
		Occasions	Proportion (%)				
Zone							
Zone 4	284	62	21.8	2.90	1.57	5.38	0.001
Zone 3	343	28	8.2	0.92	0.44	1.93	0.830
Zone 2	459	144	31.4	2.43	1.55	3.81	<0.001
Zone 1	329	61	18.5	1.00			
Distance to edge of alluvial plains							
1-3 km	216	47	21.8	1.09	0.67	1.78	0.719
< 1km	1199	248	20.7	1.00			
Perimeter							
> 100 m	1234	278	22.5	1.61	0.30	8.76	0.583
10-100 m	152	14	9.2	0.73	0.12	4.45	0.733
< 10 m	29	3	10.3	1.00			
Habitat types							
brick or sand pits	5	1	20.0	0.40	0.06	2.83	0.361
cattle troughs	9	1	11.1	0.53	0.04	7.79	0.644
pool	59	14	23.7	1.02	0.39	2.67	0.961
water channel	69	1	1.4	0.15	0.02	0.96	0.045
stream fringe	387	74	19.1	0.90	0.47	1.71	0.736
puddles or tyre tracks	6	1	16.7	4.31	0.30	62.43	0.284
floodwater	425	57	13.4	0.57	0.29	1.12	0.101
rice fields	375	120	32.0	2.10	0.60	7.38	0.248
edge of floodwater	80	26	32.5	1.00			
Landcover							
Mangrove	76	2	2.6	0.22	0.02	1.99	0.178
Reeds	391	58	14.8	0.53	0.07	3.88	0.529
Sea-purslane	9	1	11.1	1.03	0.11	9.40	0.982
Bush	36	6	16.7	0.50	0.05	5.62	0.578
Sedge	250	58	23.2	1.30	0.18	9.09	0.794
Rice	328	107	32.6	0.73	0.08	6.89	0.785
Grass	308	60	19.5	0.85	0.12	5.82	0.869
Barren floodplain	17	3	17.6	1.00			
Culicines							
present	346	213	61.6	18.86	13.23	26.89	<0.001
absent	1066	79	7.4	1.00			

In the upland area during the rainy season (Table 2.3) the most frequently recorded aquatic habitats were pools (n = 152), cattle troughs (n = 140), and puddles (n = 115). The majority of *Anopheles* records were taken from pools (n = 80), cattle troughs (n = 42), puddles (n = 21), and rice fields (n = 21). Risk factor analyses showed that habitats larger than 10 m in perimeter were three times more often associated with anopheline larvae than smaller ones ($P = 0.009$) but the risk of finding *Anopheles* larvae in the most frequently encountered aquatic habitats in the upland was not

significantly associated with any landcover or habitat type. The presence of larvae was significantly less when aquatic habitats were more than 3 km away from the edge of the alluvial plains. In the dry season aquatic habitats were rarely encountered in the upland. Out of 214 sampling events, only 14 had *Anopheles* larvae, 64% of these were found in cattle troughs and the rest in puddles and pools.

Table 2.3 Factors associated with the presence and absence of anopheline larvae in the upland area in the rainy season. C.I. is 95% confidence interval.

Factor	Number of visits (n)	Anophelines present		Odds ratio	Lower C.I.	Upper C.I.	P
		Occasions	Proportion (%)				
Zone							
Zone 4	411	234	56.9	0.55	0.37	0.83	0.004
Zone 3	346	122	35.3	0.24	0.16	0.37	<0.001
Zone 2	469	243	51.8	0.26	0.17	0.38	<0.001
Zone 1	304	205	67.4	1.00			
Distance to edge of alluvial plains							
1-3 km	142	45	31.7	0.42	0.27	0.64	<0.001
< 1km	1388	759	54.7	1.00			
Perimeter							
> 100 m	1285	696	54.2	6.81	1.72	26.92	0.006
10-100 m	222	101	45.5	3.20	0.82	12.55	0.095
< 10 m	23	7	30.4	1.00			
Habitat types							
brick or sand pits	10	8	80.0	0.56	0.13	2.49	0.450
cattle troughs	8	4	50.0	0.67	0.14	3.28	0.625
pool	165	105	63.6	0.93	0.46	1.86	0.831
pond	14	10	71.4	1.42	0.19	10.62	0.730
water channel	33	8	24.2	0.56	0.20	1.57	0.266
stream fringe	295	125	42.4	0.49	0.25	0.95	0.036
puddles or tyre tracks	50	23	46.0	1.17	0.42	3.32	0.762
footprints	5	1	20.0	0.13	0.02	0.82	0.030
floodwater	439	190	43.3	0.56	0.31	1.01	0.056
rice fields	413	272	65.9	0.62	0.27	1.43	0.265
edge of floodwater	98	58	59.2	1.00			
Landcover							
Mangrove	39	3	7.7	0.59	0.15	2.42	0.467
Reeds	298	107	35.9	1.78	0.71	4.44	0.220
Sea-purslane	20	4	20.0	0.58	0.12	2.76	0.490
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Sedge	260	138	53.1	3.11	1.26	7.63	0.013
Rice	355	238	67.0	3.33	1.09	10.16	0.035
Grass	476	280	58.8	2.92	1.25	6.84	0.013
Barren floodplain	30	5	16.7	1.00			
Culicines							
present	656	556	84.8	18.35	13.19	25.54	<0.001
absent	859	233	27.1	1.00			

Rice fields and pools were most frequently found, especially during the rainy season (Table 2.1-2.3), but differed greatly in the area they covered (Table 2.4). Rice fields stretched in total over approximately 2150 ha (21.5 km^2) whereas pools covered less than 1 ha within the entire study area. In comparison, the edge of floodwater was approximately 500 ha.

Independent of location and season, there was a very strong positive association between the presence of anophelines and the presence of culicines in the aquatic habitats (Table 2.1-2.3, Figure 2.3).

Table 2.4 Sampling frequency and size of major anopheline breeding habitats

Habitat	Frequency	Area (ha*)				
		Zone 1	Zone 2	Zone 3	Zone 4	Total
Rice fields	20% (1234/6083)	647	256	116	1136	2155
Pools	16% (997/6083)	0.225	0.040	0.388	0.161	0.814
Edge of floodwater	5% (296/6083)	155	75	145	125	500

*1 ha=10,000 m^2

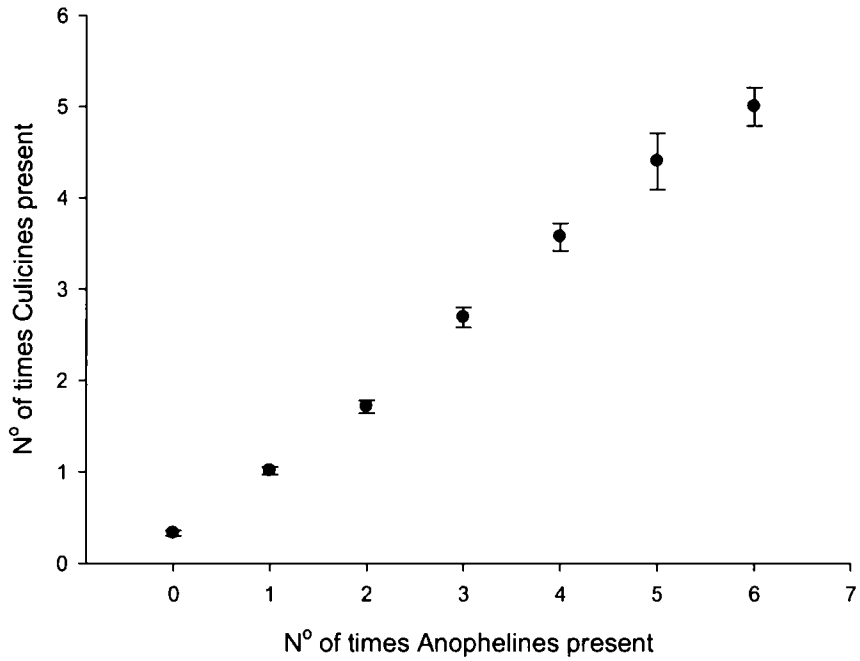

Figure 2.3 Relationship between the frequencies at which culicine and anopheline larvae occurred together. Bars represent standard errors.

PCR analysis

Of a sub-sample of 124 anopheline habitats, PCR analysis conducted on 1401 samples showed that 52% of these habitats were occupied by *An. gambiae s.l.* (35% *An. gambiae* s.s., 11% *An. melas*, 6% *An. arabiensis*). Most *An. arabiensis* (86%) and *An. gambiae s.s.* (58%) were found in rice fields, and in pools. *An. melas* was predominantly found in floodwater and edges of floodwater (57%, Figure 2.4). Binary logistic regression revealed no significant difference between characteristics of habitats occupied by *An. gambiae s.l.* and those of other anophelines.

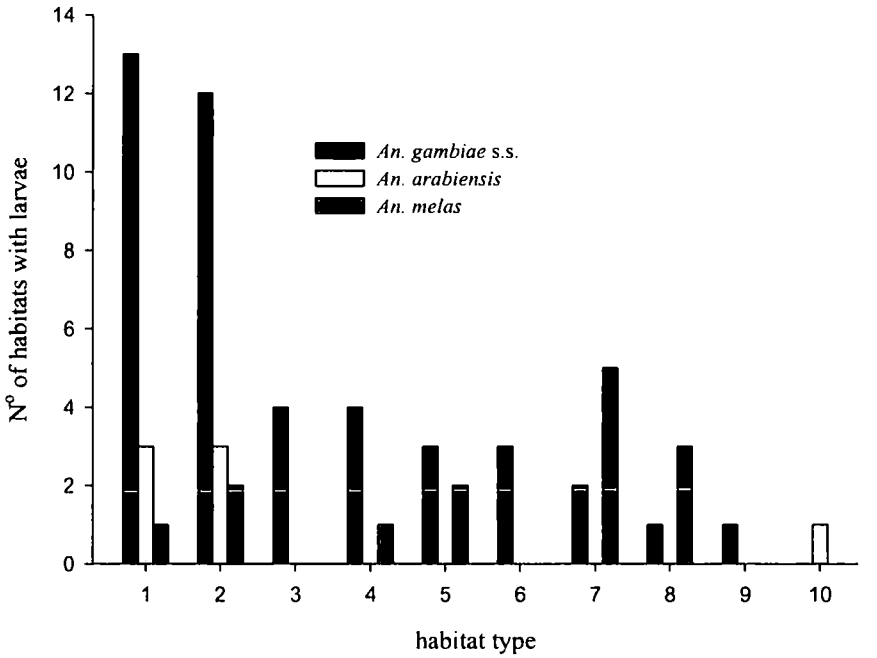

Figure 2.4 Frequency of *An. gambiae s.l.* in different habitat types where 1: pool, 2: rice fields, 3: pond, 4: puddles and tyre tracks, 5: stream fringe, 6: constructions, 7: floodwater, 8: edge of floodwater, 9: brick or sand pits, and 10: water channel

Discussion

This study represents the most comprehensive survey of mosquito larvae in The Gambia and is of relevance to other parts of the Sahel, where large river systems dominate the local malaria ecology. Aquatic habitats were mapped in an area of approximately 400 km^2 over two years, including both floodplain and upland areas during the dry and wet seasons. Whilst it was attempted to achieve full coverage of the study area, some sites may have been missed in deeper water close to the river. This study is unique in that it covers such a large area over an extended time period in

contrast to the majority of published ecology studies which were small-scale in space and time (Minakawa et al. 1999, Mutuku et al. 2006a, Mwangangi et al. 2007, Sogoba et al. 2007). Only large-scale studies allow making generalisations about the larval ecology of malaria vectors relevant for operational larval control programmes. Specifically, this research was carried out to determine whether it is possible to identify habitat characteristics associated with the presence of anopheline larvae using practical operational tools. It was hoped that any such characteristics could be used to guide interventions to target larval control at specific sites or time periods.

Most anopheline breeding habitats were confined to the floodplains, in agreement with previous studies (Bøgh et al. 2003, Thomas and Lindsay 2000). These habitats are created by flooding from the river and heavy rainfall in the rainy season and persist due to the high water table and impervious clay ground, unlike the dry and porous sandy upland (Dunsmore et al. 1976). The importance of naturally flooded areas for mosquito proliferation is supported by earlier studies in The Gambia where the salt water malaria vector *An. melas* was associated with *Avicennia* mangrove in flooded areas of the River Gambia (Giglioli 1965). Similarly *An. gambiae* s.*l.* was associated with flooded areas in Liberia (Gelfand 1955) and Nigeria (Barber et al. 1931, Chwatt 1945).

The risk of finding anopheline larvae in the floodplains during the rainy season was increased when habitats were located within 1km of the landward edge, were large in size (100 m or more in perimeter) and located in areas where grassy vegetation (including rice and sedge) dominated the land cover. This includes over 80% of all habitats encountered during the two years of rainy season surveys and does not represent selection criteria which could easily be used to guide anti-larval interventions. One exception might be the distance from the edge of the alluvial plains. The farther away from the edge and the closer to the river the more difficult it is to access habitats and to implement anti-larval interventions unless aerial spraying is used. Specifically the application of larvicides becomes difficult in these highly tidal environments. For operational reasons it would be wrong to target larviciding operations only at the landward edge of the floodplains because mosquitoes found further into floodplains would be missed.

During the dry season the small water bodies in the uplands dried out, leaving those in the floodplains as the main refugia for anophelines. Hence the probability of finding anopheline larvae during the dry season was reduced compared to the rainy

season. Presumably this was a consequence of the lower water level and the reduction in habitat availability. In the floodplains, habitats suitable for larval development were found everywhere irrespective of landcover, habitat type or size.

There were fewer aquatic sites in the upland areas compared with the floodplains. Specific risk factors for finding sites which could be targeted for anti-larval interventions were not identified. Even though fewer in number, these upland habitats are important for malaria transmission in The Gambia due to their closeness to human settlements. Mosquitoes emerging from these sites are more likely to feed on people and become infected with malaria parasites than mosquitoes that have to fly far to reach people. The upland sites, unlike the extensive breeding sites in the floodplains, could be reduced by filling unused pits and pools and ensuring that pooling does not occur around water pumps and cattle troughs. However, larval control cannot be successfully targeted only at the upland sites close to human settlements because of the greater propensity of larvae to be found in the floodplains. Adult studies also show a gradient indicating that the majority of adults emerge from the landward edge of the floodplains (Bøgh et al. 2007, Thomas and Lindsay 2000).

These findings are consistent with the hypothesis that blood fed mosquitoes have a long flight range in The Gambia, a situation typical of sparsely populated savanna (Gillies and DeMeillon 1968, Service 1997, Thomas and Lindsay 2000). Unlike urban or densely populated areas where flight range for anophelines is often around 1km (Service 1997, Trape et al. 1992a) settlements in rural Gambia are tightly clustered and not widely dispersed as in many other African countries. Thus, for a blood-questing mosquito in the floodplain it would appear to be more difficult to locate a blood meal than elsewhere, particularly as people living close to the floodplains are more likely to use bednets (Clarke et al. 2002). Whether in these areas mosquitoes will change their biting times or switch to other hosts has not yet been investigated in The Gambia. This implies that larval control cannot focus on breeding sites close to human settlements alone, but must also attack those farther away.

Even though rice fields presented as much a risk factor for *Anopheles* breeding as any other large water body with grassy vegetation, it is important to emphasise that rice fields were by far the most common aquatic habitats, covering a surface area of over 20 km^2 in the study area. Most of these are found in the floodplain, although rice is also grown in valley depressions in the uplands. It is well known that rice cultivation encourages mosquito production (Ijumba and Lindsay 2001, Service 1989, Snow

1983), although most studies describe the importance of irrigated rice, rather than the traditional 'swamp' rice grown in floodplains of the River Gambia. During the rainy season when rice was cultivated, a high proportion of all rice fields (66-68%) were colonised by anopheline larvae. Although rice fields cannot be singled out as preferred *Anopheles* larval habitats in The Gambia, their sheer abundance and the ease of recognising them in the field, makes them an important target for anti-larval interventions. Nevertheless, given the large variety of suitable water bodies for anopheline development, targeting rice fields exclusively might not be enough to reduce malaria transmission to such an extent to be cost-effective.
The large areas covered by rice fields as well as the fact that one in four habitats colonised by anophelines in the upland close to human settlements is man-made emphasises the importance of breeding sites created by people in the ecology of malaria in The Gambia.

Anopheline larvae were predominantly found in habitats covered by relatively short vegetation such as (early stage) rice, grass and sedge in accordance to larval ecology studies elsewhere in Africa (Briet et al. 2003, Fillinger et al. 2008, Klinkenberg et al. 2005, Lindsay et al. 1991b, Mwangangi et al. 2006, Snow 1983). These types of vegetation allow water bodies to be exposed to sunlight, a situation preferred by ovipositing mosquitoes (Muirhead-Thomson 1951), unlike tall and thick vegetation such as reeds and mangrove.

The finding that large water bodies are more important than small ones for mosquito breeding in both the floodplains and upland areas in the rainy season contradicts a common view, held since the 1950s, that small water bodies are typical habitats for *An. gambiae* (Holstein 1954, Muirhead-Thomson 1951). Indeed the lack of enthusiasm for anti-larval measures for malaria control in SSA was due partly to the idea that such small sites were too common and difficult to locate (Najera and Zaim 2002).

Anopheline larvae were rare and difficult to find in the field. Anophelines were found in only 309 site visits, after approximately 15,000 dips, during the dry season and on 992 site visits, after about 18,000 dips, during the rainy season. The small number of anophelines found is likely to be due to a combination of factors. Larvae are frequently clustered(Service 1971) and these clusters were distributed over a huge area in the floodplains making sampling challenging. Although dipping is a simple sampling tool, it is inefficient and only likely to capture a small proportion of the

mosquito larvae present in any habitat (Robert et al. 2002). Other sampling methods could be used to increase chances to capture larvae. For example metal cylinders also called area samplers give a more accurate comparative index of larval populations than dippers. Nets mounted on a strong frame can be used to sample larvae in different habitats and can be used to estimate the population size of the sampled area (Service 1977b). However these methods would be time consuming and could only be considered in experimental settings (Chapter 4).

Interestingly, anophelines and culicines were commonly found together. Similar findings have been reported in habitats in East Africa (Fillinger et al. 2004). The over-riding impression in The Gambia is that whilst some water bodies support a wide diversity of life, others are truly inimical for invertebrates. This would explain why anophelines and culicines shared the few prolific habitats available in the area. Niche partitioning, occurring at a finer spatial scale (Bøgh et al. 2003, Boyd 1949, Gimnig et al. 2001, Minakawa et al. 2004, Sattler et al. 2005, Toure et al. 1998), was only apparent in this study when the habitat preferences of different members of the *An. gambiae* complex were examined. *An. gambiae* s.s. predominated in pools, *An. arabiensis* was more common in rice fields and *An. melas* was most frequently found in floodwater, which was likely to be saline. The impact of water characteristics on mosquito production such as the conductivity, oxygen content, pH and turbidity was studied in our study area and will be published elsewhere (U. Fillinger, personal communication).

In this study the characteristics of sites with anopheline mosquitoes were described, not those specifically relating to *An. gambiae s.l.* the major malaria vector in The Gambia. This approach is relevant for determining where *An. gambiae* s.*l.* is found since members of the complex inhabited the same water bodies as other anophelines. Larval control programs mostly concentrate on monitoring the density of late stage larvae or pupae, since they represent sites most likely to produce adult vectors (Fillinger et al. 2008). However, for a large routine program, it would be too demanding to measure larval density in each habitat and pupae are rare and difficult to sample with a dipper as shown in other surveys (Chapter 4). Collecting data on mosquito emergence from each habitat would present the optimal way to estimate habitat productivity but would require a lengthy and thorough study which would be difficult to implement in a routine control program. The most feasible approach under

operational conditions is to employ local residents with relevant training to collect data on presence and absence of larval stages (Fillinger et al. 2008).

In The Gambia the probability of finding late instars was positively and strongly correlated with finding early stage larvae. Thus the findings for both early and late instars of anopheline mosquitoes are also generally applicable for determining sites most commonly occupied by late stages of *An. gambiae s.l.* Furthermore, in large-scale operational programmes evidence-based decisions on re-treatment intervals need to be made instantly in the field and will therefore be based on the presence of any anopheline larva, not necessarily the presence of *An. gambiae* (Fillinger et al. 2008). The findings reported here, based on practical operational monitoring and evaluation tools, show that anopheline larvae are present in a wide variety of habitats and associated characteristics, implying that successful larval control cannot be targeted at specific habitats in The Gambia. This calls for blanket treatment of all available aquatic habitats at regular intervals and the implementation of sustainable environmental modifications where applicable.

The comparatively small number of habitats during the dry season (November-May) would in principle suggest that there could be an advantage for dry season larval control, which then might lead to a large reduction in overall population size. However, the wide distribution of few sites over a vast area of floodplains and upland without any risk factors to guide the intervention to specific sites would be logistically demanding and the overall impact on malaria transmission questionable. Nevertheless, to delay the rise of adult mosquito numbers during the rainy season (Sogoba et al. 2007) and also to allow field teams to adapt slowly to the changing environment and increasing habitat numbers, anti-larval measures should be started one to two months prior to the rainy season. Furthermore, the quickly increasing risk of vector proliferation and malaria transmission with the start of the rains makes it necessary to implement anti-larval intervention throughout the wet season.

Since mosquito habitats are distributed over a large area and involve extensive water bodies situated far away from human settlements, larval control will be logistically demanding. However these sites are largely accessible and less effort is needed to control larvae in moving and deeper water bodies covered by tall reeds or mangrove forest. The long flight range of *An. gambiae* in this country means that larval control activities would have to be carried out over large areas to reduce the likelihood of adults flying into control areas from surrounding locations.

Although there would be considerable advantage in targeting larval control to specific breeding sites if they would be identifiable as the most productive habitats for malaria vectors (Gu and Novak 2005), it is necessary to be cautious about this approach since the heterogeneity in productivity of different breeding sites is not always predictable and breeding sites are highly dynamic and influenced largely by the rainfall (Killeen et al. 2006) and the fluctuation of the level of the river.

Chapter 3

Productivity of different habitats for *Anopheles* larvae and pupae in rural Gambia

Figure 3.1 Ebrima Kuyateh sampling for mosquito larvae in the floodplains of river Gambia

Productivity of different habitats for *Anopheles* larvae and pupae in rural Gambia

Abstract

Background Because anopheline larval control operations are logistically demanding, understanding which sites produce most mosquitoes should help target control and thus reduce operational costs. This study was designed to determine which habitats produced most mosquito larvae and pupae in the middle reaches of River Gambia, in order to determine whether it was feasible to target larval control at selected habitats in the area.

Methods 10 sentinel sites, representative of the main habitat types in each zone, were selected in each of four study zones (400 km^2 in area). Larval and pupal productivity was assessed using dippers in each site weekly for 19 months.

Results A total of 26,301 larvae and pupae were collected, 58% of which were early instar larvae, 35% late instars and 7% pupae. Larvae were more abundant in the rainy season than in dry season ($p < 0.001$) but pupal density did not differ between seasons. All habitats had similar larval and pupal densities, except puddles and water channels that had fewer larvae and pupae in the rainy season. *Anopheles gambiae s.l.* occurred in all habitats, except in floodwater, and were mainly associated with habitats containing sedge and grass.

Interpretation Anopheline mosquitoes in rural Gambia exploit a wide range of habitats and although the highest densities of mosquitoes are observed in the rainy season, larval and pupal development continues throughout the year. In such settings larval control cannot be targeted at selected habitats but should be applied to all areas of standing water, starting before and continuing through the rainy season.

Introduction

Successful integration of larval control into the framework of integrated vector management for malaria control will require a thorough understanding of larval ecology in areas where it is envisaged. Because larviciding campaigns are logistically demanding usually due to the short retreatment intervals (Walker and Lynch 2007), it would be cost effective to target control at selected habitats and/or at a specific times of the year in areas with seasonal malaria transmission, like The Gambia (Bøgh et al. 2003). Targeting larval control has raised debate in the malaria vector control community (Gu and Novak 2005, 2006, Killeen et al. 2006) mainly because larval ecology varies in different ecosystems making it difficult to standardise when and which habitat types should be targeted.

Although oviposition occurs in a wide variety of habitats, more than 90% of larvae do not reach the adult stage (Service 1973, 1977a). In The Gambia a study reported 47% larval mortality in puddles and 75% in rice fields (Bayoh 2001). Therefore understanding which habitats produce more late instar *Anopheles* larvae and pupae is likely to better indicate which habitats are likely to produce adult malaria vectors. A study in western Kenya showed that pupal production occurred only in a subset of habitats even when larvae were recorded in all habitats (Mutuku et al. 2006b) suggesting that larval control targeted at these habitats would reduce vectors density. In contrast a study in Tanzania did not reveal ecological characteristics of breeding sites associated with production of *Anopheles* larvae, concluding that every stagnant open water should be considered as a potential malaria vector breeding site (Sattler et al. 2005). These mixed results show the necessity of a careful study of the contribution of different mosquito breeding sites to adult mosquito production before deciding whether larval control should be targeted at restricted sites or whether it should be comprehensive and directed at all potential breeding sites.

The study reported here is part of a large-scale study looking at the ecology of the aquatic stages of anopheline mosquitoes in rural Gambia in preparation for a larviciding programme in the area (Chapter 5). A parallel study has shown that most habitats were colonised during the rainy season giving possibilities of targeting larval control at the end of the dry season and continued throughout the rainy season (Chapter 2). However it also showed that mosquitoes colonised a wide range of habitats therefore suggesting that targeted larval control to selected habitats might not be successful in this setting (Chapter 2). Since all colonised habitats are not equally

productive of adult mosquitoes, this study investigated the differences in habitat productivity of late instar larvae and pupae between the seasons in order to assess whether productivity of habitats is high at a restricted time of the year and/or in selected habitats in order to inform a larval control programme of possibilities of targeted control.

Methods

The study area is described in full in Chapter 2. Briefly, the study was carried out east of Farafenni town and run from November 2004 to April 2006 before the beginning of a larval control programme. This area has one short rainy season running from June to October. Each study zone was divided into an upland area not connected to the river and the river's floodplain. Each zone was 12 km wide, divided into a central band 4 km wide with 4 km wide buffer zones either side of the central band. Ten sentinel sites were selected randomly within the most common habitats in the central band of each zone.

Water body measurements

At each wet site the following parameters were recorded: perimeter of aquatic habitat (categorised as ≤100 m or >100 m), depth of water, and the vegetation coverage. Habitats were classified as "sparsely vegetated" when more than 50% of their water surface could be seen from top and "shaded" when they were covered with emergent vegetation and less than 50% of the water surface could be seen. Eight different aquatic habitats were surveyed and classified into the following categories (as described in Chapter 2): (1) cattle troughs, (2) pools, (3) ponds, (4) water channels, (5) stream fringes, (6) puddles or tyre tracks, (7) floodwater and (8) rice fields. Additionally, the five following dominant landcover around each aquatic habitat were recorded: (1) shrubs, (2) grass, (3) sedge, (4) rice, and (5) reeds.

Mosquito sampling

At each sentinel site 10 dips were made using a dipper in places likely to harbour mosquito larvae. Late stage anopheline larvae and all pupae were stored in 100% ethanol which was refreshed upon reaching the laboratory. Specimens were identified as members of the *An. gambiae* complex by amplification of ribosomal DNA using the polymerase chain reaction (PCR) (Scott et al. 1993b).

Statistical analysis

The mean larval and pupal density was calculated for each habitat using the Generalised Estimating Equations (GEE) by running the model without calculating the intercept. The model returned the mean of the dependent variable for each variable used as a predictor. All variables were incorporated in a mathematical model and their overall impact on the density of late instar *Anopheles* larvae and pupae tested using GEE. This model was used because it takes account of repeated measures in the analysis since the same sentinel sites were repeatedly sampled during the study. The sentinel identity (ID) was used as subject unit for repeated measures assuming an exchangeable correlation matrix. Larval and pupal density data was fitted to a negative binomial distribution with a log link function. Rice fields were used as a reference group for comparison with other habitats and sedge was the reference for the landcover type. These references were selected because they were the most frequent habitat and landcover type when both seasons were considered. Analyses were performed with SPSS version 15. Missing data were excluded from the analysis.

Results

The 40 sentinel sites contained water on 1,982 occasions during 19 months of data collection. During the rainy season habitats were dry only in 2% of the sampling occasions and in 21% of sampling occasions in the dry season. A total of 26,301 larvae and pupae were collected, with 15% of these sampled in zone 1, 13% in zone 2, 55% in zone 3 and 17% in zone 4. Early instar larvae (1st and 2nd instars) represented 58% of the total collections (15209/26301), late instar larvae (3rd and 4th instars) represented 35% (9150/26301) and pupae 7% (1942/26301) of the total collections. The data discussed below focus on late instar *Anopheles* larvae and pupae which is a useful proxy measure for the production of adult mosquitoes.

Larvae

When both seasons were considered, and after adjusting for the variation between zones, anopheline larvae were more abundant in the rainy season than in the dry season (OR = 2.42; 95% CI = 1.85 – 3.16; $p < 0.001$). The mean number of late instar *Anopheles* larval density was 2.0/dip (95% CI = 1.7 – 2.4) in the rainy season and 0.8/dip (0.6 – 1.0) in the dry season. The data were analysed separately for the rainy and dry season to assess the factors affecting the abundance of mosquito larvae at

different times of the year. Figure 3.2 shows the fluctuation in larval and pupal densities and the rainfall pattern during the study period. Larval density increased with the rains and peaked in October. High densities continued to be observed up to December then decreased for the rest of the dry season.

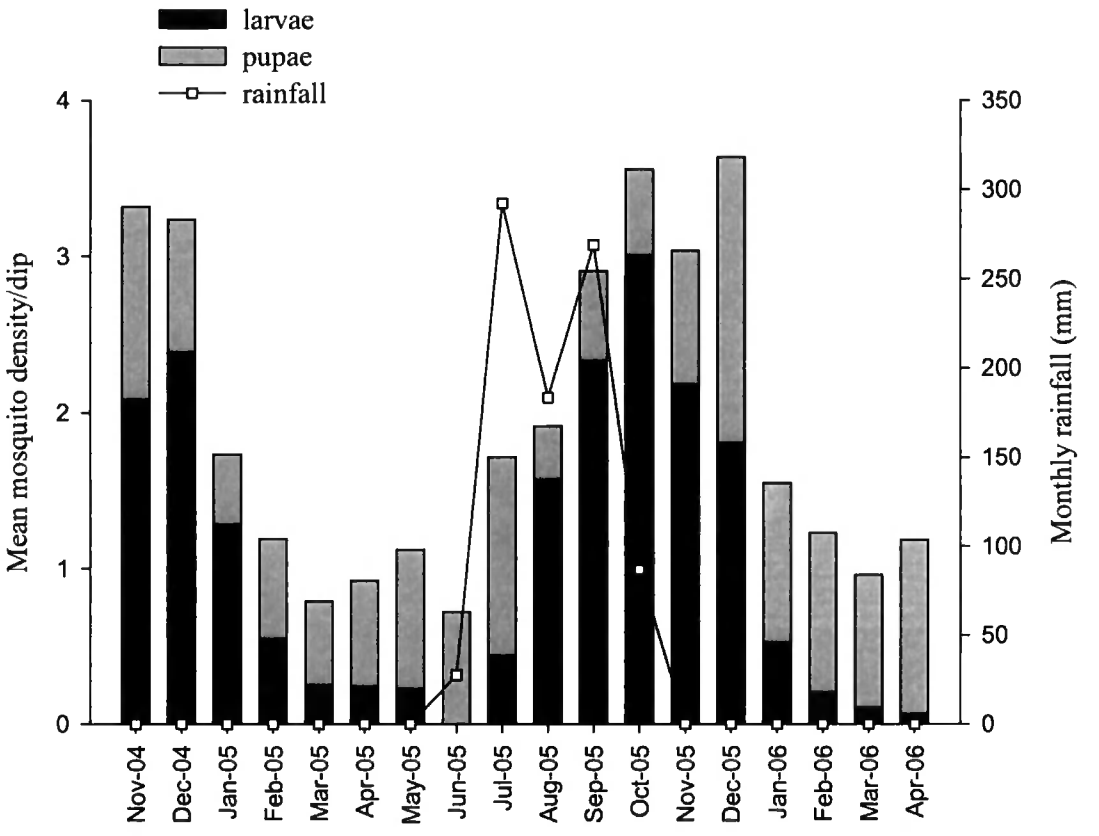

Figure 3.2 Monthly fluctuations in larval and pupal densities and rainfall pattern.

During the rainy season, and after adjusting for the variation between zones (Table 3.1), surprisingly, habitats sparsely vegetated had 31% fewer larvae than those shaded ($p = 0.011$). Larger habitats and increased water depth were positively correlated with larval abundance. Higher densities of anophelines were found in ponds, pools, stream fringes and in floodwater than they were in rice fields. However puddles had significantly fewer larvae than rice fields. Troughs, water channels and rice fields had similar larval densities. Habitats covered with tall reeds or grass as dominant landcover types had significantly fewer larvae than those covered by sedge while those covered by rice or shrubs had similar densities as habitats covered with sedge.

Table 3.1 Factors associated with abundance of late instar *Anopheles* larvae in the rainy season

Factor	Average larvae/visit	Odds ratio	Lower C.I.	Upper C.I.	P
Zone					
Zone 4	1.7	2.16	1.52	3.07	< 0.001
Zone 3	3.0	3.25	2.36	4.46	< 0.001
Zone 2	1.7	1.60	1.14	2.24	0.006
Zone 1	1.5	1.00			
Vegetation					
sparsely vegetated	2.1	0.69	0.51	0.92	0.011
shaded	1.7	1.00			
Perimeter					
> 100 m	1.9	1.51	1.07	2.14	0.019
<= 100 m	3.1	1.00			
Depth		1.02	1.00	1.04	0.041
Habitat type					
troughs	2.1	1.48	0.68	3.25	0.325
puddles	0.7	0.54	0.32	0.90	0.018
water channel	1.6	0.84	0.49	1.43	0.513
pond	4.6	2.27	1.29	4.00	0.004
pool	2.4	1.68	1.12	2.51	0.012
stream fringe	2.2	1.94	1.12	3.34	0.018
floodwater	1.8	2.08	1.09	3.97	0.027
rice fields	1.6	1.00			
Landcover type					
tall reeds	1.6	0.48	0.35	0.66	< 0.001
rice	2.0	1.36	0.79	2.32	0.265
grass	1.1	0.63	0.49	0.82	< 0.001
shrubs	2.3	1.12	0.58	2.16	0.736
sedge	2.8	1.00			

During the dry season, and after adjusting for the variation between zones (Table 3.2), there was no significant difference in anopheline density between habitats sparsely vegetated and those shaded. The size and depth of habitats had no impact on larval abundance. All water bodies were likely to have similar densities of larvae regardless the type of habitat or landcover type. Figure 3.3 shows the variation in the mean number of *Anopheles* larval density between the rainy and dry seasons. During the rainy season larval density was similar in most habitats except for puddles where it was lower and ponds where it was the highest. During the dry season larval density was consistently lower in all habitats compared to the rainy season, except for puddles where it is slightly higher during the dry season. Larval density was highest in pools during the dry season.

Table 3.2: Factors associated with abundance of late instar *Anopheles* larvae in the dry season

Factor	Average larvae/visit	Odds ratio	Lower C.I.	Upper C.I.	P
Zone					
Zone 4	0.5	1.07	0.41	2.81	0.887
Zone 3	0.7	0.68	0.20	2.33	0.541
Zone 2	0.7	0.47	0.19	1.18	0.109
Zone 1	1.0	1.00			
Vegetation					
sparsely vegetated	0.8	1.49	0.81	2.72	0.199
shaded	0.6	1.00			
Perimeter					
> 100 m	0.8	2.60	0.77	8.77	0.124
<= 100 m	0.5	1.00			
Depth		1.07	1.04	1.10	< 0.001
Habitat type					
troughs	0.5	0.66	0.22	1.99	0.463
puddles	0.6	2.57	0.47	14.18	0.278
water channel	0.4	0.41	0.09	1.80	0.236
pond	0.8	1.61	0.54	4.74	0.391
pool	2.1	2.00	0.71	5.59	0.189
stream fringe	0.7	1.11	0.32	3.88	0.868
floodwater	0.6	0.89	0.28	2.84	0.838
rice fields	0.5	1.00			
Landcover type					
tall reeds	0.7	1.34	0.67	2.67	0.405
rice	0.6	0.79	0.28	2.26	0.666
grass	0.5	0.50	0.17	1.44	0.198
shrubs	2.8	2.27	0.47	10.90	0.305
sedge	0.7	1.00			

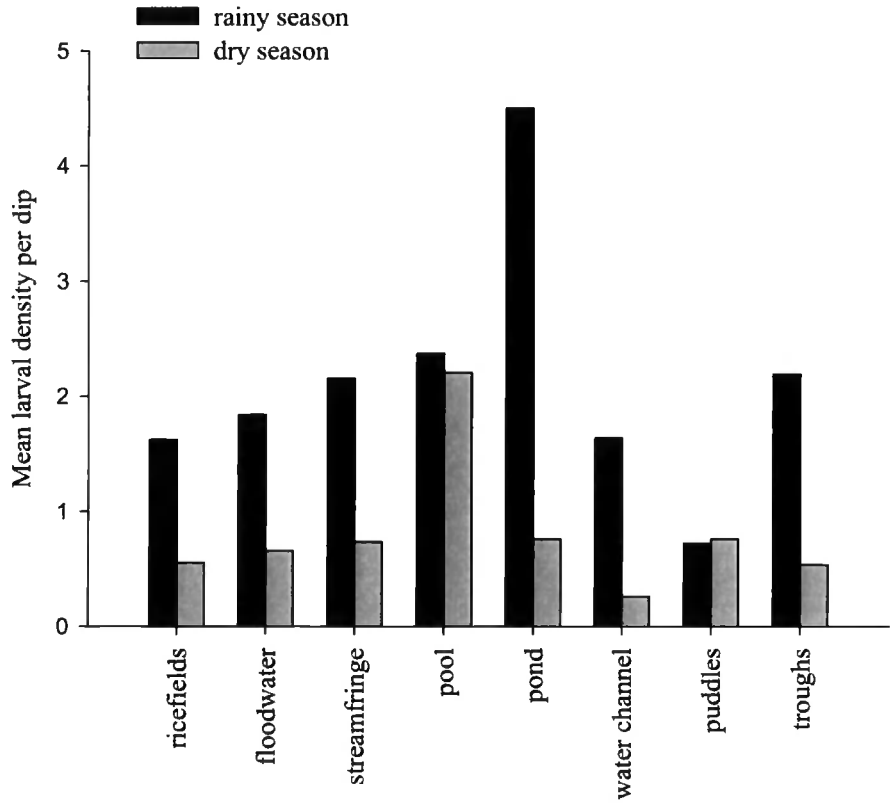

Figure 3.3 Mean *Anopheles* larval density in different habitats in the rainy and dry seasons.

Pupae

After adjusting for variation in zones, surprisingly there was no significant difference in pupal density between seasons. However the data for rainy and dry season were analysed separately to assess the factors affecting the abundance of pupae through time in order to assess possibilities of a time-targeted control.

During the rainy season and after adjusting for the variation between zones (Table 3.3) habitats sparsely vegetated were six times more likely to have high densities of pupae than those shaded ($p = 0.034$). The size and depth of habitats did not affect pupal abundance. Puddles and water channels had fewer pupae than rice fields while troughs, ponds, pools, stream fringes and floodwater habitats had similar densities of pupae as rice fields. Pupae were more abundant in habitats containing rice and grass than those covered by sedge while habitats covered by tall reeds or shrubs had similar densities as those covered by sedge.

Table 3.3: Factors associated with abundance of pupae in the rainy season

Factor	Average pupae/visit	Odds ratio	Lower C.I.	Upper C.I.	P
Zone					
Zone 4	0.7	1.19	0.15	9.22	0.867
Zone 3	1.5	3.89	0.62	24.50	0.148
Zone 2	0.3	1.03	0.15	7.12	0.974
Zone 1	0.2	1.00			
Vegetation					
sparsely vegetated	0.9	5.83	1.14	29.82	0.034
shaded	0.1	1.00			
Perimeter					
> 100 m	0.5	0.44	0.17	1.13	0.089
<= 100 m	2.8	1.00			
Depth		0.99	0.97	1.00	0.141
Habitat type					
troughs	3.6	1.33	0.42	4.21	0.622
puddles	0.1	0.06	0.03	0.12	< 0.001
water channel	0.3	0.23	0.12	0.45	< 0.001
pond	1.0	0.87	0.38	2.01	0.740
pool	1.4	1.55	0.54	4.47	0.419
stream fringe	0.4	1.59	0.75	3.37	0.224
floodwater	0.0	0.19	0.02	2.10	0.176
rice fields	0.7	1.00			
Landcover type					
tall reeds	0.2	3.67	0.70	19.31	0.125
rice	0.8	2.31	1.39	3.86	0.001
grass	1.0	1.96	1.30	2.96	0.001
shrubs	1.0	0.85	0.23	3.14	0.812
sedge	0.4	1.00			

During the dry season and after adjusting for the variation between zones (Table 3.4), habitats sparsely vegetated surprisingly had 49% less pupae than those shaded ($p = 0.016$). The size and depth of the water bodies was not related to pupal abundance. Troughs and pools were more likely to have high densities of pupae, while puddles had significantly fewer pupae. Water channels, ponds, stream fringes and floodwater had similar densities of pupae as rice fields. Pupal density was significantly less in habitats covered with tall reeds, rice and grass than in habitats covered with sedge. The density of pupae in habitats covered by shrubs was similar as habitats covered by sedge. Figure 3.4 shows the variation in mean number of pupal density between the rainy and dry seasons. Surprisingly pupae were less abundant in the rainy season compared to the dry season. In both seasons pupal density was highest in troughs and pools.

Table 3.4 Factors associated with abundance of pupae in the dry season

Factor	Average pupae/visit	Odds ratio	Lower C.I.	Upper C.I.	P
Zone					
Zone 4	1.1	55.02	16.91	179.00	< 0.001
Zone 3	3.3	38.27	16.83	87.04	< 0.001
Zone 2	0.2	4.10	1.92	8.78	< 0.001
Zone 1	0.1	1.00			
Vegetation					
sparsely vegetated	1.7	0.51	0.30	0.88	0.016
shaded	0.2	1.00			
Perimeter					
> 100 m	0.8	1.86	0.97	3.55	0.061
<= 100 m	4.5	1.00			
Depth		1.02	1.00	1.05	0.113
Habitat type					
troughs	5.3	4.08	1.50	11.12	0.006
puddles	0.4	0.39	0.16	0.96	0.041
water channel	1.7	0.81	0.23	2.90	0.745
pond	3.0	1.69	0.54	5.28	0.367
pool	4.3	5.28	2.15	13.01	< 0.001
stream fringe	0.4	0.67	0.21	2.21	0.514
floodwater	0.1	0.61	0.10	3.50	0.576
rice fields	0.6	1.00			
Landcover type					
tall reeds	0.1	0.35	0.17	0.68	0.002
rice	0.2	0.16	0.05	0.46	0.001
grass	1.3	0.39	0.16	0.92	0.031
shrubs	6.4	1.49	0.53	4.17	0.453
sedge	1.2	1.00			

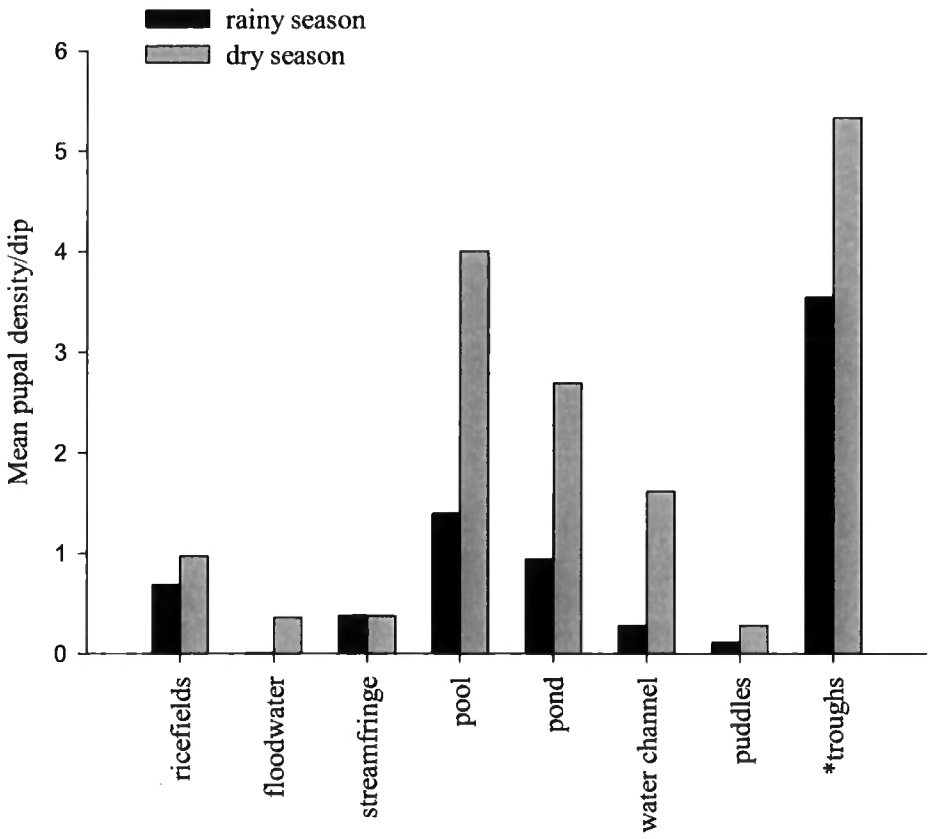

Figure 3.4 Mean pupal density per dip in different habitats in the rainy and dry seasons. *The GEE model could not return the mean for troughs because pupae were found in troughs at very few sampling occasions therefore the arithmetic mean is presented.

Anopheles gambiae complex

A subsample of late instar *Anopheles* larvae and pupae was subjected to PCR for species identification. Out of 644 samples processed 215 (33%) did not amplify either as a result of failure in the DNA amplification process or species not belonging to the *An. gambiae* complex; 217 (34%) belonged to the *An. gambiae* complex and 212 (33%) were other *Anopheles* species. Of the *An. gambiae* complex 86 (40%) were *An. gambiae s.s.*, 5 (2%) were *An. arabiensis* and 126 (58%) were *An. melas*.

Although most samples processed (31%) came from rice field habitats, only 7% of these samples were *An. gambiae s.l.* The proportion of *An. gambiae s.s.* was highest in troughs, stream fringes and water channels where the landcover type was predominantly sedge. *An. arabiensis* was relatively rare, but when it was found it was more abundant in ponds and where the landcover types were dominated by sedge. The

highest proportion of *An. melas* was found in puddles and water channels where the landcover types were predominantly sedge and grass. Other *Anopheles* that were identified in the area are *An. coustani* complex, *An. pharoensis*, *An. rufipes*. These were distributed in all habitats sampled but were most abundant in troughs. Figure 3.5 and 3.6 shows the proportion of *An. gambiae* species in different habitats and landcover types respectively. The proportion of *An. gambiae s.s.* and *An. arabiensis* was highest in ponds, and in water channels for *An. melas*. All three species were found in high proportion in habitats covered with sedge, grass and rice.

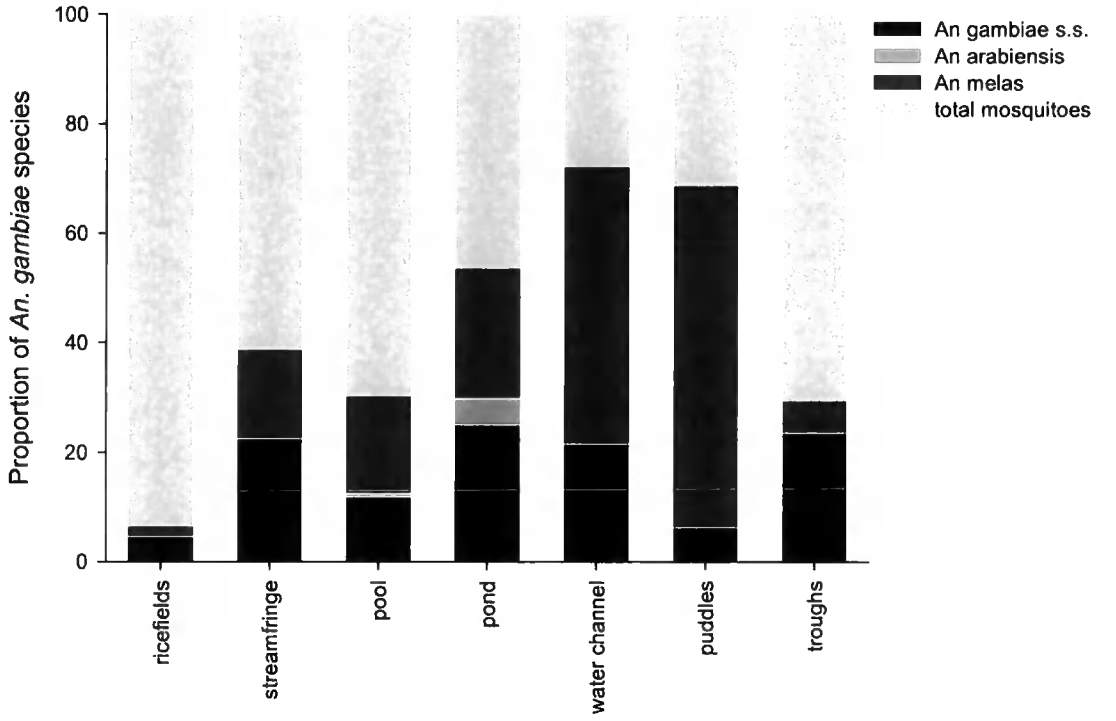

Figure 3.5 Proportion of *An. gambiae* species relative to the total number of mosquitoes in different habitat types.

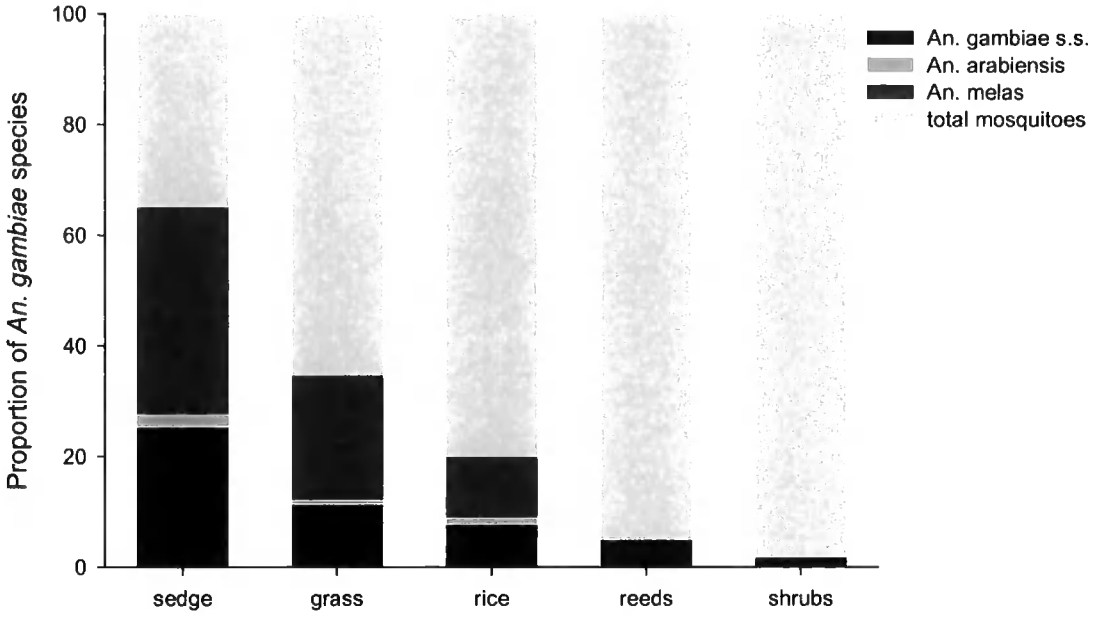

Figure 3.6 Proportion of *An. gambiae* species relative to the total number of mosquitoes in different landcover types.

Discussion

This study surveyed populations of immature mosquitoes over a 19 month period. This is to my knowledge the first study investigating population dynamics of immature mosquitoes routinely for such a long period in the Sahel region of Africa. The findings are relevant for programmes planning larval control in the Sahel region. One of the factors determining the level of malaria transmission is the density of vectors in the area, and this is a result of availability of breeding sites and their productivity (Fillinger et al. 2004, Zhou et al. 2007). The low densities of *Anopheles* larvae obtained in this area are similar to densities observed in market-garden wells in Senegal (Robert et al. 1998) and in rice fields in Mali (Klinkenberg et al. 2003)

As observed in Chapter 2, other studies have shown that anopheline mosquitoes exploit a wide range of habitats (Fillinger et al. 2004, Sattler et al. 2005) and concluded that all potential breeding sites should be exhaustively targeted in larval control programmes. However other studies argue that not all habitats where oviposition takes place are likely to produce adult mosquitoes. It has been suggested that it is not necessary to manage all aquatic habitats to obtain a significant reduction in malaria incidence (Gu and Novak 2005). In rural Kenya pupae mostly occurred in

burrow pits and streambeds and it was concluded that larval control should target those habitats producing pupae (Mutuku et al. 2006b).

This study revealed that *Anopheles* larvae are found throughout the year and their densities are three times greater during the rainy season compared to the dry season. Although the rains start usually in June, larval density peaks later in the season (September-October) when most sites are flooded. This could be due to the fact that the water in habitats on the floodplain is still saline, but as the rains increase creating or expanding more freshwater habitats, larval density increases and peaks in October. Although the rains stopped in October, high densities of larvae continued to be found up to December. This could be due to the fact that unlike breeding sites in the upland that dry out quickly due to the sandy texture of the soil (Chapter 2), habitats in the floodplain where most sentinel sites were located are wet for a longer time. These sites might sustain larval production for a long period before the water is too salt to allow larval development (Bøgh et al. 2003). Despite these high densities observed after October, adult densities show a decline to low densities from November onwards during the dry season (Lindsay et al. 1993). This indicates that although larvae are found during the dry season, their development to adult stage is reduced at this time.

It was surprising that habitats sparsely vegetated had fewer anopheline larvae during the rainy season since it has been a common belief that in West Africa anopheline larvae prefer sun exposed water bodies (Muirhead-Thomson 1951, Taylor et al. 1993). This could be an artefact, explained by the fact that habitats sparsely vegetated had a large surface of open water without vegetation, and larvae usually collect next to vegetation rather than the open water. It has also been observed in this area that larvae prefer vegetated areas where there are fewer predators (fish) than in open waters (Bøgh et al. 2003). Moreover, the difference observed in habitat occupancy between seasons could be due to the difference in species composition over time. Apart from the *An. gambiae* complex, members of the *An. coustani* complex and *An. pharoensis* were found in the study area (Fillinger et al., in prep). These species have different habitat requirements for larvae, with *An. gambiae* often found in open sunlit habitats, *An. pharoensis* in habitats with emergent vegetation, and *An. coustani* can develop in shaded or non-shaded habitats (Horsfall 1955).

Large water bodies had higher densities of larvae and this was in line with the findings in Chapter 2 showing that these habitats were more likely to be colonised by *Anopheles* larvae than small ones. However during the dry season all habitats were

similarly colonised by *Anopheles* larvae. The lack of rain and the changing environment during the dry season might affect larger water bodies in such a way that they offer similar conditions as small habitats for larval development. Increasing depth was positively correlated with presence of larvae. In hot areas like The Gambia where outside maximum temperatures can reach more than 40°C (Chapter 4) it is likely that shallow water bodies heat up and hamper larval development. Laboratory studies showed that adult mosquitoes did not emerge when water temperature was beyond 34°C and larval survival was shortest between 38-40°C (Bayoh and Lindsay 2003, 2004).

During the rainy season, most habitats have a similar and relatively high productivity in mosquito larvae, except in puddles. It is likely that in puddles larvae are flushed out during heavy downpours (Paaijmans et al. 2007) and do not survive in these habitats. Although rice fields are important breeding sites for mosquito larvae (Chapter 2), and a source for mosquito production (Ijumba and Lindsay 2001, Service 1989, Snow 1983) other habitats in this area had higher larval densities than rice fields. Ponds had a high larval density, probably because ponds do not dry out during the dry season and are relatively undisturbed by human activities and might sustain more nutrients for larvae than seasonal rice fields. This could be explained by the fact that rice fields in this area are very extensive therefore larvae might be dispersed over a large area. In such case large habitats might have been under-sampled since a similar number of ten dips were made in each habitat therefore the importance of rice fields should not be overlooked. In a similar ecosystem in Mali, rice fields were an important breeding site for *An gambiae s.s.* during the early growing stage (Klinkenberg et al. 2003)

During the dry season although pools seem to have the highest larval density, the model suggest that all habitats are likely to have similar densities of *Anopheles* larvae. It is interesting to realise that although mosquitoes drop significantly in numbers during the dry season all sampled habitats can sustain some level of productivity throughout the dry season. This implies that although in this area the rainy season is the main malaria transmission season (Thomson et al. 1999) larval production is sustained throughout the year. Earlier studies in The Gambia showed that although *An. gambiae s.s.* decrease substantially in the dry season, *An. melas* maintain their population throughout the dry season (Giglioli 1964). Although mosquito densities might not be sufficiently high to sustain malaria transmission during the dry

season, it would be beneficial to control these populations and delay the rise in density observed in the rainy season. A larval control programme should start in the dry season when there are fewer sites (Chapter 2) before the rise in larval density observed in the rainy season. Most habitats were equally productive for *Anopheles* larvae during the rainy season and the same pattern was observed in the dry season. Therefore targeting larviciding to some habitats only is unlikely to succeed in this setting.

Pupae are the last stage before metamorphosis into adult mosquitoes and in the absence of adult emergence studies this is a good indicator of habitats contributing to adult emergence (Mutuku et al. 2006b). Although pupae are difficult to sample with a dipper (Chapter 4) a good number of pupae were sampled in this study and their habitat preference assessed. It was surprising that pupae were not more abundant in the rainy season than the dry season, and the proportion of pupae in all habitats seem to be higher in the dry season. It is possible that the pupal stage is longer in the dry season and sampling at that time is likely to pick the pupae and/or pupae might have reduced movements during the dry season and therefore become easier to sample with dippers.

Unlike larvae, pupae were more abundant in habitats sparsely vegetated during the rainy season, but this was the reverse during the dry season. Although during the rainy season shaded habitats had high densities of larvae, a high proportion did not reach the pupal stage. During the dry season larvae similarly exploited sparsely vegetated as well as shaded habitats but it is likely that those developing in shaded habitats were more protected from predators (Bøgh et al. 2003) therefore could reach the pupal stage. Most pupae were found in pools which are usually situated in bushy environments in the upland area and these are often shaded.

The size and depth of a water body did not affect pupal productivity in both the rainy and dry seasons. Most habitats in the rainy season were similarly productive of pupae, except puddles and water channels that were less productive. As observed for larvae, heavy downpours in the rainy season that flush out larvae reduce their chances of development into pupal stages in the puddles. Because water channels have running water especially in the rainy season they might not be able to support pupal development. In the dry season puddles were less productive possibly due to the fact that they dry out quickly (Chapter 2) and do not keep water for long enough to support larval development. Another reason could be that water in puddles heats up quickly and high temperatures might reduce mosquito development (Bayoh and Lindsay 2003). Troughs and pools were more productive for pupae both in the rainy and dry

season. These habitats are discrete and not under the river's influence therefore are subjected to less or no fish predation. In addition these habitats are closer to human settlements and might contribute greatly to adult mosquitoes reaching homes.

An. gambiae s.s is the main malaria vector in The Gambia (Hemingway et al. 1995, Lindsay et al. 1993) and shows a high plasticity in its ability to exploit a wide range of habitats. Results reported in Chapter 2 have shown the importance of floodplains for mosquito distribution and an earlier suggested that *An gambiae* was almost exclusively confined to the alluvial floodplains (Bøgh et al. 2003). However *An. gambiae s.s.* was also found in troughs which are in the upland area, closer to human settlements. In line with previous studies (Bøgh et al. 2003) *An. gambiae* s.s. and *An melas* overlapped in their habitat requirements and were both associated with sedge and grass. Although *An. melas* is more salt tolerant, *An gambiae* s.s. shows a high salt tolerance and could be found in waters up to 30% sea water (Bøgh et al. 2003). Both species were found in all the habitat types sampled, except floodwater habitats where mosquito predators are usually abundant (Chapter 2). Despite narrowing the investigation to malaria vectors only, this study illustrates the difficulties of finding risk factors associated with mosquito productivity in different aquatic habitats of rural Gambia.

In conclusion, mosquitoes in The Gambia exploit a wide range of habitats. Although the highest densities of adult mosquitoes are observed in the rainy season, larval and pupal development continues throughout the year. In such settings larval control targeted at few habitats is unlikely to be successful thus a comprehensive control starting before and continuing through the rainy season might reduce vectors density.

Chapter 4

Agriculture and the promotion of insect pests: swamp rice cultivation and malaria vectors in The Gambia

Figure 4.1 Lamin Jarju emptying a mosquito trap in a rice field

Agriculture and the promotion of insect pests: swamp rice cultivation and malaria vectors in The Gambia

Abstract

Background *Anopheles gambiae*, the principal mosquito vector of malaria in Africa, frequently breeds in ricefields. Here we explore how 'swamp rice' cultivation in the floodplains of the Gambia River, an ancient agricultural practise in West Africa, affects the production of anopheline mosquitoes during the rainy season, the period of peak malaria transmission.

Methods Routine surveys were carried out from June 2005 to January 2006. 500 m long transects crossing rice fields from the landward edge of the floodplains to the river were surveyed weekly. Aquatic invertebrates were sampled using area samplers and emergence traps and fish sampled using caste nets. Semi-field experiments were used to investigate whether the presence of nutrients commonly found in natural rice fields affected larval abundance.

Results At the beginning of the rainy season rice is grown on the landward edge of the floodplains. This is the first area to flood with fresh water and it is rich with dung left by cattle feeding on the rice stubble from the previous year's harvest. Later, rice plants are transplanted close to the river, the last area to dry out on the floodplain. Nearly all *An. gambiae s.l.* were collected <100 m from the landward edge of the floodplains, where immature rice plants were grown. These paddies contained stagnant freshwater with high quantities of nutrients, and mosquito larvae were protected from insectivorous fish found outside the paddies. Semi-field trials showed that cattle faeces were associated with high mosquito numbers.

Interpretation Rice fields close to the landward edge were most commonly colonised during the rainy season, since they were large areas of standing freshwater, rich in nutrients, were protected from fish, and were situated close to human habitation, where egg-laying mosquitoes from the villages had a short distance to fly.

Whilst people exploit the ecology of the floodplains for rice cultivation in West Africa, these sites are also exploited by malaria vectors. As the demand for locally-produced rice gathers pace, rice farming communities must be protected against malaria.

Introduction

It is ironic that the world's huge agro-ecosystems designed to feed the ever increasing human population also provide a habitat for a far greater number of insects to exploit and to thrive in. Each year nearly 18% of the world's crops are damaged or consumed by insect pests (Oerke 2006). Agro-ecosytems also provide ideal breeding habitats for many insects. For example, irrigation is associated with the production of vectors that transmit pathogen to humans, including those responsible for malaria and Japanese B encephalitis (Oomen et al. 1988). In order to manage vector populations it is important to know which specific human practices promote those pests.

It is well known that rice cultivation leads to increased mosquito production in Africa (Ijumba and Lindsay 2001, Lacey and Lacey 1990, Muturi et al. 2006, Service 1989, Surtees 1970). Increases in the number of *Anopheles gambiae s.l.*, the major malaria vectors in Africa, typically correspond with the beginning of rice cultivation, when the paddies are first flooded and rice is short (Klinkenberg et al. 2003, Lacey and Lacey 1990, Lindsay et al. 1991b, Muirhead-Thomson 1951, Mwangangi et al. 2006, Snow 1983). Most research on rice and malaria focuses on irrigated rice production (Audibert et al. 1990, Doannio et al. 2002, Faye et al. 1995, Klinkenberg et al. 2003, Lindsay et al. 1991b, Robert et al. 1992, Snow 1983) and not traditional practices (Dossou-Yovo et al. 1998, Gbakima 1994).

Rice has been grown in The Gambia for many centuries (Parks 1799). Traditional lowland rice is grown in the floodplain of the Gambia River, and is known locally as '*bafaro*' or 'swamp rice'. However as the population increase, local rice production cannot meet the demand and the country has to rely increasingly on imported rice (Figure 4.2). Swamp rice production is one of the oldest forms of rice cultivation in West Africa, with approximately 200,000 ha under cultivation in Guinea, Guinea Bissau, Senegal, Sierra Leone and The Gambia (Agyen-Sampong 1994). During the rainy season in The Gambia, a combination of heavy rainfall and a rising river level results in the Gambia River flooding extensive parts of the alluvial floodplain. It is here the rice fields are constructed during the rainy season. Rice is cultivated between 110-290 km from the river mouth, where the river is still tidal and often salty, which reduces rice yield but also limits the growth of weeds and, consequently reduces the amount of weeding needed in the fields (Webb 1992). A recent comprehensive study of larval habitats over a 400 km^2 area along the middle reaches of the River Gambia demonstrated that lowland rice fields on the edge of the

floodplains were an important breeding habitat of anopheline larvae (Chapter 2). Here we investigated the reasons for this observation in the hope that we could improve malaria control in this environment.

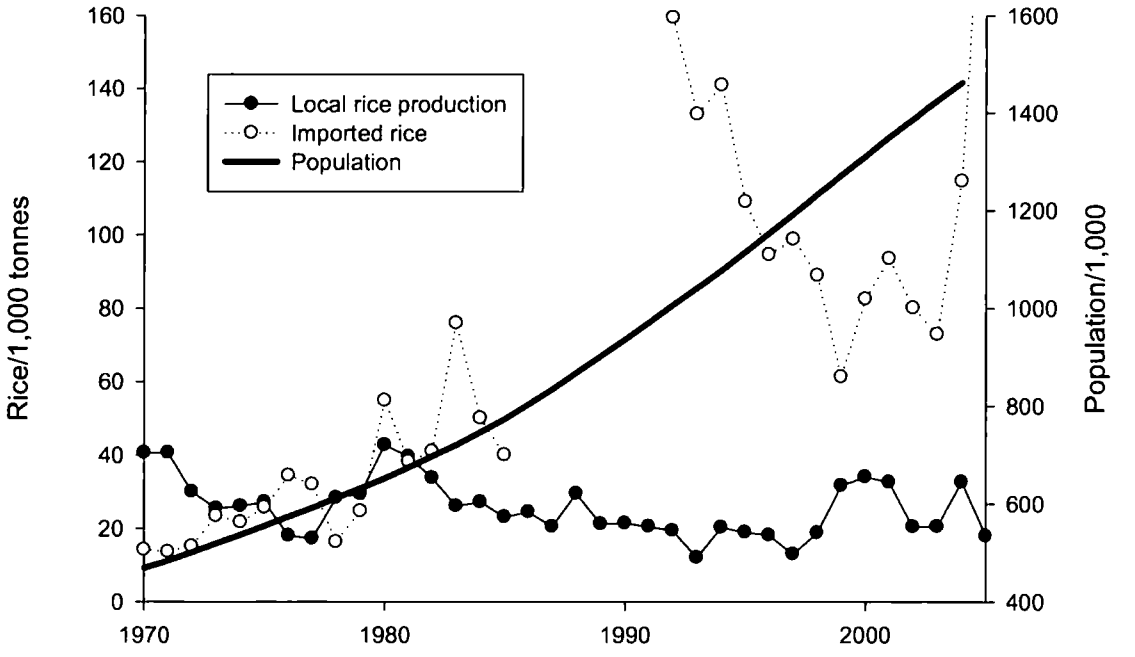

Figure 4.2 Rice imports and exports in relation with the rising population in The Gambia. No data on imported rice prior to 1990. Data from FAO (faostat.fao.org) and Webb 1992 (Webb 1992).

Methods

Study area

The transect study was carried out near Tamba Koto Village (UTM coordinates: 1331776N, 1530990W) 10 km east of Farafenni town (UTM coordinates: 1500200N, 435500E), close to the Gambia River (Figure 4.3). The population of the village was about 215 inhabitants(Anonymous 2003), predominantly Mandinka. The area is generally flat, open farmland and sparse woodland, typical of Sudan savannah. The major crops are rice, cultivated on the floodplains, and groundnuts, millet, sorghum and vegetables, which are grown inland. The area has a short rainy season from June to October, followed by a long dry season from November to May. The semi-field study

was carried out at the Medical Research Council Field Station, in Farafenni. Rainfall data were also collected at the Station.

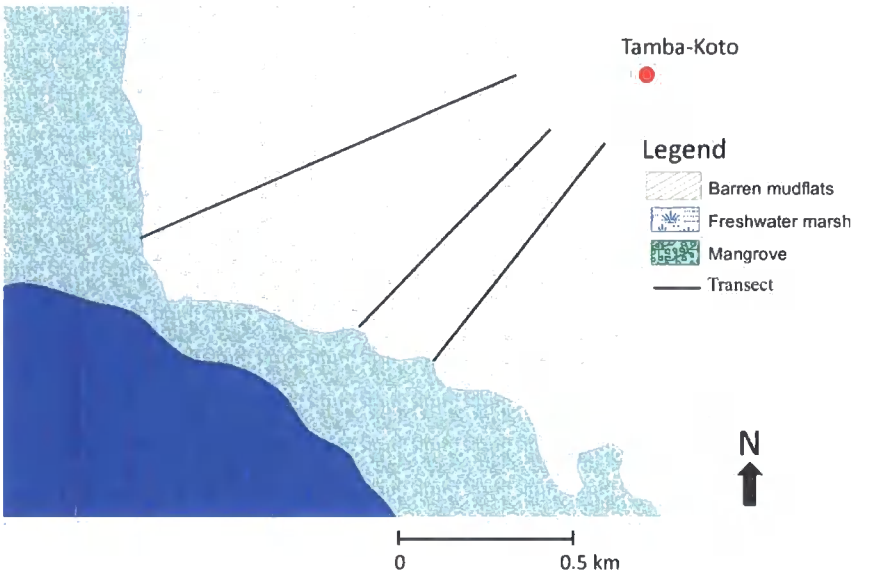

Figure 4.3 Map of study area.

Aquatic surveys

Three parallel transects each approximately 500 m in length, 20 m wide, and 200 m apart were sampled weekly from June 2006 to January 2007. Transects were situated across the alluvial floodplains of the Gambia River, in an area of swamp rice cultivation, starting on the landward edge near the village and ending near the river (Figure 4.3). Since we had evidence that many mosquitoes were breeding on the edge of the floodplains (Chapter 2; (Bøgh et al. 2003) we concentrated our sampling here. Larvae were sampled at 0 m, 25 m, 50 m, 75 m, 100 m, 200 m, 300 m, 400 m and 500 m along each transect (Figure 4.4). At each distance three samples were made with an area sampler (AS; (Service 1977b)) one at the centre and two at opposite ends of the paddy. The AS was a 39.5 cm long aluminium tube, with serrated teeth around the bottom lip to grip into the substrate. They had an upper diameter of 47cm and a lower one of 40cm (surface area of 0.126 m^2). The AS were plunged quickly into water bodies most likely to contain larvae (i.e. edge of water or near emergent vegetation (WHO 1992b)) and left for 30 seconds to allow the water to settle and larvae to come to the surface. A standard 350 ml dipper was used to empty the water from the AS and transferred into a white plastic bowl containing clear water. Excess water was carefully removed to concentrate any organisms present in the bowl. All mosquito larvae and

other specimens were collected and placed in 100% ethanol before being transported to the laboratory for identification.

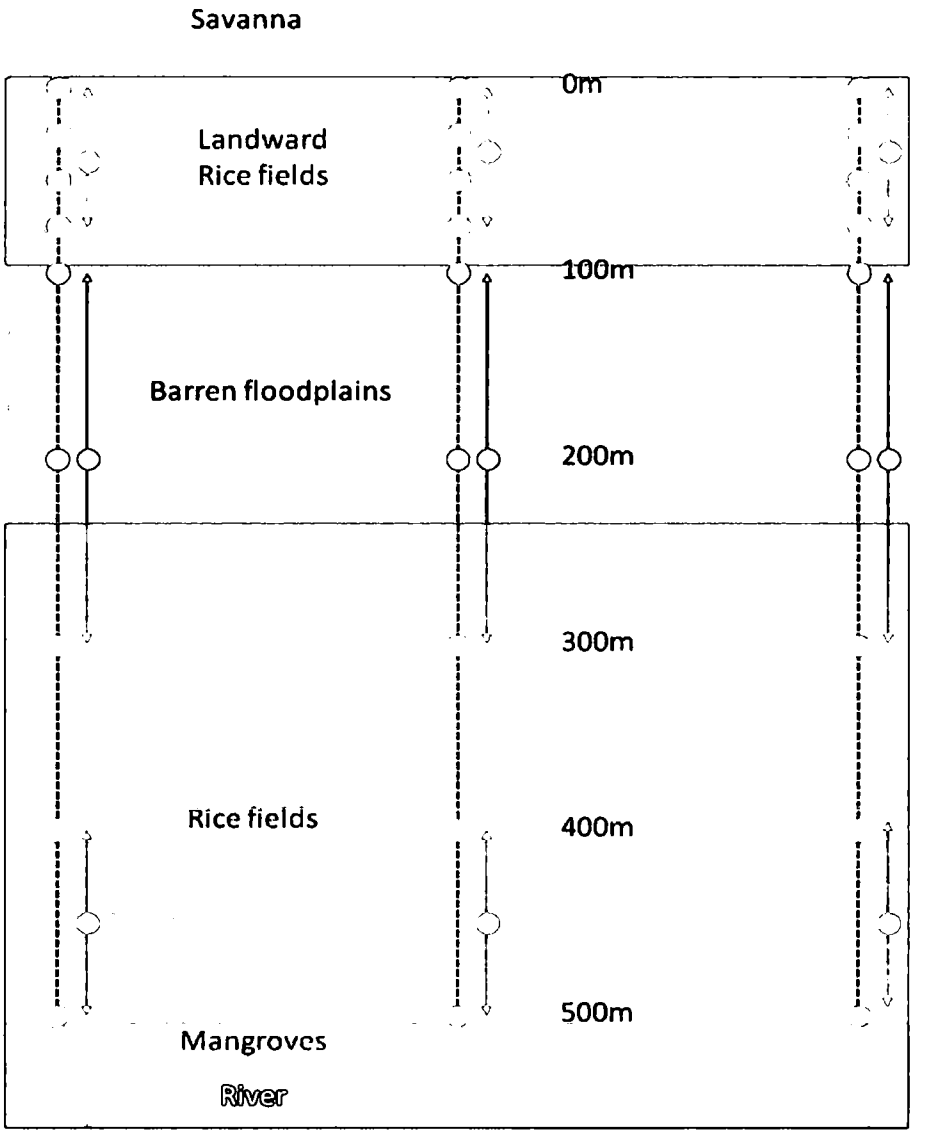

Figure 4.4 Schematic representation of sampling frame in the study area. Where broken lines represent the three transects, red circles represent weekly sampling points for aquatic invertebrates and blue circles weekly sampling points for emerging invertebrates.

Emergence fauna

Floating emergence traps were used to sample adult insects continuously for the entire study period (LeSage and Harrison 1979, Southwood 1978, Stagliano et al. 1998). Emerging invertebrates were sampled in three zones along each transect: 0-75 m from

the landward edge, 100-300 m and 400-500 m at weekly intervals from June 2006 to January 2007 (Figure 4.4). Traps were positioned 4 m either side of each transect over water bodies thought likely to contain larvae. In each zone samples were made: (1) <1 m from the edge of the field nearest the village, (2) in the centre of the field, and (3) in the same field near the edge furthest from the village. This procedure was repeated in all zones along each transect. Emergence traps were designed to collect positively phototaxic arthropods that emerged from the water. They were constructed from conical metal frames 1 m in height and 1 m in diameter (0.786 m^2 surface area) and were covered in transparent nylon netting which reduced any shading of the water surface which might reduce catches of emerging invertebrates (Davies 1984). Traps were made buoyant by attaching plastic 1L bottles to the base with wire to allow the water to flow undisturbed under the trap. Each trap was tethered to a wooden stake anchored to the ground allowing the trap to rotate freely. Traps placed over mature rice plants were not tethered.

The top of each cone opened into a plastic insect collection chamber (Bioform, Germany). The chambers were filled with 250 ml of 60% glycol in order to kill and preserve flying insects that collected there. A netting sleeve on the side of each trap allowed flying insects caught within the netting cone to be removed with an aspirator. Insects were removed weekly and transported to the laboratory for invertebrate identification. Traps were moved every week from its previous sampling position to a new location where larvae had recently been found.

Specimen identification

All insects, excluding mosquitoes, were separated into the following taxonomic groups: dragonfly larvae (Odonata; sub-order Anisoptera), damselfly larvae (Odonata; sub-order Zygoptera), beetle larvae (Coleoptera), adult beetles (Coleoptera), mayfly larvae (Ephemeroptera), water measurer adults (Hydrometridae), greater water boatman adults (Notonectidae), lesser water boatman adults (Corixidae), water scorpion adults (Nepidae), pond skater adults (Gerridae), creeping water bug adults (Noucoridae), pigmy backswimmer adults (Pleidae) and broad-shouldered water striders (Veliidae). Mosquito larvae were counted and identified as anopheline and culicine in the field. In the semi-field studies, 1st and 2nd stage larvae were recorded as early instars and 3rd and 4th stage larvae as late instars. Pupae were removed from the bowls, counted and transferred into separate cages for each treatment where adult

mosquitoes emerged. All mosquitoes were identified with the aid of morphological keys and members of the *An. gambiae* complex identified by PCR analysis (Scott et al. 1993a).

Fish sampling

Fish were sampled using a cast net (diameter: 230 cm, mesh size: 10 mm) and a hand net (25 x 17 cm, mesh: 2 mm) at 50 m intervals, as in the larval sampling, once along each transect in August. At each sampling point three cast-net throws were made at three different locations within 10 m of either side of the transect point. Five cumulative minutes of sweeping were also undertaken with the hand-net within the same sampling area, with only enough time between sweeps and net throws to remove the fish from the net. Fish were identified to species using Paugy and co-workers (Paugy et al. 2003).

Physical measurements

At each mosquito survey point a number of physical measurements were made. Water depth was measured with a metre rule at three different locations within the sampling area and averaged. We recorded whether the water was under the influence of the tides by visual inspection. Water conductivity, pH, temperature and dissolved oxygen content were measured with a multi-parameter probe (350i WTW, Germany) and water turbidity with a turbidity meter (HANNA, USA). All samples were taken between 07:00 and 14:00 h. Rice height was measured from the water surface to the maximum vertical height of the plant. During the long, hot dry season cattle are allowed to graze freely in the floodplains. Counts of cow dung were made at 0 m, 25 m, 50 m, 75 m, 100 m, 200 m, 300 m, 400 m and 500 m along each transect in June, before the start of the rainy season.

Semi-field experiments

We investigated whether nutrients commonly found in ricefields affected mosquito production. 12 plastic bowls filled with 20L of tap water served as artificial breeding sites for mosquitoes. The bowls were arranged in open grassland in a grid of parallel rows, with each bowl 1 m from its neighbour. Each bowl had a surface area of $0.21m^2$. Three small holes, 1 cm in diameter, were made at the top of each bowl and covered with netting to prevent rain water washing larvae out of the bowls. The bowls were 25

cm deep and were sunk into the ground with 5 cm remaining above the ground. Approximately 5g of alluvial soil from the River Gambia floodplain was added to each bowl in order to provide the biotic and abiotic conditions suitable for mosquitoes (Chapter 5, (Fillinger et al. 2003).

Each bowl had one of the following treatments: cow dung (20 g), urea (200 g, 46% nitrogen, grain size 0.85mm, Honorich Technology Co., China) and tap water served as a control. The amount of cow dung and urea was roughly approximated to conditions seen in the field. Treatments were allocated to bowls in a balanced design. For each of the four trials, each treatment was randomly allocated a different bowl number. Bowls were covered for two days to allow the soil to settle and then left open for wild mosquitoes to lay their eggs for seven days. Larval sampling was done using a standard 350ml dipper. Five scoops of water were taken from the surface of each bowl, four on the edges and one in the centre. This was done daily for 14 days over a 12 week period. Anopheline larvae were counted and returned to the bowl. All pupae were removed to avoid emergence of mosquitoes.

Nutrient analysis

In the semi-field trial nutrients were measured in 9 bowls, each containing the three different treatments (i.e. 3 bowls/treatment) and sampled after 1 day, 4 days and 7 days. 250ml water samples were filtered using 0.45μm cellulose acetate membrane filters and analysed spectrophotometrically for filterable reactive phosphorus (FRP – PO_4^{3-}) following the ascorbic acid method (Clesceri et al. 1998), for filterable reactive nitrogen (FRN – NO_3/NO_2) using the Feree method (Ferree and Shannon 2001) and ammonia (NH_4), following the Nessler method (Clesceri et al. 1998). Colour, an indirect measure of the concentration of tannins, was measured by taking a reading at 440nm (Cuthbert and Del Giorgio 1992). All measurements were carried out on the same day of collection to minimise any change in water qualities in the samples.

Statistical analysis

Non-normal data were normalised by log transformation or squared to stabilise the variance. Comparisons between normally distributed data were made using t-tests. Proportions were compared using chi-square analysis. The number of insect taxa found was counted. All variables were incorporated untransformed in a mathematical model and their overall impact on the presence or absence of anopheline larvae or adults

tested using Generalised Estimating Equations (GEE). This was a logistic model and adjusted for repeated measures. GEE were also used to examine the relationship between larval numbers and treatment group, adjusting for repeated measures. Analyses were performed with SPSS version 15 and EpiInfo version (TM). Missing data and data from emergence traps that were not fully working were excluded from the analysis.

Results

Meteorology

Total rainfall during the 2006 rainy season was 807.9 mm. Rain started in the beginning of June and ended in the middle of October. The rainfall was similar to the mean annual rainfall of 772.8 mm (95% Confidence intervals = 694.9-850.7 mm) for the period 1990-2005.

Flooding patterns in the river floodplains

The profile of the landscape from Tamba Koto village to the River Gambia is characterised by upland agricultural fields, the tree-lined fringe of the upland savannah, followed by an area of barren mud, before the first rice fields and the large areas of floodwater beyond. Further into the floodplain tall reeds are found before reaching the second area of rice fields close to the river and the mangrove forest fringing the banks of the river. Different parts of the floodplain are subjected to different patterns of flooding (Table 4.1). Rice fields within the first 100 m from the landward edge were the first to flood in August, filled by rain water. In September the rice fields close to the river were flooded due to a combination of rainfall and rising river levels. Whilst those paddies close to land dried out by the middle of October, those near the river were more permanent and did not completely dry until January.

Table 4.1 Seasonality of flooding in study area. Grey fill represents flooding in at least one of the three transects each week.

Month	Week	Distance from landward edge (m)								
		0	25	50	75	100	200	300	400	500
Jul	1									
	2									
	3									
	4									
Aug	1									
	2									
	3									
	4									
Sep	1									
	2									
	3									
	4									
Oct	1									
	2									
	3									
	4	no data collection								
	5									
Nov	1									
	2									
	3									
	4									
Dec	1									
	2									
	3									
	4									
Jan	1									
	2									
	3									
	4									
	5									

Rice cultivation

Fields closer to the upland were divided into small fields with high embankments built to help conserve rain water, whilst those close to the river were less clearly demarcated. Rice cultivation started in June when farmers ploughed their fields. Nerica rice, an African-Asian hybrid, was sown twice on raised nursery beds close to the landward edge of the alluvial floodplains. The first seedlings were transferred to the fields closest to the landward edge by the end of August. Here the rice was grown to maturity, even though some of the fields were not water-logged later in the season. The second batch of seeds were sown in the nursery beds in early August and transplanted

to the fields near the river from late September to the end of October. Urea was applied to fields by hand when transplanting rice plants in September or shortly afterwards at a dose of 25Kg of urea to $50m^2$. Rice was harvested from December to January, starting with paddies closer to the landward edge.

Physical measurements

Water in the fields on the landward edge of the floodplain was stagnant, shallower, warmer, with a lower conductivity and pH and richer in cow dung (80% of deposits) than water in fields close to the river (Table 4.2).

Table 4.2 Characteristics of water and distribution of invertebrates along the transects obtained during larval surveys. Values shown are means after the data were normalized [a]by log transformation (ln(x +1)), or [b]by squaring values. Figures in parenthesis represent 95% confidence intervals for abiotic variables and proportion of sites with specimens for biotic variables.

Variables	Distance from landward edge		P
	0-100m (N = 140)	>100m (N = 415)	
[a]Depth (cm)	9 (8.4-9.7)	10.7 (10.1-11.2)	0.001
[a]Turbidity (ntu)	107.9 (86.9-133.9)	129 (120.8-137.8)	ns
[b]pH	7 (6.8-7.3)	7.6 (7.5-7.7)	<0.001
[a]Conductivity (mS/cm)	1 (0.9-1.2)	2.5 (2.3-2.7)	<0.001
[a]Temperature °C	30.2 (29.8- 30.6)	28.6 (28.3-28.9)	<0.001
Oxygen content units (mg/L)	5.6 (5.2-6.1)	6 (5.8-6.2)	ns
Presence of moving water (%)	4.30%	99.00%	<0.001
Height of rice (cm)	17 (12.9-21.1)	39.1 (35.5-42.7)	<0.001
Cow dung samples/site	132 (N = 638)	33 (N=802)	<0.001
Total anopheline larvae	349 (58/638)	26 (13/789)	<0.001
Anopheles gambiae s.l.	66 (19/638)	14 (9/802)	0.011
Anopheles gambiae s.s.	15 (3/638)	8 (2/802)	ns
Anopheles arabiensis	30 (6/638)	6 (2/802)	ns
Anopheles melas	21 (4/638)	0 (0/802)	0.038
Culicine larvae	423 (53/638)	19 (7/802)	<0.001
Other aquatic insects	912 (19/638)	532 (9/802)	<0.001
Mean no. invertebrate taxa, excluding mosquitoes	3.1 (2.6-3.6)	0.9 (0.8-1.0)	<0.001
Mean no. fish species/sample	0	1.19 (0.68-1.59)	

Aquatic invertebrates

375 anopheline larvae and 442 culicine larvae were collected from 555 samples during the study. There were 80 *An. gambiae s.l.* of which 45% were *An. arabiensis*, 29% *An. gambiae s.s.* and 26% *An. melas*. This is equivalent to 1.14 *An. gambiae s.l.*/m^2. Members of the *An. gambiae* complex were found shortly after the fields were first flooded in August (Figure 4.5), but their numbers fell to zero in early November coincident with the drying out of the fields close to the landward edge (Table 4.1) and increased height of rice. Most aquatic invertebrates were sampled within 100 m from the landward edge of the alluvial floodplains (i.e. 83% anophelines, 96% culicines and 63% of other invertebrates). Even though the first 100m of each transect were sampled more intensively than sites further away, the sites closer to land dried out more quickly (i.e. 140 vs 415 water bodies sampled). After adjusting for differences in flooded sites, 93% of *An. gambiae s.l.* larvae were found in the first 100 m of each transect.

Multivariate modelling revealed that the presence of all anophelines and *An. gambiae s.l.* larvae was highest within rice fields less than 100 m from the landward edge of the floodplains (Table 4.3). Within each paddy larvae were more common along the edge than in the centre. The presence of rice in area samplers and culicine larvae were also positively associated with the presence of anopheline larvae and those of *An. gambiae s.l.* There was also a direct relationship between the abundance of *An. gambiae s.l.* larvae and number of insect taxa ($r^2 = 0.19$, $F = 123.5$, $P<0.001$).

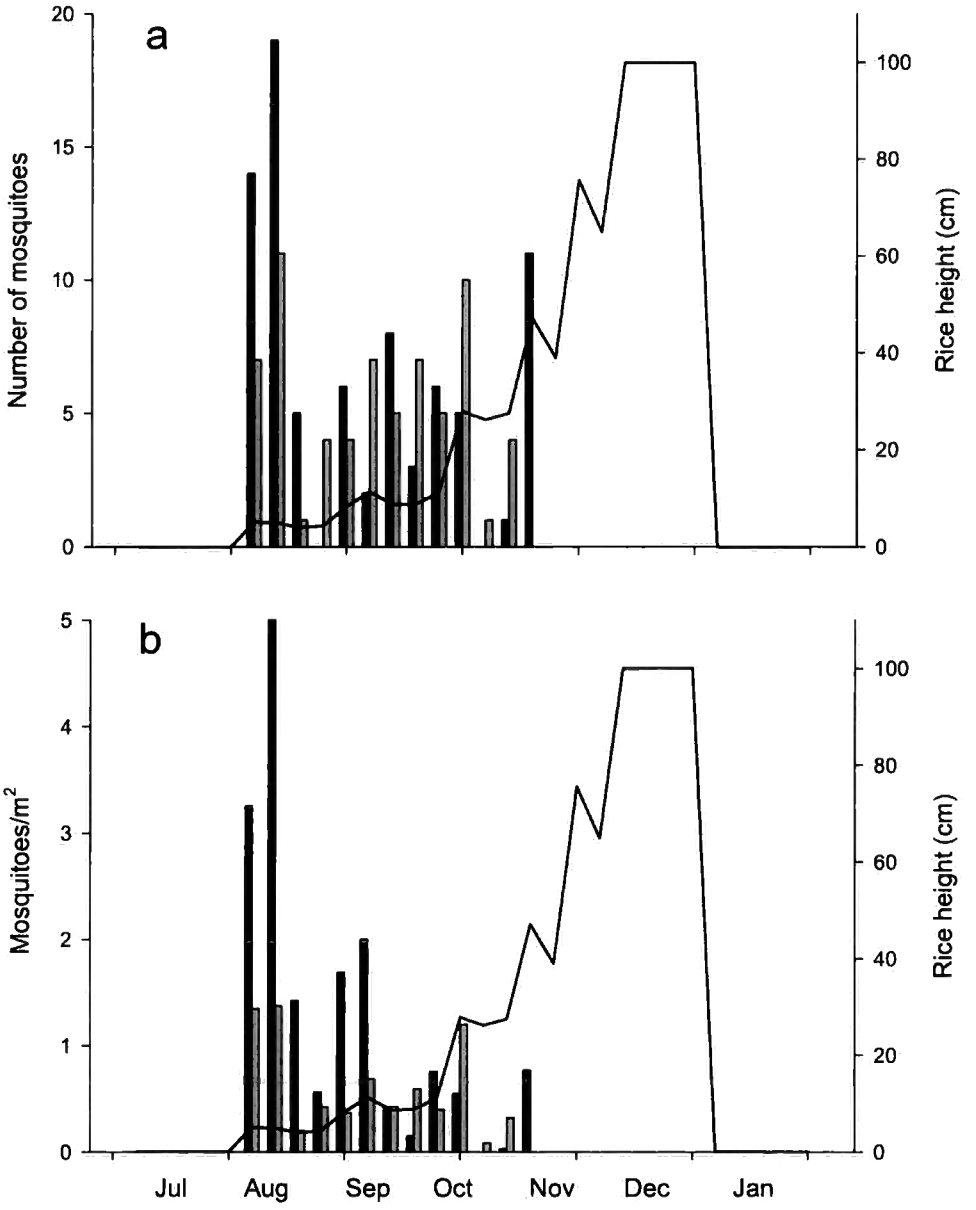

Figure 4.5 Seasonality of anopheline larvae and adults in ricefields. **a** is the total number of anophelines collected, whilst **b** is anopheline density. Where black bars are weekly larval collections, grey bars are total number of adults collected weekly and the solid line is the average height of rice.

Table 4.3 Factors associated with the presence or absence of anopheline larvae.

Variables	All anophelines			*An. gambiae s.l.*		
	Wald	Odds Ratio (95% CI)	*P*	Wald	Odds Ratio (95% CI)	*P*
Spatial measurements						
Distance from landward edge of transect						
1-100m		1			1	
>100m	40.5	0.04 (0.01-0.10)	<0.001	13.15	0.13 (0.04-0.39)	<0.001
Position of sampling point within rice field						
Landward edge		1			1	
Centre	4.97	0.23 (0.06-0.84)	0.026	5.75	0.1 (0.02-0.66)	0.016
Riverside edge	3.95	0.31 (0.10-0.98)	0.047	2.42	0.33 (0.08-1.34)	0.081
Biotic measurements						
Rice in area samples						
Absent		1			1	
Present	13.78	28.25 (4.84-164.85)	<0.001	7.01	10.53 (1.84-60.16)	0.008
Culicine larvae						
Absent		1			1	
Present	9.72	2.78 (1.46-5.30)	0.002	14.23	6.06 (2.38-15.44)	<0.001
Insect biodiversity	2.92	2.16 (0.89-5.20)	0.087	0.17	1.21 (0.50-2.95)	ns

Mosquito adult emergence

90 anopheline and 140 culicine adults were collected from 279 samples made with emergence traps during the study (Table 4.4). There were 66 *An. gambiae s.l.* of which 92% were *An. arabiensis*, 6% *An. gambiae s.s.* and 2% *An. melas*. This is equivalent to 0.30 *An. gambiae s.l.*/m^2. Adults of the *An. gambiae* complex were collected when the rice fields were first flooded in August (Figure 4.5), but the last adult was collected in early November coincident with the drying out of the fields close to the landward edge (Table 4.1) and increased height of rice. Most invertebrates were collected within 100 m from the landward edge of the alluvial floodplains (i.e. 94% *An. gambiae s.l.*, 100% of other anophelines, 95% culicines and 60% of other invertebrates) even though more than twice as many samples were made in the middle and far zone combined, compared with the near zone alone (i.e. 188 *vs* 91). After adjusting for differences in flooded sites in different parts of each transect, 97% of *An. gambiae s.l.* adults were found in the first 100m of each transect. The water within the first 100m of each transect was shallower, non-tidal and had smaller, and therefore, younger rice plants compared with the fields further away which were characterised by deeper, tidal water where taller, more mature rice plants were transplanted.

Table 4.4 Characteristics of water parameters and distribution of adult mosquitoes and invertebrates along the transects. Values shown are mean values after the data were normalized [a]by log transformation (ln(x +1)). Figures in parenthesis represent 95% confidence intervals for abiotic variables and proportion of sites with specimens for biotic variables.

Variables	Distance from landward edge		P
	0-100m (N=91)	>100m (N=188)	
[a]Depth (cm)	9.8 (9.2-10.5)	11.9 (10.3-11.5)	0.032
Presence of moving water	10/91	188/188	<0.001
Height of rice (cm)	17 (12.9-21.1)	39.1 (35.5-42.7)	<0.001
Anopheles gambiae s.l.	62 (36/91)	4 (3/188)	<0.001
Anopheles gambiae s.s.	4 (4/91)	0 (0/188)	0.01
Anopheles arabiensis	57 (34/91)	4 (3/188)	<0.001
Anopheles melas	1 (1/91)	0 (0/188)	ns
Other anophelines	24 (12/91)	0 (0/188)	<0.001
Culicine adults	133 (49/91)	7 (6/188)	<0.001
Other aquatic insects	353 (69/91)	234 (88/188)	<0.001
Insect families	2.2 (1.8-2.5)	0.9 (0.7-1.0)	<0.001

Multivariate modelling demonstrated that the emergence of *An. gambiae s.l.* adults was most common within rice fields less than 100 m from the landward edge of the floodplains, particularly along the edge of the fields (Table 4.5). The emergence of anopheline adults was associated with shorter rice plants and the simultaneous emergence of culicine adults. In the final model insect biodiversity was of borderline statistical significance, suggesting that *An. gambiae s.l.* adult emergence was associated with higher biodiversity in general. This is also indicated by the direct relationship between the abundance of *An. gambiae s.l.* adults and biodiversity (Figure 4.6; $r^2 = 0.43$, $F = 62.2$ $P<0.001$).

Table 4.5 Factors associated with the presence or absence of anopheline adult emergence.

Variable	*An. gambiae s.l.*		
	Wald	Odds Ratio (95% CI)	*P*
Spatial measurements			
Distance from landward edge of transect			
1-100m		1	
>100m	4.1	0.23 (0.05-0.95)	0.042
Position of sampling point within rice field			
Edge		1	
Centre	4.9	0.23 (0.06-0.84)	0.026
Biotic measurements			
Height of rice (cm)	3.4	0.99 (0.98-1.00)	0.024
Culicine adults	36.8	2.22 (1.72-2.88)	<0.001
Insect richness	3.4	1.78 (0.97-3.28)	0.065

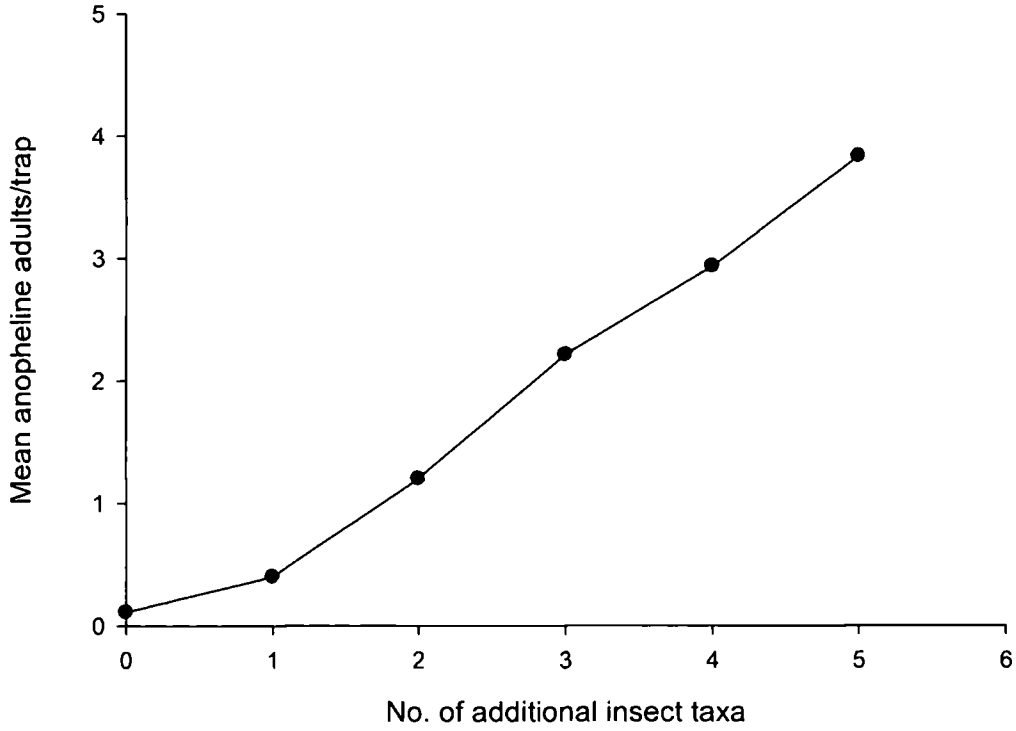

Figure 4.6 Relationship between anopheline mosquitoes and diversity of other emergent insects.

Fish sampling

Four species of fish were collected: 17 *Periophthalmus barbarus,* 12 *Tilapia quineensis,* 1 *Epiplatys spilargyreius* and 1 *Porogobius schlegelli.* No fish were collected within 100m from the landward edge of each transect, whilst 1.19 species of fish were caught on average every sampling occasion further away (Table 4.2).

Semi-field trials

A total of 6,233 anophelines and 11,234 culicine were collected during the four trials. Of the 135 members of the *An. gambiae s.l* complex collected 55% were *An. arabiensis* (n = 74), 44% *An. gambiae s.s.* (n=59) and 1% *An. melas* (n=2). Multivariate modelling revealed that the presence of cow dung in water significantly increased the number of anopheline and culicine larvae (Tables 4.6 and 4.7).

Table 4.6 GEE analyses of different treatments adjusting for trial.

	Anopheline larvae				Culicine larvae			
	Early instars		Late Instars		Early instars		Late Instars	
Explanatory variables	Odds Ratio (95% CI)	*P*	Odds Ratio (95% CI)	*P*	Odds Ratio (95% CI)	*P*	Odds Ratio (95% CI)	*P*
Trial								
1	1		1		1		1	
2	15.82 (8.30-30.15)	<0.001	3.91 (1.75-8.72)	<0.001	2.61 (1.61-4.25)	<0.001	1.39 (0.64-3.01)	0.400
3	14.53 (7.80-27.05)	<0.001	0.83 (0.36-1.91)	0.661	4.27 (2.88-6.34)	<0.001	0.78 (0.38-1.60)	0.498
4	1.47 (0.59-3.64)	0.405	0.96 (0.25-3.70)	0.956	2.37 (1.63-3.46)	<0.001	1.2 (0.49-2.89)	0.691
Treatment								
Water	1		1		1		1	
Urea	0.96 (0.56-1.67)	0.899	0.631 (0.30-1.35)	0.234	0.72 (0.48-1.07)	0.101	1.57 (0.78-3.13)	0.203
Cow dung	1.73 (1.20-2.50)	0.003	2.38 (1.12-5.09)	0.025	1.49 (0.97-2.28)	0.068	4.11 (2.17-7.78)	<0.001

Table 4.7 Mean number of larvae per bowl and treatment.

	Mean number of larvae/bowl in different treatment (95% CI)		
	Water	Urea	Cow dung
Anopheline			
Early instars	4.75 (2.85-7.93)	4.15 (2.36-7.28)	7.67 (4.97-11.83)
Late instars	0.92 (0.48-1.79)	0.59 (0.28-1.23)	1.77 (1.11-2.81)
Culicine			
Early instars	6.84 (4.62-10.12)	5.63 (4.03-7.85)	11.94 (8.30-17.19)
Late instars	1.48 (0.89-2.46)	2.25 (1.35-3.74)	5.76 (3.57-9.31)

FRN, FRP, NH_4 and colour differed significantly between treatments (Figure 4.7). There was 80% more nitrogen, 76% more phosphorous and 33% more NH_4 in the cattle dung treatment compared to the control. Urea contained 76% more NH_4 than the control. The water had 97% higher tannin content in the cattle dung treatment and 13% less in the fertiliser treatment (borderline significance) compared to the control.

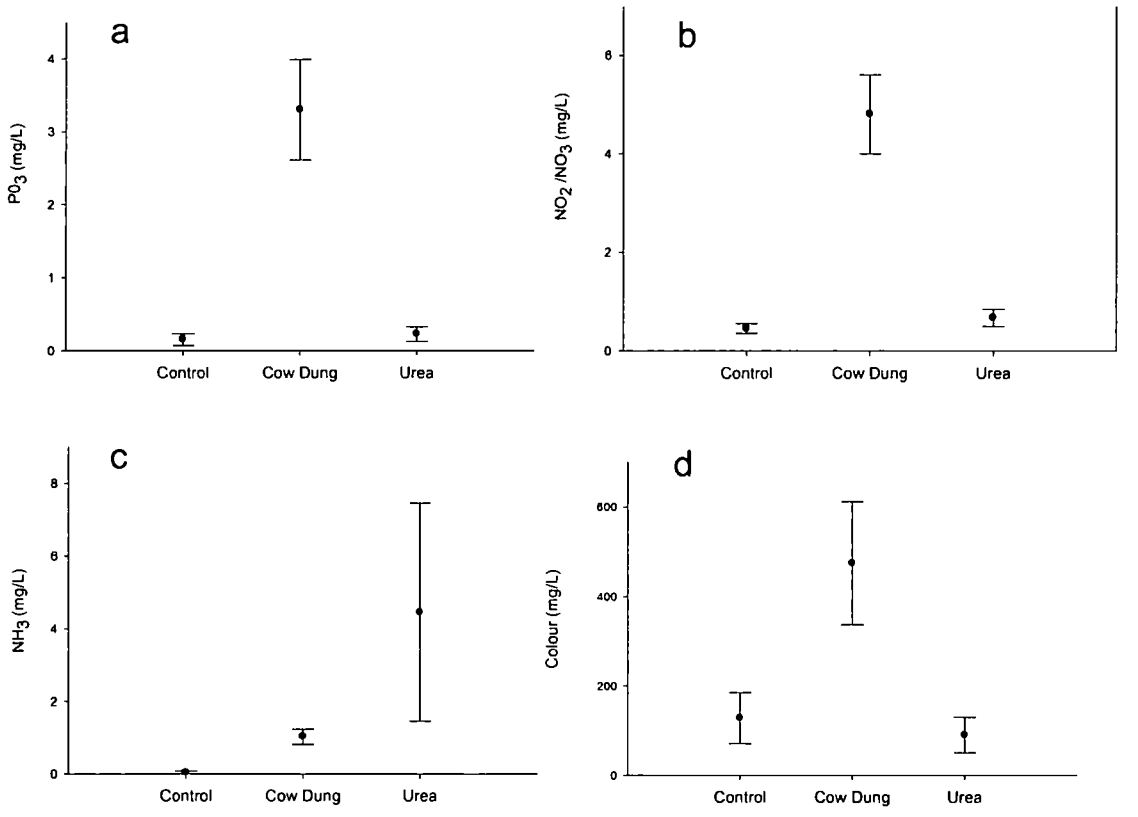

Figure 4.7 Means and 95% C.I.'s of (a) FRP, (b) FRN, (c) NH3 and (d) colour for the different treatments.

Discussion

Here we describe the dynamic process of swamp rice cultivation in wetlands bordering the River Gambia where rice production follows the changing pattern of surface water over the rainy season and demonstrate how the cultivation of rice increases the production of malaria vectors. This is the first estimate of mosquito production in a rice growing area and confirms our earlier conclusions that rice fields on the edge of the floodplains are a major site for malaria vectors in the middle reaches of The Gambia (Chapter 2; (Bøgh et al. 2003). Our findings suggest that a 100m strip of

ricefields along the edge of the floodplains, 1km in length, can generate 86,500 *An. gambiae* adults/week during the rainy season.

After the seasonal rains start in June, women plough their fields in the floodplains of the Gambia River and prepare raised beds for growing rice. The floodplain marks the edge of the open savannah and is an area of flat open land. Two sets of young plants are transplanted to the fields. The first are transplanted early on in the rainy season in paddies close to the landward edge of the floodplain in August. These are the first areas to flood after the soil becomes saturated following several heavy downpours. The water in these fields is fresh, non-tidal and contains the greatest abundance of insect life on the floodplain. These paddies are clearly demarcated by raised embankments that help keep the fresh water in the fields for as long as possible. However, by October, they are drying out and the second set of young rice plants are transplanted to fields close to the river from late September to early October. Here the water is largely flowing in from the River Gambia. It is slightly salty and tidal, but the fields are flooded and will remain so for several months, enough for the rice to mature and be cultivated.

Although it is well known that rice fields are often prolific sources of mosquitoes (Ijumba and Lindsay 2001, Service 1989, Surtees 1970), the density of *An. gambiae s.l.* produced in this study was relatively low with 1.14 larvae/m^2 and 0.30 adults/m^2. Yet the area of ricefields bordering the river is huge covering many hectares. In the rice fields, *An. arabiensis* was the most common member of the *An. gambiae* complex. This species is frequently associated with rice fields in The Gambia (Chapter 2; (Bøgh et al. 2003) and elsewhere (Ijumba et al. 2002, Mukiama and Mwangi 1989, Mutero et al. 2000). Quite why this should be so remains unexplained. Nonetheless, this is not always the case since *An. gambiae s.s.* can also predominate in irrigated rice fields (Klinkenberg et al. 2003, Lindsay et al. 1991b).

Overall 93% of *An. gambiae* s.l. larvae and 97% of adults came from rice fields close to the landward edge of the floodplains, even though many more samples were made further away. There are a number of explanations for this finding related to (1) the geographical position of the fields, (2) their water characteristics, (3) the presence or absence of fish and (4) the presence or absence of nutrients.

A rice field close to the landward edge of the floodplain represents the shortest flight distance for an ovipositing female leaving a village to lay her eggs. Previous studies have demonstrated that rice fields and habitats close to the landward edge were

associated with an increased risk of finding anopheline larvae (Chapter 2; (Bøgh et al. 2007). The phenomenon of finding larvae on the edges of rice fields has been shown before (Minakawa et al. 2002) and other habitats close to human habitation has also been recorded (Staedke et al. 2003, Trape et al. 1992b). We also found evidence from this study that this effect may exist at a finer spatial scale since within individual fields, higher numbers of larvae were found at the edge closest to the land compared to the edge nearer the river. In common with other studies, larvae were less likely to be found in the centre of the field compared with the edge (Andis and Meek 1984).

Rice fields on the landward edge of the floodplain were situated in an area of undisturbed water that was warm, fresh and exposed to sunlight, providing conditions ideal for *An. gambiae s.l.* and many other species of aquatic insect. It was here that the majority of anophelines, culicines and other insect life were dominant. The association found between anopheline and culicine larvae has been seen before in The Gambia (chapter 2) and Kenya (Chandler and Highton 1975, Mwangangi et al. 2006). However, it was surprising to find anophelines sharing habitats with a diverse taxa of insects since it has typically been thought that many species of invertebrate were important anopheline predators (Service 1973). However, fish never occurred where anophelines were found suggesting their importance as major mosquito predators (Homski et al. 1994, Linden and Cech 1990, Louca et al. 2008, WHO 2003b).

Lastly, our findings from the semi-field trial illustrate that cattle dung increases the number of larvae found in breeding sites. Nutrient analysis showed that the water was rich in reactive nitrogen and phosphorous, and ammonium radicals. These nutrients are key drivers of invertebrate abundance in aquatic systems (Sanford 2005) and presumably they provide the nutrients for the organisms upon which mosquito larvae feed early in the rainy season. The reason for the large concentration of cattle dung close to the landward edge is a result of transhumance. In the dry season cattle are grazed on the grass and rice stubble found in the floodplains. Most grazing occurs on the landward edge of the floodplain where the water is less salty for the cattle to drink in this part of the country. During the rainy season the cattle are grazed elsewhere. Unlike an earlier study in Kenya (Mutero et al. 2004) we did not find that nitrogenous fertilizer increased larval numbers.

The phenology of adult mosquitoes in relation to rice production has been well documented (Chandler and Highton 1975, Klinkenberg et al. 2003, Snow 1983), but fewer studies have described the dynamics of larval populations (Muturi et al. 2007a,

Mwangangi et al. 2006). In this study larvae colonised rice fields shortly after flooding and remained there until the rice grew tall and/or the fields dried out. It has been shown previously that few, if any, larvae are found in dense growths of rice, since vegetation prevents mosquitoes from ovipositing on water (Muirhead-Thomson 1951). Our findings clearly indicate that mosquito production was seasonal and highly associated with rice cultivation.

Whilst an increased production of malaria mosquitoes is an inevitable consequence of rice production, swamp rice cultivation is likely to increase in the future. Rice is the staple food in The Gambia and locally produced rice has failed to keep up with the demand for more rice for the growing population, with imports soaring (Figure 4.1), straining the countries meagre financial resources. Since the world's consumption of rice outstrips production rice prices are expected to double in the next two years (www.warda.org: accessed 26/7/7). Thus local production of rice must increase, to offset the rapidly increasing cost of imports. Increasing acreages of rice will increase the vector population. Quite what this will mean for the level of malaria in the country is uncertain, since generally in sub-Saharan Africa increasing transmission associated with rice irrigation does not necessarily lead to more malaria(Ijumba and Lindsay 2001). Nevertheless, this is not a time for complacency and it will be essential to ensure that local communities near rice-growing areas are protected with long-lasting insecticide treated nets and effective antimalarials. Moreover, since we have demonstrated that in this setting vector production is limited to one area of the ricefields it raises the possibility that in the future control activities could be targeted at these sites. At a time when there is a growing threat of food shortages around the world rice production will need to expand. Ensuring that the farmers and their families remain healthy is a pressing priority.

Chapter 5

Microbial larvicides for malaria control in The Gambia*

Figure 5.1 Setting up standardised field experiments for testing microbial larvicides.

*This chapter appeared as a paper, in a modified format with the same title by S. Majambere, S.W. Lindsay, C. Green, B. Kandeh and U. Fillinger in *Malaria Journal* 6:76

Microbial larvicides for malaria control in The Gambia

Abstract

Background Mosquito larval control may prove to be an effective tool for incorporating into integrated vector management (IVM) strategies for reducing malaria transmission. Here the efficacy of microbial larvicides against *Anopheles gambiae s.l.* was tested in preparation for a large-scale larviciding programme in The Gambia.

Methods The impact of water-dispersible (WDG) and corn granule (CG) formulations of commercial *Bacillus sphaericus* strain 2362 (*Bs*; VectoLex®) and *Bacillus thuringiensis* var. *israelensis* strain AM65-52 (*Bti;* VectoBac®) on larval development were tested under laboratory and field conditions to (1) identify the susceptibility of local vectors, (2) evaluate the residual effect and re-treatment intervals, (3) test the effectiveness of the microbials under operational application conditions and (4) develop a method for large-scale application.

Results The major malaria vectors were highly susceptible to both microbials. The lethal concentration (LC) to kill 95% of third instar larvae of *Anopheles gambiae s.s.* after 24 hours was 0.023 mg/l (14.9 BsITU/l) for *Bs* WDG and 0.132 mg/l (396 ITU/l) for *Bti* WDG. In general *Bs* had little residual effect under field conditions even when the application rate was 200 times greater than the LC_{95}. However, there was a residual effect up to 10 days in standardised field tests implemented during the dry season. Both microbials achieved 100% mortality of larvae 24-48 hours post-application but 3rd instar larvae were detected 4 days after treatment. Pupal development was reduced by 94% (95% CI = 90.8-97.5%) at weekly re-treatment intervals. Field tests showed that *Bs* had no residual activity against anopheline larvae. Both microbials provided complete protection when applied weekly. The basic training of personnel in identification of habitats, calibration of application equipment and active larviciding proved to be successful and achieved full coverage and control of mosquito larvae for three months under fully operational conditions.

Interpretation Environmentally safe microbial larvicides can significantly reduce larval abundance in the natural habitats of The Gambia and could be a useful tool for inclusion in an IVM programme. The costs of the intervention in this setting could be reduced with formulations that provide a greater residual effect.

Introduction

At the start of the new millennium malaria is still deeply entrenched in Africa and effective malaria control is under threat from drug and insecticide resistance (Coleman et al. 2006, Winstanley et al. 2002). In response to that, mosquito larval control has recently received renewed attention by the international scientific community (Chen et al. 2006, Fillinger and Lindsay 2006, Fillinger et al. 2004, Gu and Novak 2005, Killeen et al. 2002a, Mutuku et al. 2006b, Shililu et al. 2003, Vanek et al. 2006, Yohannes et al. 2005) and recent attempts to develop integrated vector management (IVM) strategies for different eco-epidemiological settings re-consider mosquito larval control as one of the tools to reduce malaria transmission (WHO 2003a).

Promising new formulations of the microbial larvicides *Bacillus sphaericus* (*Bs*) and *B. thuringiensis var. israelensis* (*Bti*) have recently been shown to give excellent control of the major vectors of malaria in Africa (Fillinger et al. 2003, Fillinger and Lindsay 2006). Use of these biological control agents is better than chemical larvicides since they are very species specific, environmentally safe (WHO 1999) and appear not to induce resistance when used together(Mulla et al. 2003). It is envisaged that the utilisation of such biological control agents may be best carried out using a vertical approach that actively involves local communities (Mukabana et al. 2006, Vanek et al. 2006). The national strategy for malaria control in The Gambia includes larval control (NMCP 2002), yet there has been no detailed evaluation of this methodology. Whilst *Bs* and *Bti* have been tested in different ecological settings in Africa (Fillinger and Lindsay 2006, Hougard et al. 1993, Karch et al. 1992, Karch et al. 1991, Nicolas et al. 1987, Ravoahangimalala et al. 1994), the riparian habitats found in The Gambia represent a novel habitat for investigating these microbials. In the present study the efficacy of microbial larvicides was tested against malaria vectors in The Gambia, West Africa, to identify the optimal formulations, dosages and application methods in order to prepare for a large-scale larviciding programme.

Material and methods

Study area

The study was based in and around Farafenni town (UTM zone 28, 1500200mN, 435500mE), in the central part of the country, about 100 km from the coast (Figure 5.2). Laboratory and standardised field tests were carried out at Farafenni Field Station of the Medical Research Council (MRC) Laboratories. Field tests were implemented

near Tamba-Koto village, 10 km east of Farafenni. The area is predominantly flat farmland and woodland savannah. The main upland crops are sorghum, millet, groundnut and pumpkin and in the floodplains swamps rice is grown during the rainy season. The villages in the area are discrete clusters of houses and are not scattered as seen in many parts of Africa.

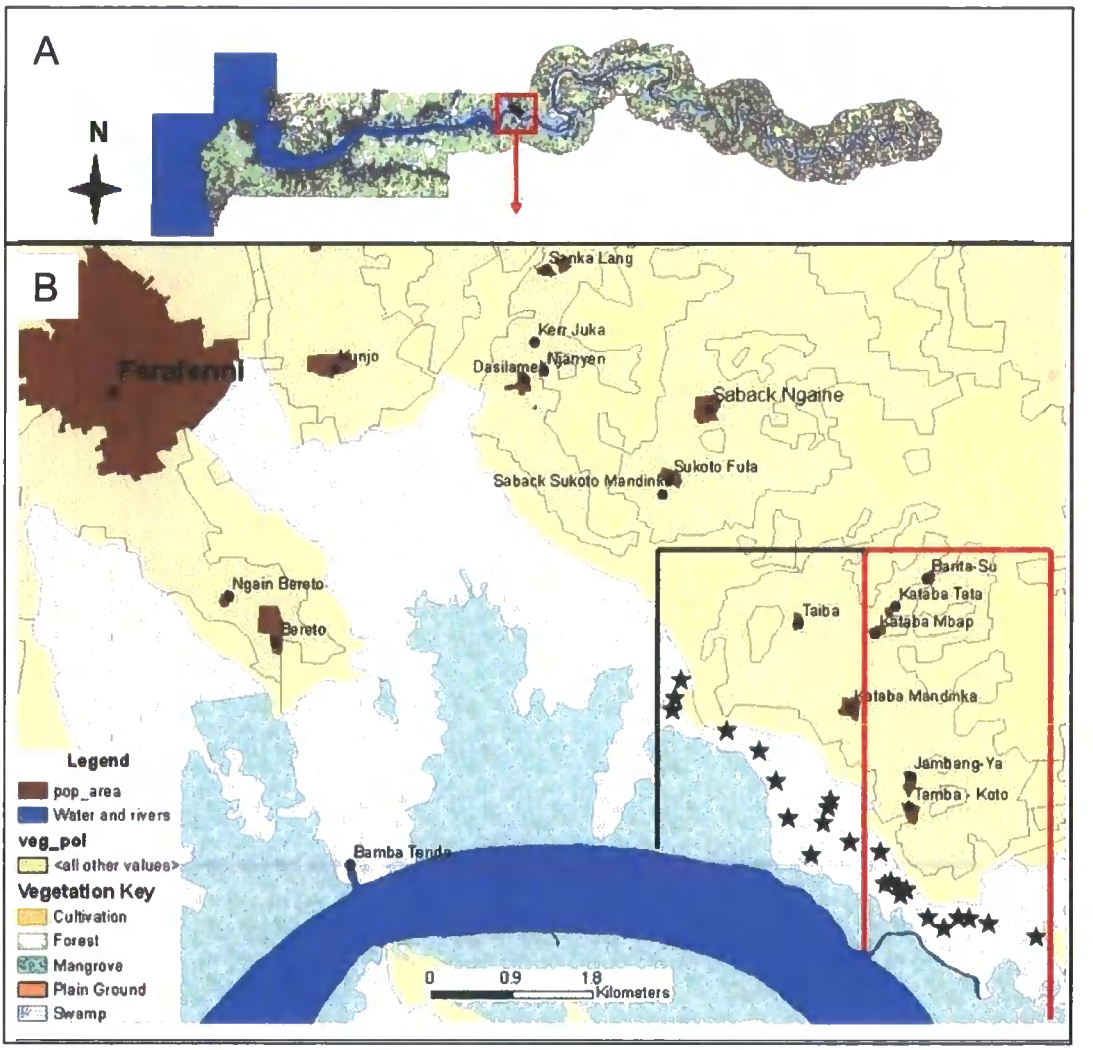

Figure 5.2 Map of The Gambia, West Africa (A) and the study area (B). The black line encloses the control, the red line the intervention area. The 24 sentinel sites for larval surveys are marked as stars.

Climate

Data on daily minimum and maximum temperatures were available from the meteorological station at Kaur 30 km from Farafenni town. Rainfall was collected with a rain gauge at the MRC station, Farafenni.

Larvicides

Water-dispersible granular formulations (WDG) of the commercial strains of *Bs* (VectoLex® strain 2362, Lot number 115-498-PG, 650 International Toxic Units, ITU/mg) and *Bti* (VectoBac® strain AM65-52; Lot number 114-114-32, 3000 ITU/mg; Valent BioSciences Corporation, Illinois, USA,) were tested in the laboratory and under field conditions, in a similar manner to that described by Fillinger *et al.* (Fillinger et al. 2003) in Kenya, in order to make direct comparisons between West and East Africa. WDG formulations were applied as liquid with handheld or knapsack sprayers. *Bs* (VectoLex®, Lot number 117-999-NB, 50 ITU/mg) and Bti (VectoBac®, Lot number 131-661-NB, 200 ITU/mg) corn granule (CG) for hand application or motorised granule spreaders was evaluated under field conditions only.

Laboratory assays

Laboratory assays were conducted to assess the susceptibility of the principal malaria vector in The Gambia, *An. gambiae s.s.*, to microbial larvicides. Laboratory assays were carried out with a colony of insectary-reared larvae originated derived from wild-caught mosquitoes collected from Saruja in The Gambia and maintained at the MRC Laboratory in Farafenni since 2002. All mosquito larvae used in the laboratory experiments were reared at a room temperature of 28°C, 80% relative humidity and an approximate 12 hour light : 12 hour dark cycle. Larvae were reared in transparent, 1.5 L capacity plastic containers (24x17x8 cm) filled with 1 L tap water that had been left in the insectary for at least 48 hours to equilibrate. Larvae were fed by adding a pinch of crushed Tetramin® (Tetra, Germany) fish food spread evenly on the water surface twice daily.

Assays were performed with the WDG formulation of VectoLex® and VectoBac® to determine their minimum effective dosages following the standard testing procedures for microbial tests (WHO 1999). Fifty third instar larvae were randomly collected for the experiment from several bowls to compensate for size differences that could have reflected the amount of food available (Koenraadt et al. 2004a) and transferred to new 1.5 L plastic containers filled with 1 L of the test solution or distilled water only (control). On every test date a fresh stock solution of 100 mg/l WDG was prepared and test aliquots made up to 1 L with distilled water. After range finding tests (WHO 1999), five to six different test concentrations were

chosen for each experiment. Test concentrations ranged between 0.001 and 0.1 p.p.m for *Bs* and between 0.001 and 0.016 p.p.m for *Bti*. Each experiment contained an untreated control. The experiment was run in three replicates at the same time and the entire experiment carried out on five occasions. Larvae were not fed during the experiments and all tests were run at ambient temperature ranging between 21 and 34°C. Larvae were counted and mortality scored after 24 hours. Where mortality exceeded 10% in the controls, the experiment was discarded and repeated.

Standardised field trials

Standardised field trials were conducted at the MRC field station in Farafenni during the rainy (September to October 2004) and dry season (December 2004 to May 2005) to identify the optimum dosages of *Bs* and *Bti* required under field conditions and to evaluate the residual effect and re-treatment intervals for the test microbials. Artificial ponds were created following the experimental design of Fillinger *et al.*(Fillinger et al. 2003). Eighteen light blue plastic tubs (0.5 m diameter) were buried into an open sunlit field in three lines of six tubs (distances between tubs was approximately 2 m). The tubs were filled with approximately 6 kg of top soil from the experimental area to provide the abiotic and biotic conditions suitable for mosquitoes. Tubs were filled with tap water and maintained at a depth of 0.4 m. Overflow holes were created at the 0.4 m level and screened with nylon netting to allow excess water to leave the tubs during heavy rainfall and prevent larvae from being washed over the edges. The habitats were left open for mosquito oviposition. Experiments were implemented eight to nine days after the tubs were set-up to allow third and fourth instar larvae to develop. Water temperatures during the experiments ranged between a minimum of 23°C and a maximum of 40°C. Acknowledging the hazard artificially created breeding sites present, all habitats were carefully screened for pupae twice daily with a dipper and visually and any pupae were removed to prevent the emergence of malaria vectors.

Of the 18 artificial habitats, six served as untreated controls and two treatments (six tubs each) were allocated to the remaining 12. Treatment and control ponds were selected randomly using the web-based randomisation tool at http://www.randomization.com. Treatment concentrations were calculated on the basis of a standard water depth of 0.1 m and fixed surface area (Ragoonanansingh et al. 1992, Schnetter et al. 1981) irrespective of the actual water depth to simulate operational procedures. Both microbial larvicides were tested in this set up at the

following concentrations: *Bs* WDG at 0.5, 1.0, 2.5 and 5 mg/l (0.5, 1, 2.5 and 5 kg/ha), and, *Bti* WDG at 0.2 mg/l (equivalent to a surface application of 0.2 kg/ha). Each concentration was tested in six habitats at a time and repeated once (i.e. 12 habitats in total). The first round of tests with *Bs* were implemented during the rainy season 2004 and replicated during the dry season 2005. *Bti* was tested during the cold dry season, in December, and repeated during the hot dry season in May (Figure 5.3). Liquid formulations were sprayed evenly over the entire water surface of the habitats using a 250 ml handheld sprayer. Each day the average number of larvae and pupae per dip (350ml capacity dipper, Clarke Mosquito Control Products, Illinois, USA) was determined by taking five dips from four different directions of each pond close to the edge and one from the middle. Mosquito larvae were classified as anophelines or culicines and recorded as early (1st and 2nd) or late (3rd and 4th) instars. After counting, larvae were returned to the water and pupae removed. Treatment was done once at day zero. The experiment was terminated when the difference between late instar and pupal density was no longer statistically significant between control and treatment tubs. A sub-sample of 69 *Anopheles* adults were allowed to emerge from pupae collected from the control and identified morphologically; rDNA-PCR markers were used for species determination of adults of the *An. gambiae* species complex (Scott et al. 1993b).

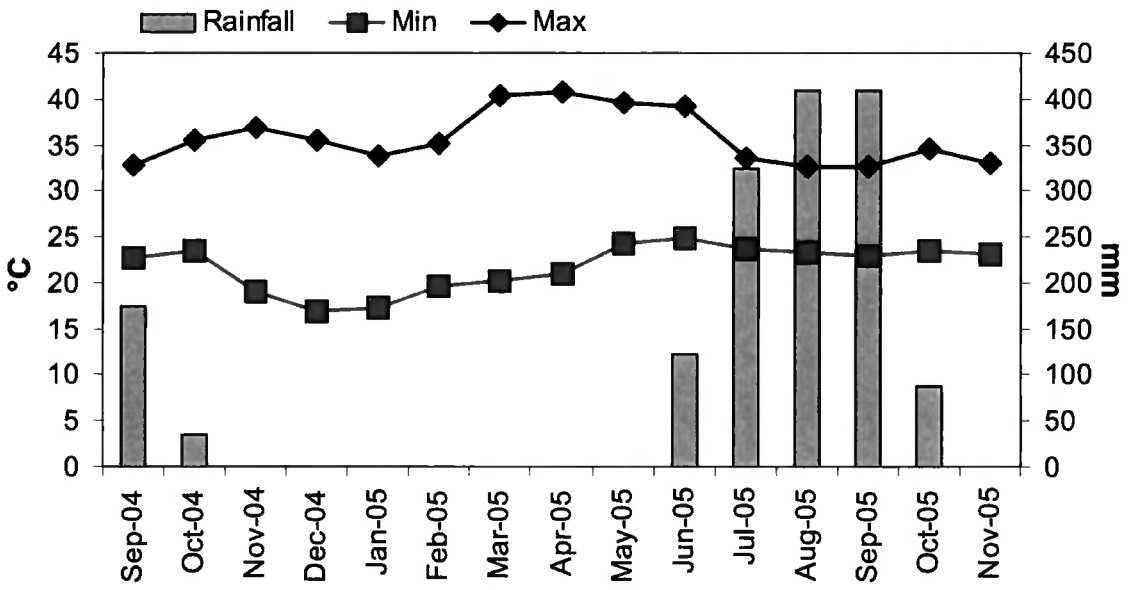

Figure 5.3 Temperature (^{0}C) and rainfall pattern (mm) during the study period.

Field trial

Based on the results from the laboratory and the standardised field tests a pilot-scale field operation was designed and implemented between August and November 2005 to test the efficiency and life span of the larvicides under natural conditions in representative habitat types in the floodplains of the River Gambia. The field tests served to identify (1) the operational requirements e.g. time needed per surface area treated, equipment and manpower needed, (2) the optimal microbial and (3) the best formulation in preparation for large-scale larviciding campaigns scheduled for the following rainy season 2006.

Liquid (WDG) and granule (CG) formulations of both *Bs* and *Bti* were tested. Liquids were applied using 5 L capacity compression sprayers (Mesto Resistent No. 3600, Freiberg, Germany) or 15 L capacity diaphragm knapsack sprayers (SOLO® 475, Sindelfingen, Germany). Both sprayers were operated at an average pressure of 4 bar. Corn granules were either applied by hand carrying the granules in a 5 L bucket on a carrying strap over the shoulder or were spread with 13 L backpack power chemical applicators (MD 150DX-13 Maruyama, Tokyo, Japan) covering a swath width of 10-15 metres.

The pilot zone was situated 10 km east of Farafenni and had an area of 24 km^2. The area included the major breeding habitats for anophelines in this region of The Gambia: extensive rice fields, pools that were people-made and natural, and large floodwater areas interspersed with grass. Notably, aquatic habitats harbouring anopheline larvae in the Gambia might be described as 'atypical' when compared with other parts of Africa. The habitats are water-fed primarily through the flooding of the river and are additionally under tidal influence leading to flooding and contraction of the habitats which are usually shallow but can be extensive in size and are probably fairly typical for many large rivers in the Sahel.

After mapping all aquatic habitats in the pilot zone the area was divided into an intervention and a non-intervention zone (Figure 5.2). In the intervention zone, 6 km^2 was routinely larvicided. In each zone 12 sentinel sites were randomly selected from the total list of habitats for measuring mosquito larval density. The sentinel sites were located in rice fields and floodwater habitats covered with grass and sedge. Larviciding was implemented under operational conditions by a team of four men from the National Malaria Control Programme who had undergone two weeks of training prior to the field trial. The monitoring of the intervention's impact in the 24 sentinel sites

was implemented independently by the research team. The larviciding teams were unaware of the location of the sentinel sites.

Bs treatments were applied at rates of 1 kg/ha for WDG and 15 kg/ha for CG; dosages proven to be effective from the standardised field trials and previous experiences (Fillinger et al. 2003, Fillinger and Lindsay 2006). *Bs* WDG was tested for two consecutive weeks and followed by *Bs* CG for one week. This allowed the authors to train larviciding staff how to use different application equipment and assess whether the two formulations performed differently under field conditions. Larval density was surveyed using the standard dipping technique (WHO 1992a). Ten dips were taken at each sentinel site to determine the larval density at the day of the first treatment (day 0) and at day two, four and seven after treatment for three consecutive weeks. Purposive sampling was done to maximise the sensitivity of collections. Re-treatments took place on a weekly basis if late instar larvae occurred at day four.

Following the *Bs* field test, operational application of *Bti* was evaluated at dosages of 4.0 kg/ha for CG for two weeks, followed by WDG applications of 0.2 kg/ha for seven weeks. Dosages were based on laboratory and field trial results and on previous studies (Fillinger et al. 2003, Fillinger and Lindsay 2006). During the application of *Bti* larval density was monitored once a week in the sentinel sites using the same methodology as described above. The monitoring was implemented one to three days after application. Due to the specific habitat characteristics in the tidal floodwater of The River Gambia the entire surface of all aquatic habitats was covered with larvicides.

Statistical analysis

LC_{50} and LC_{99} values were determined using log-probit regression analysis. The percentage reduction in larval mosquito densities in the standardised field trials was calculated using the formula of Mulla *et al.* (Mulla et al. 1971): % Reduction = 100 - $(C_1/T_1 \times T_2/C_2) \times 100$, where C_1 and C_2 describe the average number of larvae in the control tubs pre- and post-treatment, T_1 and T_2 describe the average number of larvae in the treated tubs pre- and post-treatment. The percent reduction shows for each day after treatment the proportion of larvae that have died compared to the previous day. Mean number of larvae and pupae per dip in control and treatment sites in field tests were compared using non-parametric Mann-Whitney tests. The tests were implemented separately for each sampling day comparing mean numbers of immature

stages in the controls with treatments. When multiple comparisons of more than one treatment and control were made the Bonferroni correction was used to define the alpha cut off value. The corrected significance levels are presented with the figures. All analyses were carried out using SPSS version 11.0.

Results

Climate

Figure 5.3 summarises average minimum and maximum temperatures and the monthly rainfall during the study period from September 2004 to November 2005. The dry season extended from November 2004 to May 2005 and can be portioned into a 'cold dry season', from November to February, and a 'hot dry season', from March to May. The rainy season is characterised by more constant temperatures with little difference between minimum and maximum values. Rain fell only once during the experiments on day 4 of the rainy season test of low Bs WDG dosages (Figure 5.5A), but did not appear to influence the results.

Laboratory assays

After 24 hours exposure of third instar larvae of *An. gambiae s.s.* to *Bs* WDG (VectoLex®, 650 BsITU/mg), a concentration of 0.004 mg/l (2.6 BsITU/l) caused 50% mortality (LC_{50}) and a concentration of 0.023 mg/l (14.9 BsITU/l) caused 95% mortality (LC_{95}). *Bti* WDG (VectoBac®, 3000 ITU/mg) concentrations of 0.039 mg/l (117 ITU/l) killed 50% of the larvae and 0.132 mg/l (396 ITU/l) 95% (Table 5.1).

Table 5.1 Laboratory bioassays results of *Bs* and *Bti* WDG against third instar larvae of *Anopheles gambiae s.s.* after 24 h exposure (lethal concentrations (LC) in p.p.m.)

WDG Formulations	LC_{50} (95% CI)	LC_{95} (95% CI)	Slope (SE)	c^2 (d.f.)
VectoLex	0.004	0.023	2.208	123.518
(650 BsITU/mg)	(0.003<LC<0.005)	(0.016<LC<0.042)	(0.112)	(25)
VectoBac	0.039	0.132	3.110	140.513
(3000 ITU/mg)	(0.033<LC<0.047)	(0.100<LC<0.199)	(0.141)	(23)

CI, confidence interval; SE, standard error; d.f., degrees of freedom

Standardised field trials

Throughout the year, oviposition occurred soon after the artificial habitats were set up and immature stages of anopheline and culicine mosquitoes detected after four to five days. Overall *Anopheles* larvae accounted for 40% of larvae collected during the trials. 69 *Anopheles* adults that emerged from pupae collected from the control tubs were identified to species level. 36 *Anopheles* adults belonged to the *An. gambiae s.l.* species complex and PCR analyses revealed that the tubs contained a mix of *An. arabiensis* (66%), *An. gambiae* s.s. (30%) and *An. melas* (4%). Since there were no differences of the impact of the larvicides on anophelines and culicines in the standardised field trials the data were pooled for all analyses and presentation.

***Bti* WDG** Field trials with *Bti* WDG were implemented with the minimum dosage (Becker and Rettich 1994) required to cause 100% mortality within 24-48 hours after application as identified in the laboratory assays. Since no improvement of the impact or activation of any residual effect was expected (Charles and Nielsen-LeRoux 2000) higher dosages were not tested after the minimum dosage of 0.2 kg/ha (10 times the LC_{95} to accommodate for differences between laboratory and field conditions) under standardised field conditions killed all larvae within 48 hours and provided therefore optimum control for the period of one week (Figure 5.4 and Table 5.2). Although reduced late instar densities were recorded up to eight to ten days after application (Table 5.2) these differences were only statistically significant up to day five (Figure 5.4) in both test periods. Late instar larvae and pupae developed in increasing numbers five to six days after *Bti* application. The seasons had little impact on the outcome of the trials.

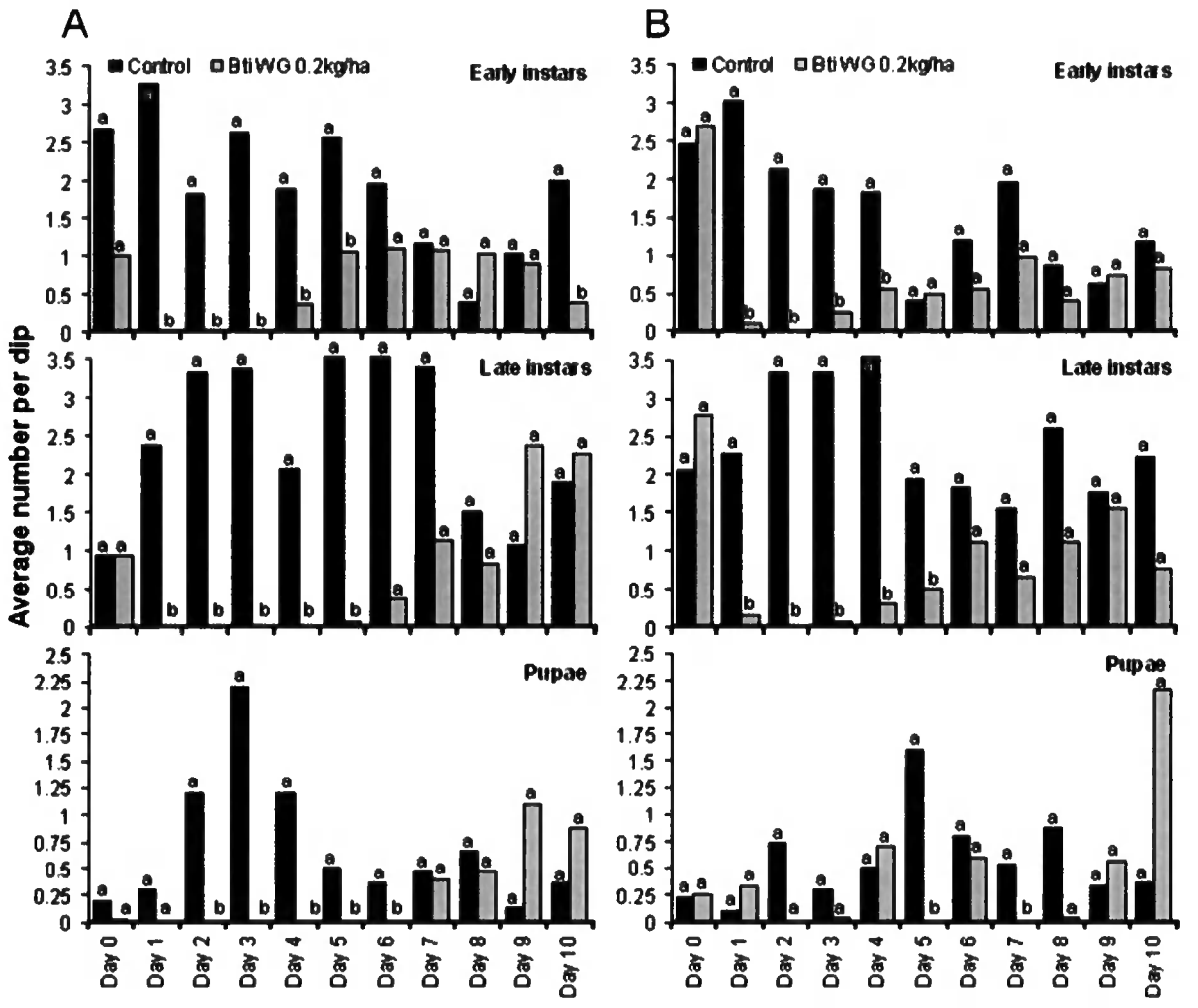

Figure 5.4 Impact of *Bti* WDG at 0.2kg/ha on early and late larval and pupae density in standardised field tests. A: during cold dry season (Dec); B: during hot dry season (May). Daily differences in immature densities were analysed using Mann-Whitney tests at a significance level of $p < 0.05$. Different letters (a,b) on top of bars indicate a significant difference at the specific sampling day.

Table 5.2 Percent reduction (%) of late instar larvae (*Anopheles* and culicines combined) after application of *Bti* WDG at 0.2 kg/ha in the cold (Dec) and hot (May) dry season

Day after application	Cold dry season	Hot dry season
1	100	95
2	100	100
3	100	95
4	100	94
5	98	81
6	90	54
7	67	68
8	45	68
9	0	33
10	0	74

Notably, pupal production could not be completely suppressed despite the well-controlled implementation of the experiment, although pupal production was more successfully suppressed during the cold than the hot dry season. The results indicate that weekly treatment intervals can reduce pupae production by 64-94%. A recent study though showed that higher rates of the WDG formulation of *Bti* may produce longer control since the WDG particles redistribute throughout the water column after application (S. Krause, personal communication) and, therefore, the effect of higher *Bti* WDG rates on field residual control of *An. gambiae* requires further study.

***Bs* WDG** Four different doses of *Bs* WDG were tested (0.5, 1.0, 2.5 and 5.0 kg/ha) and each experiment run twice to evaluate whether any residual effect of the larvicide could be detected which would allow extended re-treatment intervals. The results of the impact of the different dosages are presented in Figure 5.5 and 5.6. The results are shown separately for the replicates implemented during the rainy (A) and the dry season (B). The daily percent reduction of late instar larvae is summarised in Table 5.3.

Irrespective of dosage and season 96-100% larval mortality was achieved 24-48 hours after application. No residual effect of a single *Bs* application was detected during the rainy season at any application rate tested but was extended during the dry season for all tested dosages (Figure 5.5 and 5.6, Table 5.3). Statistically significant reductions in pupae densities were achieved up to five days post-treatment in the rainy season and up to 10 days during the dry season. There were no statistically significant differences between the different test concentrations (Figure 5.5 and 5.6). Consequently, pupae development could be reduced by over 95% when *Bs* WDG was applied at weekly intervals. During the dry season similar suppression of pupae densities could be achieved at 10-day re-treatment intervals.

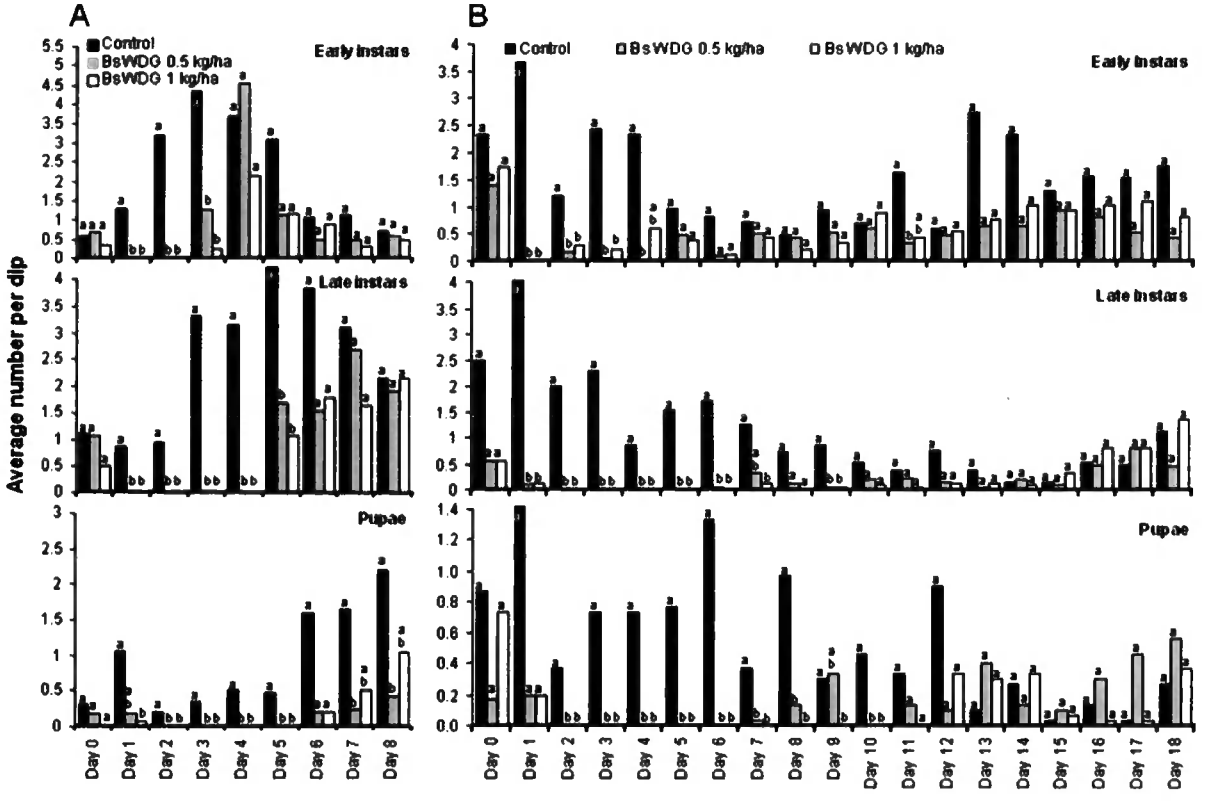

Figure 5.5 Impact of low dosages of *Bs* WDG (0.5 and 1 kg/ha) on immature mosquito density in standardised field tests. A: during rainy season; B: during dry season. Daily differences in immature densities were analysed using Mann-Whitney tests at a significance level of $p < 0.017$. Different letters (a, b) on top of bars indicate a significant difference at the specific sampling day.

Table 5.3 Percent reduction (%) of late instar larvae (*Anopheles* and culicines combined) after application of *Bs* WDG in different dosages in the dry and rainy season

	Rainy season				Dry season			
Day after application	0.5 kg/ha	1.0 kg/ha	2.5 kg/ha	5.0 kg/ha	0.5 kg/ha	1.0 kg/ha	2.5 kg/ha	5.0 kg/ha
1	100	100	100	100	96	96	100	100
2	100	100	96	100	100	100	100	95
3	100	100	89	68	100	100	100	100
4	100	100	68	85	100	100	100	100
5	60	44	52	55	100	100	88	100
6	59	0	86	69	76	100	96	60
7	11	0	62	2	4	68	89	84
8	9	0	74	0	40	100	91	94
9	-	-	70	1	54	54	58	97
10	-	-	36	0	0	17	60	86
11	-	-	-	-	0	0	4	75
12	-	-	-	-	0	48	0	70
13	-	-	-	-	0	0	0	54
14	-	-	-	-	0	0	-	66
15	-	-	-	-	0	0	-	-
16	-	-	-	-	0	0	-	-
17	-	-	-	-	0	0	-	-
18	-	-	-	-	0	0	-	-

-, test was terminated earlier

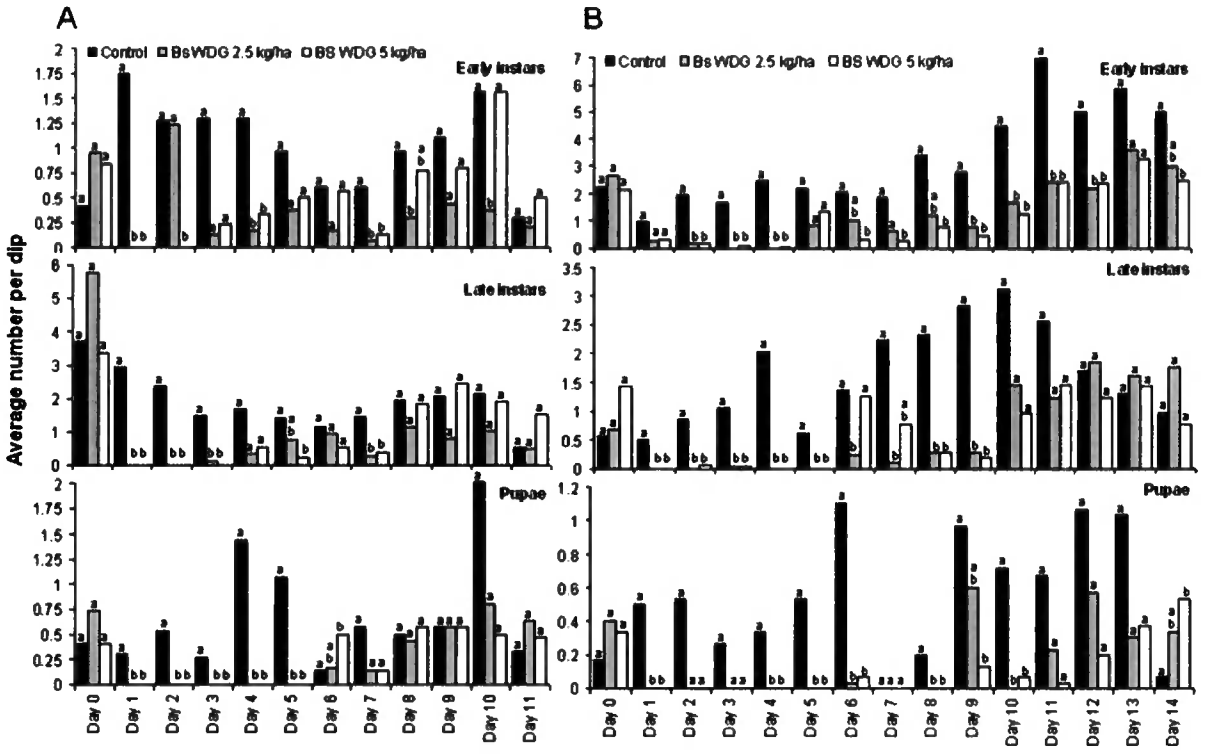

Figure 5.6 Impact of high dosages of *Bs* WDG (2.5 and 5 kg/ha) on immature mosquito density in standardised field tests. A: during rainy season; B: during dry season. Daily differences in immature densities were analysed using Mann-Whitney tests at a significance level of $p<0.017$. Different letters (a, b) on bars indicate a significant difference at the specific sampling day.

Field trial

Field trials were conducted in the floodplains of the River Gambia to confirm results from standardised field set up and to evaluate the effect of larviciding under operational conditions. The field tests were implemented during the rainy season which is the main malaria transmission season in The Gambia and the period with most larval habitats. At the start of the field trials late instar *Anopheles* larvae were found in 33% of all sentinel sites. The proportion of habitats with late instar *Anopheles* increased in the non-intervention sites with continuous rainfall to 67% in October 2005. Culicine and anopheline larvae co-existed in most of the habitats and did not show any difference in response to the larviciding. Both sub-families have, therefore, been pooled for presentation and analyses.

Bs WDG and CG formulations were evaluated to detect any residual effect of the microbial under operational application in the field. Application took place at

weekly intervals to evaluate whether continuous application might result in an increasing residual effect with time. The results of the three week trial are presented in Figure 5.7. 100% mortality of late instar larvae was achieved two days post-treatment at any application date irrespective of the formulation applied. A residual effect of the microbial which would allow re-treatment intervals greater than one week was not detected (Figure 5.7), which supports the results from the standardised field trials. Weekly application of *Bti* under operational conditions (Figure 5.8) achieved a consistent suppression of larval development over the entire nine weeks study period with minimum dosages (as identified in laboratory) irrespective of the formulation and equipment used. Surprisingly, pupae were not collected under field conditions in either the intervention or control sites and this was unexpected in the control sites. It is likely that pupae in the area are very hard to sample or are extremely rare.

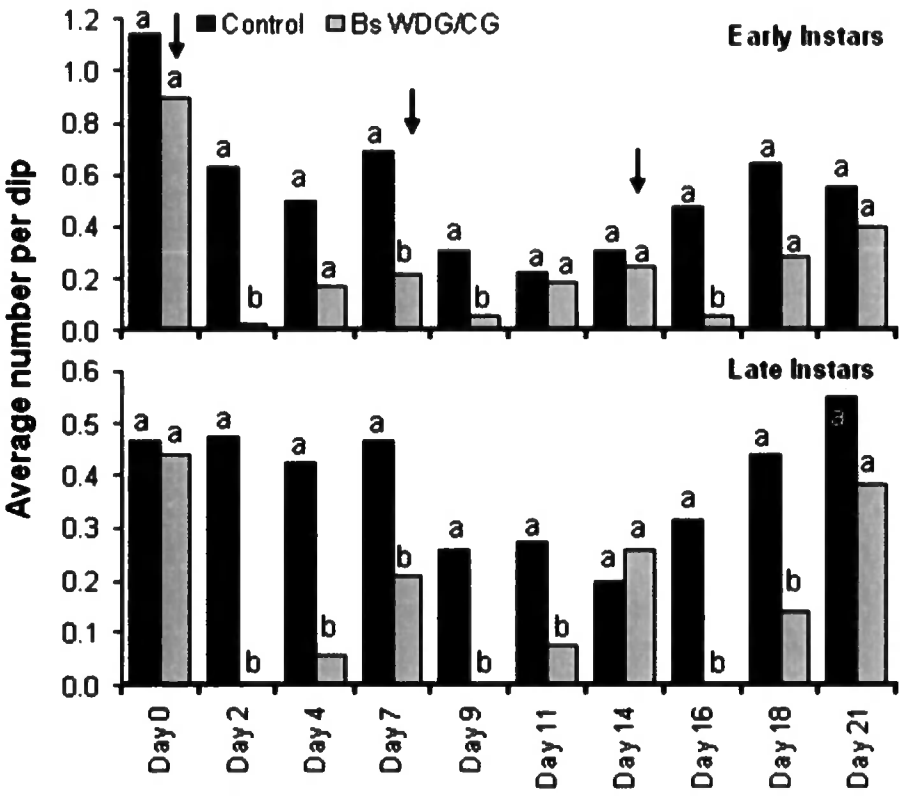

Figure 5.7 Efficiency of *Bs* treatments under operational field conditions.

Bs application took place on day 0, day 7 and day 14 (arrows). WDG formulation was applied on day 0 and 7; CG formulation was applied on day 14. Differences in immature densities were analysed using Mann-Whitney tests at a significance level of

p<0.05. Different letters (a, b) on bars indicate a significant difference at the specific sampling date.

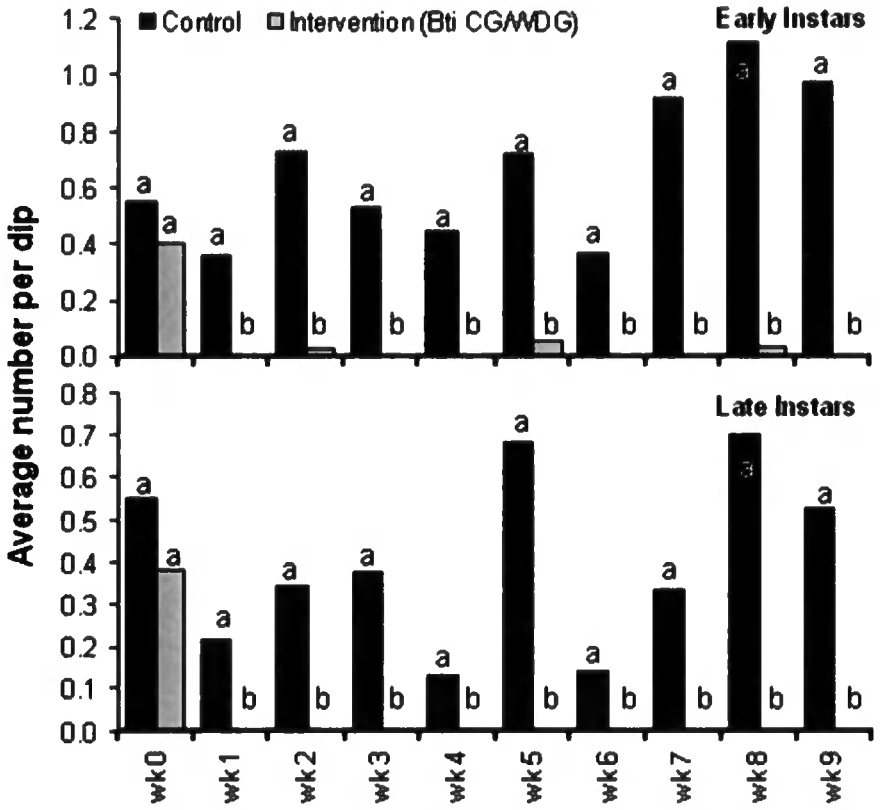

Figure 5.8 Efficiency of *Bti* treatments under operational field conditions. *Bti* application took place weekly. The monitoring of the sentinel sites was done 1-3 days after application. CG formulation was applied in week (wk) 1, 2 and 3, WDG formulation was applied from week 4. Differences in immature densities were analysed using Mann-Whitney tests at a significance level of p<0.05. Different letters (a, b) on bars indicate a significant difference at the specific sampling date.

Larvicides were applied by four men from 7:00 to 13:00 (6 hours) a day. While staff worked continuously to cover the entire study area, each habitat was spayed only once a week. All formulations could be successfully applied under operational conditions and were equally effective. Different application equipment though had an impact on the time required per surface area treated. On average seven hectares were treated per day (0.29 ha/person/hour) using 5 L compression sprayers or 13 L motorised granule spreaders; nine hectares were covered using 15 L knapsack sprayers (0.38 ha/person/hour) and 5 hectares when granules were applied by hand (0.21 ha/person/hour).

Discussion

The results show that the major malaria vectors in The Gambia are highly susceptible to *Bs* and *Bti* under laboratory and field conditions, with *Bs* even more toxic per weight applied than *Bti*. The LC values found in the laboratory experiments are very similar to those found in earlier studies (Fillinger et al. 2003, Seyoum and Abate 1997) conducted in East Africa suggesting that the susceptibility of malaria vectors to microbial larvicides is inherent to the species and not to the ecological settings of the area. *Bs* has shown residual activity for two to 10 weeks in previous studies (Hougard 1990, Lago et al. 1991, Mulla et al. 1999, Sutherland et al. 1989), with repeated applications increasing the likelihood of greater residual activity (Fillinger and Lindsay 2006, Karch et al. 1990). Larvicides with long residual activity would be advantageous for larviciding campaigns because less manpower and larvicide would be required, helping to keep down costs and increase effectiveness. However, in contrast to previous results *Bs* did not show extended residual effect under field conditions in The Gambia even after repeated treatments and when the application rate was as high as 200 times the LC_{95} (5kg/ha). Only a slightly extended residual effect up to 10 days could be detected in the standardised field tests implemented during the dry season (January to March) but not the wet season. It can be hypothesised that the different daily water temperature profile in the experimental tubs during the rainy season might be responsible for the reduced effect of the microbial. It has been observed in a study done in Kenya that the daily temperature in small pools is correlated with the amount of daily rainfall (Paaijmans et al 2008 b).

Although the average air temperatures did not differ between the experimental periods in the rainy and dry seasons the low variation between minimum and maximum temperatures during the rainy season (Figure 5.3) will have resulted in high water temperatures for longer during the rains compared with the dry season. High water temperatures result in faster destruction of the protein toxin (Rojas et al. 2001). The low residual activity could also be due to the low larval density observed in the artificial and natural habitats. *Bs* seems to persist or recycle in some environments because it rapidly increases its numbers in the midgut of killed larvae (Becker et al. 1995, Charles and Nicolas 1986, Skovmand and Bauduin 1997). Where larval densities were high the residual activity of the microbial larvicides appears to be greater (Pantuwatana and Sattabongkot 1990, Skovmand and Sanogo 1999). Dead and dying

larvae release the bacteria into the water increasing the bacterial content of the water and infecting new generations of larvae.

The presence and abundance of pupae can serve as a proxy measure for adult mosquito emergence, since pupae survive for only a few days before adult emergence. The identification of the most productive habitats for adults could help target larviciding operations especially in the extensive water surface areas of the river's floodplains. In this study no pupae were collected in the field during the pilot field tests. This unexpected finding may be a consequence of the dipping technique. Although the technique is commonly used for studying larval ecology in Africa, it appears to be inappropriate for sampling the very sensitive and agile pupae from natural aquatic habitats, particularly in The Gambia where larval densities are generally low consequently leading to even lower pupae densities. This insensitivity of the sampling technique is further compounded by the highly aggregated distribution of pupae in natural habitats compared to larvae (Robert et al. 2002, Service 1971, WHO 1992a). Even in the tubs dipping underestimated the density of pupae. Sweep nets may prove to be a better sampling tool for larvae and pupae since they collect 10 times more pupae in fewer sweeps than dips (Robert et al. 2002). Since pupal abundance is often used for establishing the 'productivity' of habitats (Mutuku et al. 2006b) further studies to develop more efficient sampling protocols are desirable. However , the absence of pupae in the control area cannot be attributed entirely to the weakness of the sampling tool since the same dippers successfully sampled pupae (Chapter 2). Other causes not investigated in this study might explain the absence of pupae.

At the end of the wet season most sites dry up quickly leaving only a few dry season refugia (Chapter 2 and 3). For these dry season sites a targeted application of *Bs* might be useful to suppress the build up of the adult population at the start of the rains. The results indicate that with commercially available microbials weekly larviciding will be necessary during the rainy season in The Gambia. In this instance the use of *Bti* products is preferred since the costs for this microbial are far lower than *Bs* (Fillinger and Lindsay 2006) and the development of resistance is unlikely(Charles and Nielsen-LeRoux 2000, Mulla et al. 2003, Zahiri et al. 2002). Very low dosages of 0.2 kg/ha (representing the LC_{99}) lead to optimal suppression of mosquito larvae and pupae which is consistent with results from East Africa (Fillinger et al. 2003, Fillinger and Lindsay 2006).

Granule and liquid formulations have proven equally effective in killing mosquito larvae but the selection of application equipment was important for the speed of coverage. The 5 L compression sprayers were easier to carry than the higher capacity sprayers, but they were slower to use because they needed to be refilled more frequently. This was exacerbated by the fact that most water bodies in the floodplains of The Gambia are shallow and muddy, and, therefore, unsuitable for water collection, which led to long distances being covered to re-fill the sprayers. Another disadvantage of the compression sprayers was that they have to be pressurised by pumping air into the tank before spraying which proved difficult on the muddy ground in the floodplains. The problem of finding suitable water sources for mixing WDG formulations and the increasing plant growth during the rainy season favours the application of granule formulations in an environment like the river floodplains.

Motorised granule spreaders provided excellent coverage and proved especially useful in areas with tall vegetation where access on foot is impossible. However, when filled with the microbials they weighed close to 20 kg and walking on soft ground became difficult and, coupled with the loud noise of the engine, made them uncomfortable for long-term use. The relatively high purchase and running costs of motorised spreaders (approximately USD300/spreader plus fuel costs) compared to knapsack sprayers (approximately USD100/sprayer) represent another disadvantage in resource poor African settings. Based on the pilot field trial, it is recommended for a large-scale larviciding programme in The Gambia to use 15 L knapsack sprayers for all large, open water surface areas, and to use granule formulations for highly vegetated areas. Hand application is the preferred method for larvicide application because it represents a low-tech and low-cost technology. Even though granule distribution does not result in an even application, as that achieved with motorised sprayers, it is easily manageable and maintenance free. Nevertheless, motorised sprayers must be used where tall vegetation dominates or access on foot is impossible due to high water level or soft underground.

The basic training of larviciding personnel in identification of habitats, calibration of application equipment and active larviciding proved to be successful and achieved full coverage and control of mosquitoes for three months under fully operational conditions. To reduce labour and management effort it would be desirable to have larvicides which would express extended efficiency under extreme climate

conditions. Microbial larvicides were chosen in this study because, in contrast to many other larval control agents, they exhibit the highest environmental safety to non-target organisms and application personnel, they are very easy to handle and are unlikely to lead to the development of resistance (Becker and Ludwig 1993, Charles and Nielsen-LeRoux 2000, Mulla et al. 2003, Zahiri et al. 2002). Nevertheless, it would be useful to explore whether greater persistence could be achieved with alternative products.

Organophosphates, like temephos, appear to be less useful since in some field studies they did not show much persistence compared with microbials (Rozendaal 1997, Shililu et al. 2003). Moreover, organophosphates can have a negative impact on non-target organisms (Anonymous 2007b, Pinkney et al. 2000) and need careful resistance management. On the other hand, the use of insect growth regulators (IGRs), like pyriproxyfen, might prove more advantageous (Yapabandara and Curtis 2004, Yapabandara and Curtis 2002, Yapabandara et al. 2001). IGRs have been highly successful elsewhere when applied at monthly intervals, although this was usually administered in highly confined habitats (Seng et al. 2006, Yapabandara et al. 2001). Whether this residual effect could be replicated in a highly mobile aquatic environment like the floodplains of The Gambia needs careful evaluation. Some of the advantages of pyriproxyfen are its photostability, which prevents it being degraded by ultraviolet light, and it can remain in waterbodies, without being degraded, for many months (Blagburn and Ann Ball 2007). The greatest disadvantage of IGRs though is the difficulty in monitoring whether they are still effective or not since larvae will always be detected in the water and the development and emergence of pupae needs to be observed, which represents a challenge given the difficulty of collecting any pupae. Moreover complicated monitoring systems using emergence cages or similar devices might not be easy to handle in a large-scale operational programme. The impact of IGRs will then have to be monitored in adult mosquito populations but the weakness of this monitoring system is that it will take longer (at least 1-2 weeks) to spot failure in the field applications and it would be too late to make the necessary changes in the field.

In conclusion the results reported here support the hypothesis that the implementation of large-scale larviciding with commercially available microbials in The Gambia will lead to a reduction in larval abundance in the natural habitats. Both microbial strains tested, can be applied successfully in extended floodplain areas either

as liquid with knapsack sprayers or as granules by hand and motorised sprayers. Due to the lack of residual effect of *Bs* products, *Bti* should be applied weekly during the rainy season. Dry season refugia should be targeted with bi-monthly *Bs* applications.

Environmentally safe microbial larvicides could be an additional tool in an IVM programme in The Gambia but due to the lack of residual effect of the microbial larvicides, there is a need to assess the costs of weekly applications in consideration of reduction in transmission intensity.

Chapter 6

Impact of larviciding on malaria vectors in The Gambia

Figure 6.1 Spraymen applying *Bti* in open water habitats

Impact of larviciding on malaria vectors in The Gambia

Abstract

Background There is growing interest in the use of anti-larval measures as a component of integrated vector management for malaria control in Africa. The application of microbial larvicides in rural areas which experience large-scale seasonal flooding and produce extensive areas of potential breeding sites for mosquitoes has not been investigated in Africa. This study aims to assess the impact of microbial larvicides on malaria transmission in the middle reaches of the Gambia River.

Method A cross-over study was carried out in four zones, each approximately 100 km^2. Baseline data were collected in 2005, whilst in 2006 *Bacillus thuringiensis* var. *israeliensis* (*Bti*, Vectobac) was applied routinely to two zones during the main period of transmission (June to November). In 2007 microbial larvicides were applied only to those zones that were untreated the previous year. Routine larval surveys were carried out using dippers and collections of adult mosquitoes made using CDC light traps.

Results Larviciding was associated with a 92 % reduction ($p < 0.001$) in the likelihood of finding water bodies colonised by late stage anophelines and culicines. Similarly, late stage larval density was reduced by 91 % ($p < 0.001$) in anophelines and 72 % ($p < 0.001$) in culicines in treated areas. Larviciding was associated with a 28% ($p = 0.005$) reduction in the number of adult female *Anopheles gambiae s.l.* found indoors in all zones, and a 42 % ($p < 0.001$) reduction when zone 4 was excluded. No significant reduction in adult culicines was observed.

Interpretation Despite successful control of larvae the reduction of adult malaria vectors was unsatisfactory for an operational programme with a high demand in resources. This relatively small reduction in adult mosquitoes may have been due to overspill of vectors from untreated areas outside the treated zones or from sites within the zones that were missed or impractical to treat. Ground application of larvicides by teams of spraymen in areas of extensive flooding cannot be recommended for malaria control in The Gambia.

Introduction

The realisation that successful malaria control and prevention cannot rely on a single tool and the need for evidence-based vector control methods has prompted the World Health Organisation (WHO) to promote a global framework for integrated vector management (IVM) (WHO 2004). This type of programme seeks to use a combination of control measures in order to achieve greater control than could be achieved by using only one intervention. Control measures such as the use of long-lasting insecticide treated nets and indoor residual spraying are effective tools for malaria control and are the mainstay of vector control in sub-Saharan Africa (SSA). However the increasing threat of malaria vectors resistance against pyrethroids in Africa (Awolola et al. 2002, Chandre et al. 1999, Hargreaves et al. 2000, Stump et al. 2004) and the capacity of adult mosquitoes to avoid control interventions (Killeen et al. 2002b) has prompted the need to also attack the larval stages of mosquitoes. This has lead to a renewed interest in the use of larval source management (LSM) for inclusion in IVM programs for malaria control in Africa. Larval control works well in urban areas with high population densities and relatively fewer breeding sites for vectors (Keiser et al. 2004, Walker and Lynch 2007) and in rural areas where breeding sites are aggregated (Fillinger and Lindsay 2006, Shililu et al. 2003).

Over the past five years a series of pilot studies have been undertaken to investigate the efficacy of LSM in different biomes in SSA: in urban areas (Fillinger et al. 2008), rural townships (Fillinger and Lindsay 2006), highlands (Fillinger et al in prep), and semi-arid (Shililu et al. 2007) ecosystems. Here the efficacy of LSM in an area of intense seasonal transmission was tested, where extensive anopheline breeding sites are formed in the floodplain of the River Gambia during the rainy season (Bøgh et al. 2003) as observed in Chapter 2.

The river is brackish up to 200 km from its mouth (Bøgh et al. 2003) due to salt intrusion from the Atlantic Ocean but during the rainy season fresh water dilutes the river. The flooding caused by the Gambia River has created a well defined floodplain where large expansive water bodies are under the influence of the tides. Upland areas are sandy and have fewer breeding sites, but these may well be important for malaria transmission, since they are closer to the villages (Chapter 2).

The floodplain area is extensive and includes a number of different types of habitats that are interconnected and ill-defined. However most of these extensive areas are accessible around their edges where mosquito larvae are usually found (Chapter 2),

(Bøgh et al. 2003) therefore making it possible to control those (Chapter 2). Here mosquitoes are found in low densities, but occur over extensive areas. Since these habitats would be difficult to refill or alter in order to eliminate standing water, larviciding is an appealing method for reducing larvae in this setting. Previous studies showed that commercial microbial larvicide *Bacillus thuringiensis* var. *israelensis* (*Bti*) were effective in killing mosquito larvae under field conditions in rural Gambia (Chapter 4).

Malaria transmission is highly seasonal in The Gambia (Thomson et al. 1999) being confined largely to the rainy season which lasts from June to October. Since the density of mosquitoes is greatly reduced during the dry season, larval control was targeted by starting the application of microbials at the end of the dry season and continued weekly application throughout the rainy season. It was unfeasible to target specific habitats since anopheline larvae were found in a wide range of different water bodies (Chapter 2). Moreover, since this was an operational programme, it was important to keep instructions simple for the spray teams with limited training in larval ecology. For both reasons it was considered that blanket coverage of all potential breeding habitats was most appropriate in this setting.

The aim of this study was to assess the impact of larval control with microbial larvicide *Bti* on the aquatic mosquito populations and on adult mosquito populations in rural Gambia.

Methods

Study area

The study was carried out east of Farafenni town (UTM zone 28 1500200mN, 435500mE) in The Gambia from May 2005 to November 2007. Four zones, each approximately 100 km^2 in area, were selected two on the north bank and two on the south bank of River Gambia. Each zone was 12 km wide and was divided in 3 subzones: a 4 km wide central band and 4 km wide buffer zones either side of the central band (Figure 6.2). Sampling of adult mosquitoes was confined to villages located in the central band. The 4 km buffer zones were thought to be sufficiently wide to prevent overspill of mosquitoes from untreated sites into treated zones, since earlier studies in the area showed that only few mosquitoes fly further (Bøgh et al. 2007, Lindsay et al. 1991a). The study area has been extensively described elsewhere

(Chapter 2). All study villages within the study zone were located between one and eight kilometres from the River Gambia.

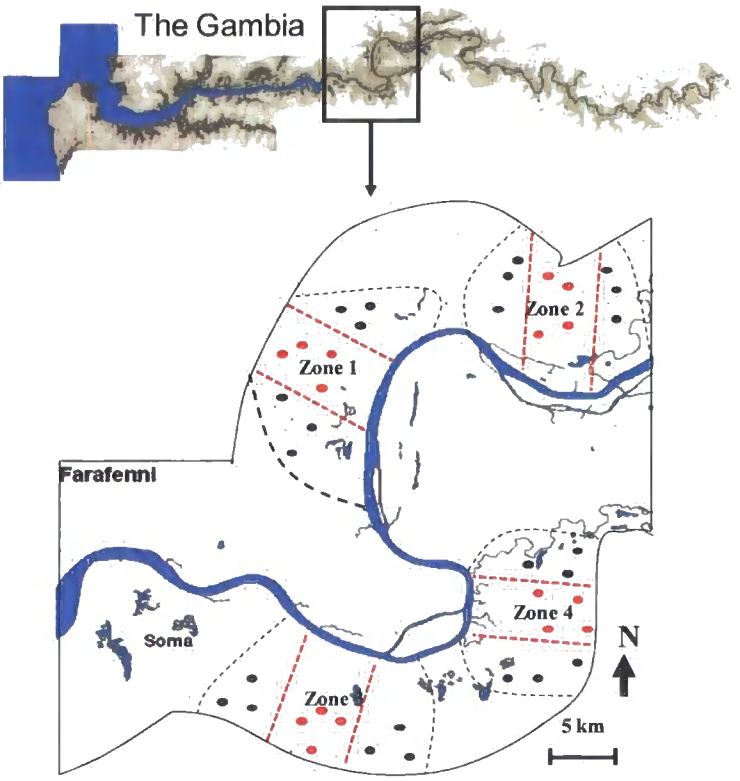

Figure 6.2 Map of study area. Red dotted lines sketch the zonal subdivision and study villages are shown in red plain circles and other villages in plain black circles.

Study design

A cross-over design was used for applying larvicides. Baseline data were collected in 2005 when no larvicides were applied and operational larviciding covered the rainy seasons for two consecutive years (2006-2007). In 2006 larvicides were applied in zones 1 and 3. These zones were selected since they were close to Farafenni station which allowed easier access and planning and helped to build up experience of running a larviciding programme. In 2007 zones 2 and 4 were treated and zones 1 and 3 left untreated.

Larval distribution

During the baseline year all water bodies in each zone were continuously sampled from May 2005 to November 2006 using a 350 ml dipper (Clarke Mosquito Control Products, Illinois, USA). In each water body 10 dips were done purposively in parts of

the water body most likely to harbour mosquito larvae, such as the edges around tufts of vegetation or floating debris in shallow water bodies. During the intervention years (2006 and 2007) sampling for presence of mosquito larvae was done daily in 40 sites randomly selected out of 1076 total sites mapped in the four zones before the larviciding period. In the zones where larviciding was ongoing these sites were not known to the spraymen and served as sites to monitor whether the larviciding team had successfully covered all water bodies. In very few cases where sites were found containing late instar larvae because they had been missed, spraymen were alerted and the sites were immediately treated. The random selection of sites for spot-checking was stratified in such a way that sampling would evenly cover alternatively all the three subzones in each zone within a week.

Larval density

After four months of mapping all water bodies in the area, 10 sentinel sites were randomly selected from each zone. These sites represented the most common habitats in each zone; where larval density was measured weekly. In these sites purposive sampling was done by making 10 dips in areas likely to harbour mosquito larvae with a dipper and the number of anopheline and culicine larvae and pupae recorded. Late instar anopheline larvae and all pupae were stored in 98% ethanol and brought back to the laboratory for species identification with PCR (Scott et al. 1993b). Larval sampling during the dry season was scaled down to all field teams to take their annual leaves. In this thesis only rainy season data is reported for larvae.

Adult sampling

Sentinel houses were randomly selected proportional to population size with one to three traps per village in each central subzone where adult collections were made. During the rainy season sampling was done every fortnight and every month during the dry season. Houses with open eaves, thatch roof, no ceiling and where a single person slept were selected. Mosquitoes were sampled using standard miniature CDC light traps (Model 512; John W. Hock Company, Gainesville, Florida, USA) positioned one meter above the floor at the foot end of the bed next to a man sleeping under an untreated bed net. Traps were set at 7:00 pm and collected at 7:00 am the following morning. When room occupants moved house the traps were moved to the nearest similar house in the same village. When occupants did not spend a night in the

selected room or when the trap was faulty these data were excluded from the analysis. Mosquitoes were taken to the laboratory for species identification and were scored as blood fed, unfed, gravid or semi-gravid *An. gambiae s.l.* females, *An. gambiae* males, other *Anopheles* females and males, and other nuisance mosquitoes. Blood fed *An. gambiae s.l.* females were subsequently subjected to an ELISA analysis (Burkot et al. 1984) for sporozoite rates identification.

Larviciding

Microbial larvicide *Bacillus thuringiensis* var. *israelensis* (*Bti*, VectoBac®, Valent Biosciences Corporation, Illinois, USA) was applied weekly from June to November in 2006 and May to November in 2007. Where the vegetation was low ($\leq$30 cm) and more than 50% of water surface could be seen through the vegetation, dissolved water dispersible granular (WDG) formulation of *Bti* was used at a dosage of 0.2 kg/ha. Spraying was done using 15 L capacity diaphragm knapsack sprayers (Solo 475, Sindelfingen, Germany) for WDG formulations. Where the vegetation was more than knee-high and covering more than 50% of the water surface, granular formulations (CG) were applied by hand using 5 L buckets held with a strap around the waist or neck. In areas where access on foot was difficult or when there was need to spread the granules at a larger distance, 13 L capacity motorised sprayers (MD 150DX-13 Maruyama, Tokyo, Japan) were used (Chapter 4).

Microbial larvicides were stored in three houses in the three subzones of each intervention zone. After calibrating the pace of spraymen and the knapsack output, WDG formulations were divided into 286g sachets, that were easy to carry, and mixed with 15 L of water in a knapsack and stirred to ensure complete dispersal. Each morning spraymen were supplied with enough *Bti* to last the day and were dispatched to the intervention area. Teams of three to four spraymen walked abreast 8m apart. Each sprayman covered a 180° swath in front as he walked while spraying from the beginning of a water body to the end of it or until progress was impossible in deep water bodies close to the river or its major tributaries. Once the spraying mixture was exhausted, a new mixture was made from the microbial aliquot mixed with water from the field. Flags were used as landmarks to help spraymen remember where spraying had stopped on a previous visit. Any previously mapped or new water body reached by spraymen was treated on a weekly basis.

Statistical analysis

The proportion of sites colonised by anophelines and the mean number of mosquito larvae and adults was calculated for each zone using the Generalised Estimating Equations (GEE) by running the model without calculating the intercept. The model returned the mean of the dependent variable for each variable used as a predictor. The impact of larviciding on presence and density of mosquito larvae was assessed using GEE after adjusting for the zone and year. The habitat identity (ID) was used as subject unit for repeated measures assuming an exchangeable correlation matrix. The impact of larviciding on adult mosquito density was assessed using GEE after adjusting for the zone, the distance of a village to the edge of alluvial floodplains and the use of ITNs in study villages. The trap ID was used as subject unit for repeated measures assuming an exchangeable correlation matrix.This model was used because it takes account of repeated measures in the analysis since water bodies and sentinel houses were repeatedly sampled during the study. Binary larval data was fitted to a binomial distribution with a logit link function. Count data for mosquito larvae and adults were fitted to a negative binomial distribution with a log link function. All analyses were performed with SPSS version 15.

Results

Spraymen were recruited and trained one month before the start of the larviciding campaign. They covered the intervention zones (200 km^2) weekly working from 7:00 am to 1:00 pm. In the first year of intervention, 60 spraymen applied 4,933 Kg of *Bti* WDG and 2,712 Kg of *Bti* CG to zones 1 and 3 from June to November 2006. In the second year 64 spraymen applied 6,705 Kg of *Bti* WDG and 7,553 Kg of *Bti* CG to zones 2 and 4 from May to November 2007.

Habitats colonisation

A total of 1,155 visits were made in the four zones from May to November during the baseline year 2005, 1,109 during the first year of intervention (2006) and 1,092 in the second year of intervention (2007). Annual rainfall collected at Farafenni Field Station was 858.3 mm in 2005, 807.9 mm in 2006 and 751.4 mm in 2007. During the baseline year there was no difference in the likelihood of finding anopheline larvae between the peripheral subzones but the central subzone was 54% less likely to be colonised by anophelines than the peripheral subzones (OR = 0.46; 95% CIs: 0.36-0.58; $p < 0.001$).

During the intervention years, there was no difference in the likelihood of finding anopheline larvae in all subzones.

Larviciding reduced significantly the proportion of sites colonised with *Anopheles* larvae in the four study zones (Figure 6.3). During the baseline year when no larviciding was done, the proportion of sites with *Anopheles* larvae was 41% (626/1537). In the first year of larviciding only 2% (28/1526) of sites were colonised with *Anopheles* in zones where larviciding took place and 45% (647/1425) in the untreated zones. In the second year of larviciding 6% (88/1437) of sites had *Anopheles* larvae in the zones where larviciding took place while 17% (254/1506) of sites had *Anopheles* in the untreated zones.

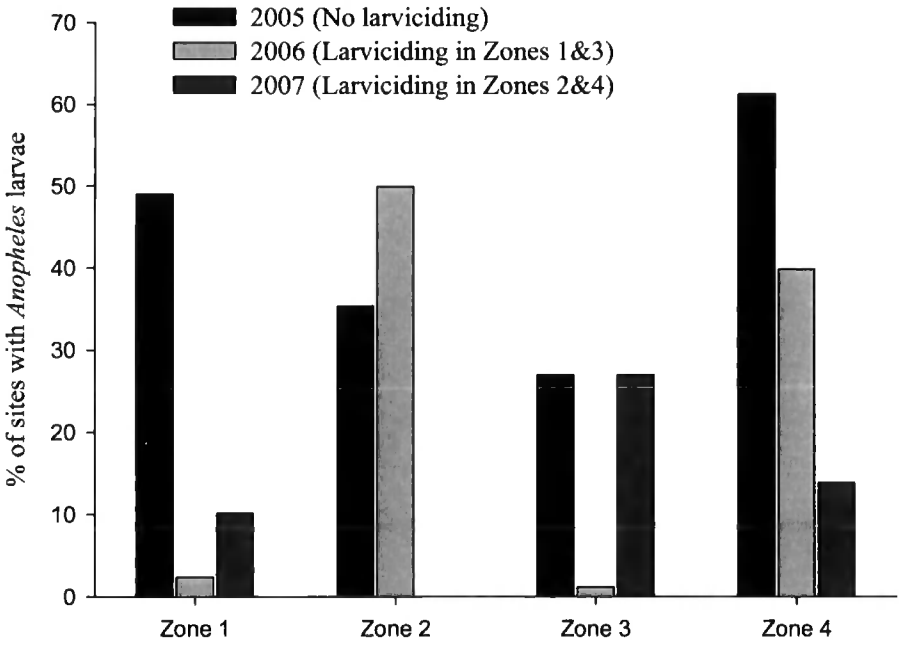

Figure 6.3 Proportion of sites colonised with *Anopheles* larvae during the rainy season

GEE modelling after adjusting for the year and the zones revealed that larviciding was associated with a 92 % reduction in the likelihood of finding water bodies colonised by late stage anophelines (OR = 0.08; 95% CIs = 0.06 – 0.10; $p < 0.001$) and culicines (OR = 0.08; CIs = 0.07 – 0.11; $p < 0.001$).

Larval density

A total of 993 sampling occasions were done at 40 sentinel sites selected in the four zones from May to November during the baseline year (2005), 1,107 occasions during the first year of intervention (2006) and 1,190 during the second year of intervention (2007).

The mean of late immature *Anopheles* density per dip was significantly reduced in all zones during the larviciding period although the reduction in zone 4 was not as dramatic as that observed in the other zones (Figure 6.4). GEE modelling after adjusting for the year of intervention and the zones showed that larviciding reduced the risk of finding higher densities of late instar *Anopheles* larvae by 91% (OR = 0.09; CIs = 0.05 – 0.16; $p < 0.001$) and culicines by 72% (OR = 0.28; CIs = 0.19 – 0.42; $p < 0.001$). During year two of intervention no late instar larvae or pupae were found in zone 2.

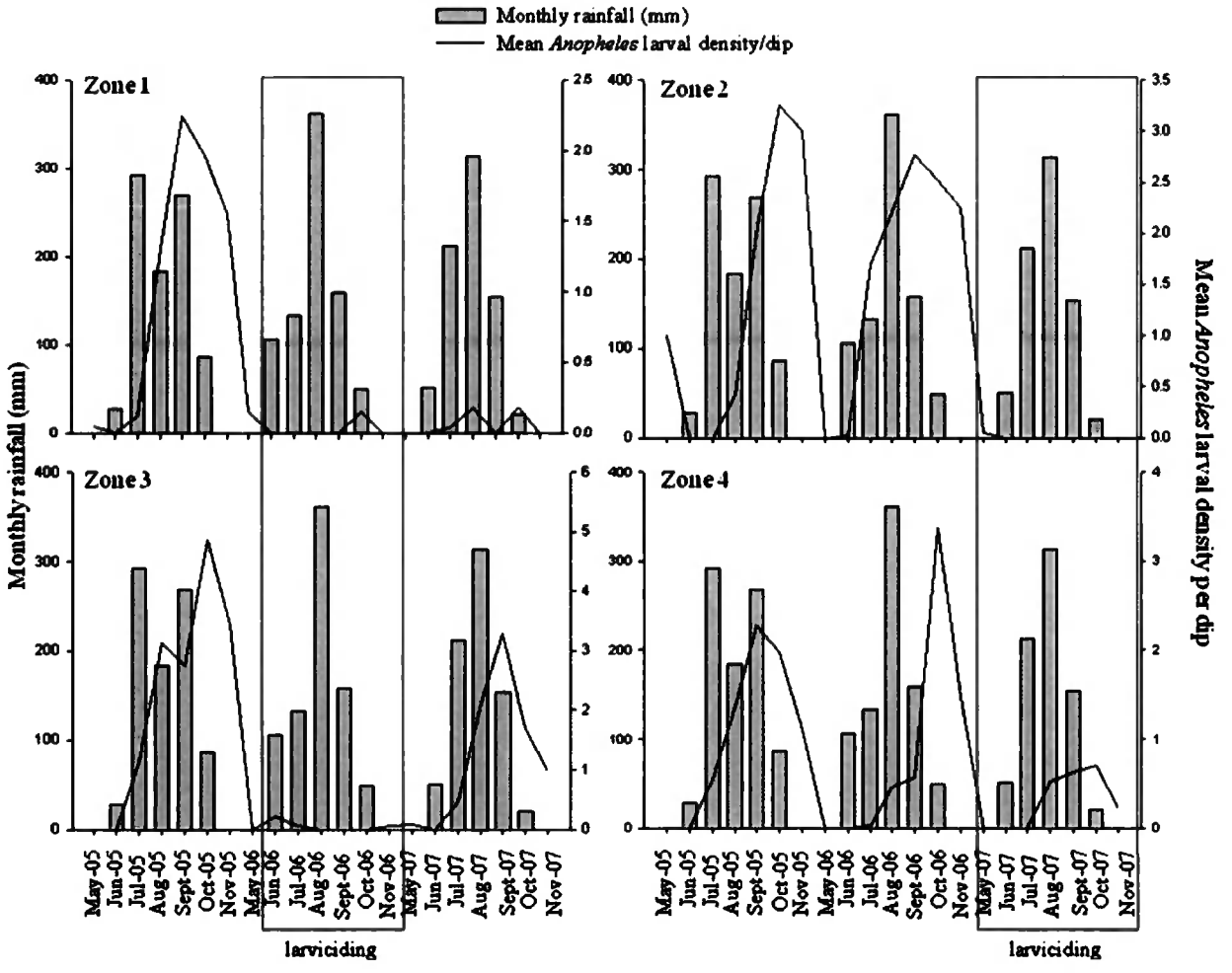

Figure 6.4 Mean *Anopheles* larval density per dip in the four zones and rainfall pattern.

Adult mosquito densities

A total of 61 CDC light traps were set in 40 villages in the four study zones each year. Traps were sampled on 2,248 occasions and on 27 occasions traps were not working properly or the human subject had moved away and these results were excluded from the analysis. The total number of adult mosquitoes sampled for the three consecutive years is shown in Table 6.1. Of all mosquitoes collected indoors 47% were *An. gambiae s.l.* in 2005, 51% in 2006 and 30% in 2007. The mean number of female *An. gambiae s.l.* and the sporozoite rate per zone are shown in Table 6.2. A subsample of 626 *An. gambiae s.l.* females caught in houses was subjected to PCR for species identification. 82 (13%) of these samples did not amplify and from the rest of the samples 54% were *An. gambiae s.s.*, 27% were *An melas* and 19% were *An arabiensis*. The sporozoite rate decreased in 2006 compared to 2005, and increased in 2007 compared to 2006 in all zones regardless where larviciding took place.

At baseline the risk of having a high number of mosquitoes in houses decreased by 77% (OR = 0.27, 95% CIs: 0.07 – 0.76, p = 0.016) with increasing distance from houses to the edge of alluvial swamps. This trend did not change during the larviciding period.

Table 6.1 Total number of adult mosquitoes caught indoors with CDC light traps during the study period in the four zones

Species	2005	2006	2007
bloodfed *An. gambiae* s.l.	488	595	553
unfed *An. gambiae* s.l.	22,275	14,032	20,047
gravid/semigravid *An. gambiae* s.l.	94	27	1
male *An. gambiae* s.l.	429	299	381
other female anophelines	1,674	886	1,648
other male anophelines	235	59	11
female nuisance mosquitoes	23,875	13,048	45,318
male nuisance mosquitoes	514	571	1,441
Total	49,584	29,517	69,400

Table 6.2 Mean of female *An. gambiae s.l.* (95% CI in brackets) and the sporozoite rate per zone. *indicates the zones where larviciding was done.

Factor	2005	2006	2007
Mean/trap/night			
Zone 1	5.6 (4.0 - 8.0)	1.8 (1.4 - 2.3)*	3.2 (2.2 - 4.7)
Zone 2	29.3 (23.0 - 37.4)	19.9 (14.9 - 26.6)	14.7 (11.1 - 19.4)*
Zone 3	93.0 (32.2 - 268.7)	45.4 (16.6 - 124.3)*	61.7 (35.9 - 106.2)
Zone 4	16.9 (13.8 - 20.7)	7.9 (5.5 - 11.3)	16.8 (12.0 - 23.5)*
Sporozoite rate			
Zone 1	1.1% (13/1191)	0% (0/469)*	0.37% (2/546)
Zone 2	0.2% (16/8332)	0% (0/4105)	0.08% (3/3493)*
Zone 3	0.1% (16/15136)	0.08% (7/9315)*	0.16% (25/15796)
Zone 4	0.2% (7/3008)	0.12% (4/1633)	0.14% (6/4154)*

The impact of larviciding on *An. gambiae s.l.* adult density varied between zones but was less apparent in zone 4 (Figure 6.5). Overall the mean number of *An. gambiae s.l.* adults density per trap in the non- intervention areas was 28.1 (17.2 – 45.8) and this number was reduced to 19.6 (11.1 – 34.5) in the intervention areas. Univariate analyses showed that the probability of having increased densities of adult *An. gambiae s.l.* in areas where larviciding was done is reduced by 30% (OR = 0.70; 95% CIs: 0.59 – 0.82; $p < 0.001$) compared to areas where no larviciding was done. After adjusting for ITN use in villages, the zones and the distance from houses to the edge of swamps, the model revealed that the risk of having increased densities of adult *An. gambiae s.l.* was reduced by 28% ($p = 0.005$) in larviciding areas compared to the non-intervention areas (Table 6.3). When data for zone 4 were excluded from the analysis, larviciding reduced by 42% the risk of exposure to *An. gambiae* mosquitoes ($p < 0.001$, Table 6.3). Nuisance mosquitoes caught inside houses increased in areas where larviciding was done in a univariate analysis, however after adjusting for ITN use in villages, the zones and the distance from houses to the edge of swamps, the model revealed no impact of larviciding on nuisance mosquitoes.

Table 6.3 Impact of larviciding on *Anopheles gambiae* mosquitoes adjusting for ITN use, zones, and distance between trapping house and the edge of alluvial plains

Factor	Odds ratio	Lower C.I.	Upper C.I.	P
with zone 4				
larviciding	0.72	0.57	0.90	0.005
no larviciding	1.00			
ITNs	1.00	1.00	1.00	0.002
Zone 4	8.58	4.45	16.56	< 0.001
Zone 3	13.78	6.70	28.33	< 0.001
Zone 2	7.09	5.08	9.90	< 0.001
Zone 1	1.00			
distance house-floodplains	0.39	0.26	0.59	< 0.001
without zone 4				
larviciding	0.58	0.47	0.70	< 0.001
no larviciding	1.00			
ITNs	1.00	0.99	1.00	< 0.001
Zone 3	18.48	8.90	38.40	< 0.001
Zone 2	6.82	5.06	9.19	< 0.001
Zone 1	1.00			
distance house-floodplains	0.47	0.34	0.66	< 0.001

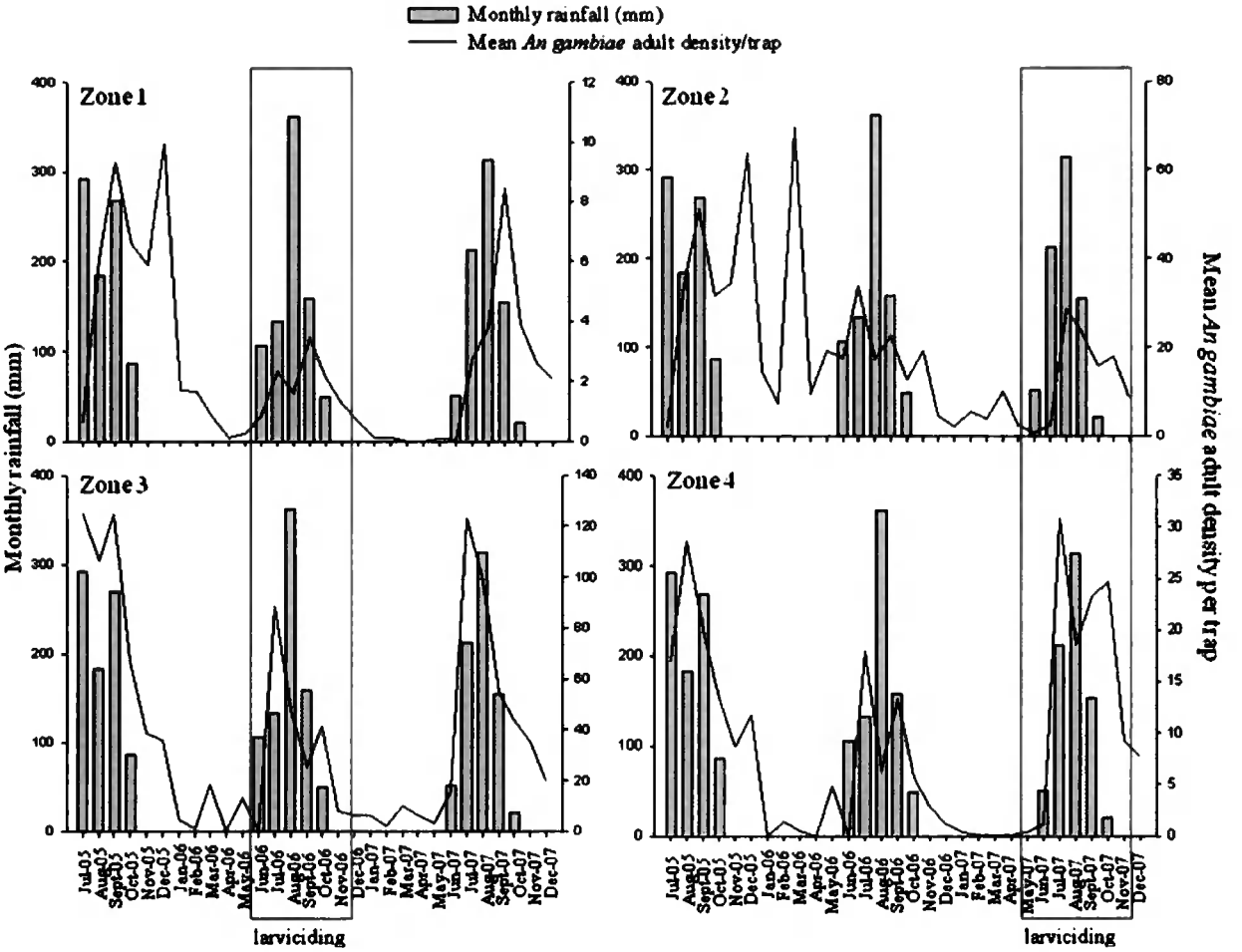

Figure 6.5 Mean *An. gambiae s.l.* adult density per trap in the four zones and rainfall pattern.

Discussion

A large and closely monitored larviciding programme was undertaken and assessed the impact of larviciding with *Bti* on malaria transmission. It is the first large-scale assessment of the impact of microbial larvicides in rural ecosystems dominated by the ecology of major floodplains like the Gambia River. Larviciding reduced by 92% the likelihood of colonisation with mosquito larvae and also reduced by 72-91% the probability of having high densities of late instar larvae or pupae. Larviciding was so successful in reducing larvae that despite a thorough sampling of ten sites daily (on average a total of 100 dips) in zone 2 no single larva was found in the whole rainy season during the intervention year. However, despite the significant resources put in its implementation larviciding reduced by only 28-30% the risk of exposure to *An. gambiae* females mosquitoes, the primary vectors of malaria in The Gambia (Lindsay et al. 1993).

The resources necessary for a successful implementation of the larviciding programme are significant. On average 60 spraymen successfully applied larvicides to 200km^2 area each week. In 2007 more spraymen were recruited compared to 2006 due to the large extent of water bodies in zone 4. Finding all water bodies and getting familiar with the intervention zone is a key to the success of the larviciding campaign (Fillinger and Lindsay 2006, Walker and Lynch 2007). It did not take too much effort for spraymen to know the area because most spraymen were recruited from within the intervention area and were already familiar with the water bodies in the area. Training spraymen how to apply larvicides took only one week and was relatively simple because the equipment used was easy to use. Spraymen worked six hours per day starting early morning to avoid the hottest hours of the day since in this area temperatures can reach 45°C (Giglioli and Thornton 1965), with high humidity close to the river making the work physically demanding. A considerable quantity of microbials was needed to cover the entire area, more so in the second year because larviciding started one month earlier than in the previous year. This was done in order to reduce the dry season mosquito density before the start of the rainy season. It was expected that this would help in delaying the rise in mosquito numbers observed at the onset of the rainy season (Lindsay et al. 1993), however although this rise was delayed in zone 2 compared to the previous year, it was not the case for zone 4.

In zone 4 larval collections show that late instars were still found in this area during the larviciding period, indicating a failure in treating all the sites. This can be explained by two main reasons: first, zone 4 has tributaries of River Gambia running in the upland area very close to human settlements (Figure 2.2). These tributaries create more breeding sites for mosquitoes than anywhere else in the study area and limit full access to spraymen in deeper areas. Second, this zone had the largest areas covered by rice fields both in the floodplains and the upland area. These extensive rice fields are so uniform and similar that it is easy for spraymen to confuse where larviciding reached or not.

There were more habitats with tall vegetation in the second year of intervention requiring more granular formulation product than the previous year. This might also imply that full coverage was not achieved since areas with tall vegetation are difficult to access. Within these areas it is likely that small pockets of sun exposed habitats go unnoticed by spraymen. *Bti* does not spread after hitting the water surface and settles

rapidly (Lacey 2007) therefore in these vegetated environments larvicides with surface films activity (Cameron and Richard 2007) might be a better option.

The system used for monitoring the success of larviciding was adequate to find sites that might have been missed by the spraying team. Spray teams did not know *a priori* where the field worker in charge of monitoring was going to survey nor did the investigator until the sites were randomly selected in advance. The routine surveillance of mosquito density in sentinel habitats and houses provided an additional robust tool to investigate fluctuations in mosquito numbers and the impact of larviciding.

The results show that *Bti* works well against immatures of malaria vectors in the floodplain ecosystem of rural Gambia as it has been observed in other ecosystems in SSA. The reduction in larvae observed in this study is supported by studies in rural Kenya where larviciding reduced mosquito larvae density by 95% and exposure of humans to mosquito bites by 92% (Fillinger and Lindsay 2006). Similarly a study done in semi-arid conditions in Eritrea found that larviciding with *Bti* and *Bs* could control mosquito populations (Shililu et al. 2003) and when coupled with environmental management reduced larval and adult mosquito production (Shililu et al. 2007).

Despite reducing mosquito larvae by a magnitude, the impact of larviciding on adult *An. gambiae s.l.*was less impressive. An overall reduction of 28% in *An. gambiae s.l.* was achieved, although this was 42% when zone 4, the area with most breeding sites, was excluded. Similar to these results, only a 31% reduction in the primary vector of malaria, was observed after one year of community-based larviciding in urban Dar es Salaam (Fillinger et al. 2008). However, it is possible that with increasing experience in larviciding and knowledge of the intervention area, results in controlling adult mosquitoes might be improved, as observed in Dar es Salaam (Fillinger, personal communication). A number of reasons might explain why the success in controlling larvae due to larviciding did not have a big impact on adult mosquito densities. First, mosquito larvae are relatively rare in the water bodies of rural Gambia (Chapter 2) compared to the huge numbers of adult mosquitoes observed in houses mainly because larvae are thinly distributed over huge areas of water bodies. This might explain why a 90% reduction in mosquito larval density due to larviciding did not translate into a similar reduction in adults because the area sampled for mosquito larvae (and where larvicides were applied) might not be the only source of adult mosquitoes. This situation is different from what is usually observed in other areas such as in East Africa where mosquito larvae are clustered in discrete habitats, usually human-made

(Fillinger et al. 2004, Mutuku et al. 2006a). In such areas, ground application of larvicides is more likely to yield better results than in extensively flooded areas like those observed in this study.

Second, the flooded area of the Gambia River is subjected to regular and strong tidal movements (Giglioli 1964) that might disperse and dilute microbials applied in the floodwater. Since spraying was done weekly, any larvae not killed within 24-48 hours of spraying are likely to develop to maturity if they do not die of other causes because *Bti* does not show any residual effect in The Gambia (Chapter 4). A study in The Gambia showed a development rate for mosquito larvae of approximately 10 days at temperatures beyond 28°C (Bayoh and Lindsay 2003) and the average water temperature in the study area was 29.9°C (95% CI = 29.3-30.5, Fillinger et al in prep). However the life cycle of mosquitoes could be shorter at high temperatures (Giglioli and Thornton 1965) and the maximum found in the study area was 42°C (Chapter 4). Mosquito eggs can survive on damp soil for up to 12 days (Beier et al. 1990) and once these sites are flooded with water, the eggs hatch and larvae develop successfully to adult stage. A similar situation might occur in The Gambia when eggs laid at low tide on damp soil remain viable for days. At high tide when these sites are flooded, the eggs might hatch and develop quickly due to the lack of residual activity of *Bti* or due to the dilution of the microbial at high tide. This is supported by previous observations where development to adult emergence took less than five days, consequently leading to failures in weekly larviciding (McCrae 1998). If this is the case for The Gambia, a successful intervention would require larviciding at shorter intervals instead of weekly application as adopted in this study. Alternatively other larvicides with a longer residual effect and not affected by the tidal movement of the water might be effective in this area. One candidate could be the insect growth regulator pyriproxifen which has a long residual effect (Yapabandara and Curtis 2002) but have not yet been tested in Africa.

Third, it is possible that mosquitoes might have invaded the study areas from non-treated sites outside the intervention areas. Earlier studies in The Gambia show that almost all mosquitoes emerge from the flooded alluvial plains, but the fact that mosquitoes can be found further away where there are very few or no breeding sites is a result of their dispersal and survival capacity (Bøgh et al. 2007). It was observed that within 4 km from breeding sites there was almost a 12-fold reduction in the mean

number of infective mosquito bites received per person (Bøgh et al. 2007). However, in these open savannah areas mosquitoes might fly further following wind gradients.

The proportions of *An. gambiae s.s.*, *An. melas* and *An. arabiensis* found indoor is similar to results obtained around Farafenni area (Kirby et al. 2008) although in the study area *An. arabiensis* was less common. These findings concur with previous studies in the area showing the role of the three species in malaria transmission in The Gambia (Lindsay et al. 1993). Although larviciding reduced malaria vectors, it is difficult to draw a conclusion about its impact on the EIR. The sporozoite rate was highly variable in the different zones and between years as observed in earlier studies in the Gambia and across SSA (Bøgh et al. 2007, Hay et al. 2000). The sporozoite rate was lower in 2006 for all the zones compared to 2005 and increased in 2007 in all zones despite larviciding occurring in zones 2 and 4. These results suggest that the EIR in the area might be influenced by other factors not investigated in this study and no direct impact of larviciding on the EIR was detected.

The results reported here revealed that *An. gambiae* production although at low density, continues during the dry season (Figures 6.4). This could be explained by the fact that breeding sites in the floodplains such as edges of floodplains and fringes of river tributaries have a permanent and semi-permanent character and do not dry out during the dry season. Although survival of mosquitoes is lower during the dry season, when breeding sites are available malaria transmission may continue at a low rate during the dry season (Lindsay et al. 1991b). The high production of mosquitoes observed in zone 2 during the months of December and March 2006 is unusual. This might be due to the tidal movement of the river creating suitable breeding sites in the area and a combination of other biotic and abiotic factors allowing mosquito production in high numbers.

Although the small reduction obtained in malaria vectors was not seen in other nuisance mosquitoes, it was not due to the difference in susceptibility between anopheline and culicine larvae to *Bti*. In semi-field and field conditions both anophelines and culicines show a high susceptibility to *Bti* (Chapter 4, (Fillinger et al. 2003)). Two main reasons might explain why larviciding did not reduce culicine mosquitoes. First, culicine mosquitoes might be breeding in the deeper waters distant from the landward edge of the floodwater, where spraymen could not reach or where the deep water might reduce microbial activity. It has been observed that in deep water *Bti* might work less efficiently because of the rapid settling of the toxins which reduces

its residual effect (Lacey 2007). Second, culicines can breed in a wide variety of water bodies covered by emergent and floating vegetation (Muturi et al. 2007b) where *Bti* might not reach the water surface successfully, unlike the open sun-lit habitats commonly known to be preferred by anophelines (Minakawa et al. 2004, Munga et al. 2006). Moreover water bodies entirely covered by grassy vegetation might not be easy to locate and missed by spraymen.

This study has shown a reduction in malaria vectors due to larviciding. However the reduction is so small that it might not translate into a large reduction in the burden of malaria in the area. Only a considerable reduction of transmission would be able to reduce the burden of malaria for the whole community on a long-term basis (Trape and Rogier 1996). In the context of the Macdonald model for malaria transmission (MacDonald 1957), larviciding cannot reduce the longevity of vectors, however a substantial reduction of the mosquito biting population through larviciding would reduce malaria transmission. Since there was no reduction in nuisance mosquitoes, it would be hard to convince the community to support such intervention over many years. The relationship between malaria, mosquito species and habitats is usually unknown in local communities (Clarke 2001, Mutuku et al. 2006a, Vundule and Mharakurwa 1996) and mosquitoes are often seen as a nuisance more than a disease vector (Adongo et al. 2005, Klein et al. 1995, Schellenberg et al. 1999). Therefore an intervention not reducing the nuisance of mosquitoes is less likely to be received with enthusiasm by the community (Stephens et al. 1995). Similar to the lack of community protection after mass administration of gametocidal drugs (von Seidlein et al. 2003), there is no record of community effect by larviciding unlike the use of bed nets (Hawley et al. 2003). In areas like The Gambia where mosquitoes have a long flight range exceeding 4 km, it would be difficult to show impact on disease transmission unless the area covered by larviciding is very big.

The ecology of mosquito larvae, the tidal movements of the river, the extent of breeding sites, the flight range of mosquitoes and lack of long residual activity for *Bti* might explain why larviciding in this area did not greatly reduce adult mosquitoes. In this study a low technology approach of ground spraying was used which was assumed to be cost-effective for majority of Africa. However in areas with extensive flooded areas and ecological settings similar to the study area, it might be better to consider aerial application of larvicides. However, the sustainability of such operations in SSA would need to be assessed.

Although this study covered a substantial area in the middle reaches of the Gambia River, the ecological settings are not similar for the whole country. Further upriver the flooded area is relatively smaller and not under tidal influence. In such area larviciding might produce better results than in the flooded area where this study was conducted.

Although larviciding is a viable tool for killing mosquito larvae in The Gambia, its impact on adult malaria vectors was minor and it had no impact on nuisance mosquitoes. The results obtained do not seem to be worth the resources involved. Therefore the effectiveness of anti-larval measures for malaria control would be questionable in extensive floodplains of The Gambia and in similar large river ecosystems of the Sahel region. Other vector control methods such as those directed to adult mosquitoes should be advocated in these settings.

Chapter 7

General conclusions

Figure 7.1 Spraymen with their certificates of completion at the end of the larviciding campaign.

General conclusions

Malaria continues to cause a huge death toll and is a serious economic burden in countries where it is endemic, mainly in SSA countries (Sachs and Malaney 2002). Many major efforts to control and prevent the disease are under way and some interventions such as the use of ITNs and IRS together with efficient drugs such as ACTs have contributed greatly to curbing the disease and a number of success stories in Africa are being reported (Wakabi 2007). However the battle is still a long way from being won and with the threat of the development of resistance against currently efficient insecticides (Santolamazza et al. 2008) and drugs (Mutabingwa 2005) there is an increasing necessity for new tools to combat this old disease.

Vector control is one of the best ways to tackle malaria (WHO 2004) and today it is recognised that not one tool can be sufficient alone to control malaria vectors in long term, therefore the WHO has proposed a global strategic framework for integrated vector management (IVM) that comprises interventions that have shown efficacy in combination or separately such as use of ITNs and IRS (WHO 2004). Among these interventions, larval source management (LSM) for malaria control is regaining ground as an efficient tool for malaria control. A number of pilot studies are taking place in Africa (Fillinger et al. 2008, Fillinger and Lindsay 2006, Shililu et al. 2007) in order to inform decision makers of the possibilities of incorporating LSM as a component of the IVM programmes. Since the efficacy of larvicides depends on the ecology of the area and the susceptibility of indigenous mosquitoes (Becker and Rettich 1994) this study assesses for the first time the impact of microbial larviciding on malaria transmission in areas of Africa with riparian habitats such as The Gambia. In this area malaria transmission is highly seasonal and peaks in the one rainy season running from June to October (Lindsay et al. 1993). The main vectors are *An. gambiae s.s.*, *An. arabiensis,* and *An. melas* all belonging to the *An. gambiae* complex (Lindsay et al. 1993). The country is bisected by the Gambia River which creates flooded areas where most breeding of mosquitoes is confined (Bøgh et al. 2003, Bøgh et al. 2007).

The goal of this study was to determine whether routine larval control with microbial larvicides in the main anopheline breeding sites will reduce mosquito densities and consequently malaria transmission in rural Gambia. Because larval

control is logistically demanding it would be cost-effective to target control at the habitats most productive of mosquitoes. This requires knowledge and characterisation of habitats in which mosquitoes occur and where they are likely to emerge from in order to inform larval control programmes on where to target control. This study describes the spatial distribution of mosquito larvae in the middle reaches of Gambia River.

The majority of water bodies that were mapped contained *Anopheles* larvae. Although *Anopheles* larvae were more likely to be found in the rainy season than in the dry season, larvae were found throughout the year mainly in the alluvial plains shaped by the Gambia River. Although adult mosquito densities are highest during the rainy season (Lindsay et al. 1993) the continuous presence of water bodies created by the tidal movements of the river sustains larval production throughout the year. The implication of these findings is that in these areas, vector control measures should not only be focused on the rainy season. Although most breeding sites for *Anopheles* were found in large habitats close to the landward edge of the flooded alluvial plains, larval control targeted at these sites alone cannot be successful because it would miss many other important habitats, such as those closer to human settlements, which although few in number are likely to contribute to malaria transmission. Therefore all standing water bodies in this area should be considered as potential breeding sites for anopheline mosquitoes. This finding is not particular to these riparian habitats alone, two studies in East Africa came to a similar conclusion (Fillinger et al. 2004, Sattler et al. 2005).

Rice is a staple food for Gambians and rice fields were the most common breeding habitat in the area. Rice cultivation is known to contribute to the proliferation of mosquitoes (Ijumba and Lindsay 2001, Service 1989, Snow 1983) implying that in such areas humans activities are contributing to the rise in disease transmission. However, increased transmission does not necessarily lead to increased malaria in local communities (Ijumba and Lindsay 2001). Reasons for this apparent paradox are uncertain, but one suggestion is that rice production can provide wealth and better nutrition leading to greater protection against this disease. As the population grow in The Gambia so does the area of rice cultivation and with that the risk of increasing malaria transmission. This real dilemma cannot be solved by reducing rice cultivation because of its importance in these poor areas. One of the solutions would be to focus malaria control activities in such areas. The ricefields can be easily defined therefore

can be targeted during larviciding programmes. However these cannot be the exclusive target since other bodies of water were equally colonised by anophelines and might contribute to malaria transmission. A significant number of habitats dry out during the dry season and the probability of finding anophelines is greatly reduced at this time, therefore it would be advantageous to start larviciding at this time before the number of mosquito habitats increase with the rains. The programme should continue throughout the rainy season when most breeding occurs and adult mosquitoes peak in number.

The hypothesis that the distribution of mosquito larvae is clustered in specific habitats does not hold. This study revealed that larval control in this area cannot target selected habitats but would have to cover all accessible water bodies. This is likely to render larval control efforts more challenging than in areas where larval habitats are discrete and clustered. The aim to describe the spatial distribution of anopheline mosquitoes in rural Gambia was achieved and it is recommended that larval control should be comprehensive and applied to all potential breeding sites. The results reported here are relevant for any programme planning to use anti-larval measures for malaria control in similar riparian habitats in the Sahel region.

Studies in East Africa have shown that not all habitats colonised by mosquito larvae produce adult mosquitoes (Mutuku et al. 2006b), suggesting that only a few habitats produce adult mosquitoes and can be targeted in a larval control programme. Therefore within the mapped habitats the next step was to describe the most productive habitats for mosquitoes in rural Gambia. Although the spatial distribution of anopheline mosquitoes is a good tool to inform larval control programmes, it is important to know which of the habitats colonised by mosquito larvae are more productive for late instar larvae and pupae. This would provide a good proxy for adult mosquito emergence and if these habitats can be identified readily, they could be targeted for larval control, resulting in a more cost-effective programme.

The results of this study show that *Anopheles* larvae were more abundant in the rainy season than in dry season, however, surprisingly pupal density did not differ between seasons. However the dry season collections may be over-estimates since the surface area of the water bodies is much reduced, making it easier to sample with a dipper. It is also possible that there were more larval predators in the rainy season than in the dry season, however further studies are required to confirm this. As observed with the distribution of larvae, larger habitats had more larvae in the rainy season but

in the dry season both large and small habitats were similarly productive. Larval and pupal densities were similar in most habitats and were low only in puddles, which are minor habitats in the area compared to the extensive and permanent and semi-permanent habitats connected to the river. The main malaria vectors in The Gambia belong to the *An. gambiae* complex (Bryan 1983, Lindsay et al. 1993). Both *An. gambiae s.s.* and *An. melas* shared the same habitats and occupied the majority of habitats sampled. This finding has serious implications for communities living close to standing water bodies in the area since these might be a potential source for mosquito breeding. Earlier studies had revealed that the risk of malaria transmission was higher for communities living closer to the alluvial plains of Gambia River than those farther away (Bøgh et al. 2007, Thomas and Lindsay 2000). This study clearly illustrates that most habitats in rural Gambia can produce mosquito larvae and pupae in both seasons therefore any targeting of larval control to a selected number of habitats is unlikely to succeed.

The hypothesis that larval and pupal production is clustered to some habitats does not hold. Most habitats are equally productive of mosquito larvae and pupae and any larval control programme should be comprehensive and cover all accessible water bodies in the area.

The aim to describe the habitats most productive of mosquito larvae and pupae in rural Gambia was achieved and it is recommended that in The Gambia and in ecosystems with extensively flooded habitats larval control should be comprehensive and applied to all potential breeding sites. This approach would be very challenging with ground application of larvicides but might be feasible with aerial application. These findings are relevant for any programme planning to use anti-larval measures for malaria control in similar ecosystems of the Sahel region.

Results from this study have shown the importance of rice fields in the production of mosquito larvae (Chapter 2). For this reason I attempted to find out how swamp rice cultivation affects the production of malaria vectors. The landward edge of the floodplains is the first area that gets flooded with freshwater at the beginning of the rainy season. This is the first area where rice is grown and it is rich with nutrients, including cow dung , which is associated with high mosquito larval densities. 93% of *An. gambiae* s.l. larvae and 97% of adults came from this area. The main reasons for this finding are that this strip of water is closest to human settlements and is the shortest flight distance for an ovipositing female. Water is relatively fresh here and

exposed to the sun, a situation preferred by *An. gambiae* mosquitoes. This area also has few fish which are known to be predators of mosquitoes. Since it is likely that rice demand will continue to increase (www.warda.org: accessed 26/7/7), it is a priority to make sure that rice farmers and populations closer to rice fields are well protected against malaria.

This study has revealed more insight in the biology of *An. gambiae* in rural Gambia and similar ecosystems. *An. gambiae* shows a high plasticity in exploiting a wide range of habitats. All standing water bodies might be breeding sites for mosquitoes. Despite a decrease in population density during the dry season, mosquito production continues throughout the year.

New formulations of *Bti* and *Bs* have shown good control against malaria mosquitoes in East Africa (Fillinger and Lindsay 2006, Shililu et al. 2007). However because their efficacy might vary in different ecological settings (Becker and Rettich 1994), it was necessary to assess their efficacy on mosquitoes in the riparian habitats of The Gambia and to determine the optimal formulations, dosage and application methods before starting a large-scale larval control programme in the area.

In the laboratory, the main malaria vectors in The Gambia showed a high susceptibility to both *Bti* and *Bs* at very low dosages comparable to the results obtained in East Africa (Chapter 4). These results imply that the susceptibility of mosquitoes to the microbials is inherent to the species and not to the ecological settings. Therefore these microbials can be recommended for mosquito control in different areas. In standardised field conditions both microbials successfully killed all larvae and *Bs* showed a short residual activity up to 10 days. However in natural field conditions although both microbials achieved full control of mosquitoes, third instar larvae appeared four days after treatment for both microbials suggesting that with available larvicides, larval control will have to be repeated at least at weekly intervals. The implication of this finding is that although mosquitoes are susceptible to microbials, the residual activity of microbials varies depending on the ecology of the area where they are applied. Application methods of microbials in the field vary in areas where water bodies are covered with a mixture of low and tall vegetation. Liquid formulations should be applied to habitats with low vegetation where the microbials can reach water surface, and in areas with tall vegetation granular formulations should be applied.

Training of field staff in recognising habitats, calibration and microbial application was relatively easy and spray teams were able to work under fully operational field conditions for three months and achieving a successful control of mosquitoes. These teams should be selected among the communities within the intervention area since they are likely to locate the breeding sites easily and would benefit financially from this work. This would help keep moral high for such a physically demanding task. Because *Bs* did not show any residual activity *Bti* should be used in those areas because although it does not have residual activity, it has no record of developing resistance despite being used for more than 30 years in the field, unlike *Bs* (Charles and Nielsen-LeRoux 2000). In the future larvicides with a longer residual effect would be more cost-effective for places like rural Gambia.

The hypothesis that microbial larvicides *Bti* and *Bs* are efficient against mosquitoes in The Gambia was correct. Full control of mosquitoes was achieved with both microbials. The aim to test the efficacy of *Bti* and *Bs* on Gambian mosquitoes was achieved and this study has shown that both microbials are efficient against malaria vectors in The Gambia.

After studying the ecology of mosquitoes in rural Gambia and testing *Bti* in the field, it was concluded that blanket coverage of all potential breeding habitats was the best larviciding approach. Routine operational larval control was implemented in the study area using this strategy. The results show that larviciding was associated with a 92% reduction in the likelihood of finding mosquito larvae in breeding sites. Larviciding also reduced the density of *Anopheles* larvae by 91%. Although this reduction is encouraging and similar to what other studies obtained in East Africa (Fillinger and Lindsay 2006), it did not translate into a similar reduction of adult mosquitoes. However whilst there was a 28% reduction in malaria vectors where larvicides were applied this reduction was not observed for nuisance mosquitoes. These findings reveal that although microbial larvicides are clearly effective against immatures of malaria mosquitoes, their application in large river ecosystems such as in the Sahel region does not translate into a major reduction of adult mosquitoes. The 28% reduction in *An. gambiae s.l.* density is not expected to have a large impact on the overall malaria burden. Only a considerable reduction of transmission would is able to reduce the burden of malaria for the whole community on a long-term basis (Trape and Rogier 1996). On the other hand, the lack of reduction in nuisance mosquitoes might cause a lack of enthusiasm and involvement of communities in larviciding operations.

The overall implication of these findings for public health is important: areas with large river ecosystems are likely to create extensive breeding sites for mosquitoes in their floodplains. In western Kenya and Tanzania where similar studies have been conducted (Fillinger et al. 2008, Fillinger and Lindsay 2006), the impact of larviciding on adult mosquitoes and malaria transmission is much greater than in The Gambia. The main difference between these sites and The Gambia is in the types of breeding sites. Areas where larval control was implemented in Kenya and Tanzania were relatively discrete habitats, and the water source was mostly rainfall or ground water not subjected to extensive flooding. These are relatively easier to access and treat with larvicides. In contrast most breeding sites in The Gambia are a result of flooding caused by rainfall or tidal movements of River Gambia that create extensive and ill-defined bodies of water. In this area mosquito oviposition seems to be overly dispersed in extensive breeding sites. This combined with the fact that mosquitoes show a rather high plasticity to exploit a wide range of habitats with different biotic and abiotic conditions implies that targeting larval control to selected habitats is unlikely to succeed. The logistics needed for a larval control programme to cover such large areas are a challenge and since the microbial larvicides available today would require a weekly application, larviciding does not seem to be a cost-effective approach in such areas.

Although LSM is a viable and efficient tool for mosquito control and is advocated to be incorporated in IVM programmes their use cannot be recommended in areas like the Sahel region of Africa with large river ecosystems that flood large areas and create extensive breeding sites for mosquitoes. Vector control in such areas could rely on other measures that have proven efficacy such as ITNs and IRS. LSM would then be used as an additional tool in areas where mosquito breeding occurs in discrete and relatively small and few water bodies of the urban or peri-urban area, or in rural upland sites.

The immensity of the area flooded by the river and the extent of breeding sites created thereof suggest that ground application of microbials in such areas is likely to miss some water bodies. If logistics allow, aerial spraying could be a better approach in such ecosystems as observed in the control of Onchocerciasis in 11 countries of West Africa (Hougard et al. 1997). However the cost-effectiveness of such an operation and its sustainability represent major challenges. Moreover a larvicide agent with a longer residual activity than *Bti* would reduce the costs of weekly applications.

In conclusion, mosquitoes in rural Gambia exploit a wide range of aquatic habitats throughout the year. Ground application of larvicides in areas with extensive floodplains is not likely to reduce substantially malaria transmission, therefore other vector control interventions directed to adult mosquitoes should be considered.

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