Utilization of 'swamp' rice fields by members of the anopheles Gambiae complex in Gambia

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UTILIZATION OF ‘SWAMP’ RICE FIELDS BY MEMBERS OF THE *ANOPHELES GAMBIAE* COMPLEX IN THE GAMBIA

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Masters of Science by Research

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CONTRIBUTIONS OF OTHER AUTHORS

The field work of all portions of this study and write up was done by me. The study design and multivariate statistical modeling was carried out with support from Professor Steve Lindsay. Entomological identification of mosquito species and Polymerase Chain Reaction was done with assistance from Dr. Clare Green, while Vasilas Loucas assisted in fish sampling, insect species identification and measurement of physical characteristic of water in study site. General study supervision was given by Silas Majambere, Dr. Matt Kirby, Mr. Musa Jawara and Mr. Balla Kandeh. I thank all of you.

MR. LAMIN B.S. JARJU

SIGNATURE

DEDICATION

This work is dedicated to my Father (Ba Seedy Jarju), my grand parents (Mbabinta Jaiteh (Senior) and Ba Foday Saidy) who all brought me up, struggled to send me to school but did not live to see and enjoy my little wealth in future. Specially dedication to my dear mother, Bintou Sawo, Kaddy Saidy (PP) and my dear uncle Njanko Kassama and his wives (Aja Mariama Minteh, Jarra Marong and Nyima Jaiteh) who have all been very supportive during my study periods. Special regards to my wives; Mbabinta Jaiteh (Junior), Fatou Fatty and my son Muhammad Jarju whose support and understanding have transformed my degree program into reality.

SUMMARY

Background

Whilst it is well known that rice production in Africa increases the production of *Anopheles gambiae* mosquitoes, most studies have investigated this in irrigation schemes. Here I examine the colonisation of 'swamp' rice fields by *An. gambiae* mosquitoes in The Gambia and examine some of the factors responsible for the presence and absence of these vectors in field and semi-field conditions. This work is of relevance to large-scale larval control programmes that have identified rice fields as a major source of malaria vectors in The Gambia.

Methods

Larval and adult mosquito surveys were carried out in rice fields near Tamba Koto village in rural Gambia from June to January, 2006, a period that included the wet season, the period of most malaria transmission. Three transects each 500m long and 200m apart situated on the edge of the River Gambia floodplains were routinely surveyed. Larval sampling using area sampler and dippers was done at regular intervals along each transect. Adult sampling with emergence traps placed over water. I compared three different water treatments that were commonly found under field conditions in Gambian rice fields: the presence of algae, cow dung and urea. The number of larvae and pupae were counted daily for 14 days in 16 artificial breeding sites of plastic bowls filled with tap water, each with a different treatment and an untreated control bowl. This trial was repeated four times over 12 weeks period.

Results

Three hundred and seventy-five (375) anopheline larvae were caught during the larval survey in the field, with 349 larvae (93%) collected within the first 350m from the landward edge of the paddy fields. Out of the *An. gambiae* complex collected, 36 (45%) were *An. arabiensis*, 23 (29%) were *An. gambiae sensu stricto* and 21 (26%) were *An. melas*.

A total of 263 adult mosquitoes were collected during the adult emergence study. Sixty - eight belonged to the *An. gambiae complex*, 139 were culicines, 30 were *Aedes*, and 26 were anophelines which could not be identified by Polymerase Chain Reaction. Out of the 68 *An. gambiae sensu lato,* 61 were *An. arabiensis,* 4 were *An. gambiae s.s.* and 1 was *An. melas.* Of the 68 *An. gambiae s.l.* caught, 62 (91.1%) were caught along the edges of rice fields. Although fish are common and evident animals in rice fields, there have been relatively few studies on the ecology of indigenous species.We did not find fish in any of the sampling points within 350m of the village, but did find them far away close to the river.

A total of 17,467 mosquito larvae were collected from 16 bowls during four months of the semi field trial. Of these 75% were early instars and 25% late instars. Of the total number of mosquito larvae sampled, 6,233 (36.7%) were identified as anopheline and 11,234 (63.3%) culicine. Of the anophelines sampled, 5164 (83%) were early instars whilst 1069 (17%) were late instars. Field surveys showed that paddies closest to the land had no fish and were rich in cow dung.

More anopheline larvae were produced from cow dung treatments (n=1,718; 33.3%) and, bowls treated with algae (n=1,453; 28.1%) compared to water alone (n=1,064; 21%). Similar numbers were found in bowls with urea (n=929; 18%) compared with water alone. Similar trends were observed for culicine larvae. Out of 8,021 early stage larvae, 2,675 (33%) were found in bowls with cow dung, 2,554 (32%) from algae, 1,532 (19%) from water alone and 1,260 from urea (16%).

One hundred and thirty-eight adult mosquitoes of *An. gambiae* complex were identified. Fifty-nine were *An. gambiae s.s.*, 74 were *An. arabiensis* and 2 *An. melas.* Out of 59 *An. gambiae s.s.*, 24 (41%) were recorded from cow dung treatments and 21 (36%) from algae treatments. Of the 74 *An. arabiensis,* 35 (47%) were recorded form cow dung whilst 28 (38%) were from algae. This result indicated that both cow dung and filamentous algae supported production and development of *An. gambiae* complex.

Discussion

Anopheles arabiensis was the dominant member of the *An. gambiae complex* in the study area. Nearly all breeding of both *An. gambiae* complex species and culicines took place in rice paddies within 350m zones from the edge of the paddies closest to human settlement. Both types of mosquitoes were collected together and were more likely to be found on the edges of paddies compared to the centres. Most aquatic invertebrates were also more frequently found in rice fields close to villages. In contrast, fish were more common in rice fields close to the river. It is impossible to be certain that fish are important predators of mosquitoes in this setting since mosquitoes may simply colonise different habitats to fishes. Here the paddies containing highest numbers of mosquitoes were situated closest to the village and were demarcated by raised embankments to prevent salt water encroachment, which could have also prevented fish from getting in them. Both cow dung (Organic manure) and algae (Biofertilizer) favoured larval breeding of both anopheline and culicine species. These results suggest that since most cow dung was found in paddies close to the settlement, this may also have contributed to higher numbers of larvae in the same areas. Larval control measures should therefore target rice fields close to human settlements and along the edges of rice paddies for successful source reduction.

Chapter 1

MALARIA AS AN IMPORTANT PUBLIC HEALTH DISEASE

The term malaria designates the diseases produced by the infection with any of the four human parasites of the genus *Plasmodium* (*P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*; Najera *et al.*, 1996). These parasites are transmitted from man to man by the bite of a female mosquito of the genus *Anopheles*, and are very selective of their vertebrate host, so that human malarias have no animal reservoir. Exceptionally, mainly as laboratory accidents, some plasmodia of monkeys have infected man, and only chimpanzees and a few South American monkeys can be infected with human parasites, to serve as laboratory animal models of the malaria infection. By extension, the term malaria is also applied to the infections produced by other species of plasmodia in their respective hosts. Congenital malaria transmission, although possible, is quite rare (Najera *et al.*, 1996).

The life cycle of malaria parasites passes through a sequence of three different types of reproduction: 1) a single period of sexual reproduction, called the "sporogonic cycle", taking place in the *Anopheles* host; 2) a period of asexual reproduction, called the "pre-erythrocytic cycle", in a liver cell of the human host; and 3) an indefinite number of cycles of asexual reproduction, called the "erythrocytic cycle", in the red blood cells of the human host. During this erythrocytic cycle some parasites develops into male and female gametocytes which, if taken in with the blood meal of an *Anopheles*, will initiate the sporogonic cycle. The sporogonic cycle takes between 9 and 30 days or longer depending on the parasite species but, even more importantly, on the temperature. The gametocytes present in the blood meal mature in the stomach of the mosquito and, after fertilization, produce a motile egg that penetrates and encysts in the stomach wall, where it divides into about 1,000 motile sporozoites, which burst into the mosquito's body cavity and invade the salivary glands, from where they are ready to infect a human host in each successive bite of the female anopheline (Najera *et al.*, 1996).

There are about 400 species of *Anopheles*, but only about 60 are vectors of malaria under natural conditions, some 30 of which are of major public health importance (Wernsdorfer & McGregor, 1988). Not all species of anophelines are vectors of malaria and, even among those that are vectors; there are great differences in their ability to transmit the disease. The habitat of the immature *Anopheles* is water. Eggs are laid on or on the edge of water and hatch in about 2-3 days into larvae, which develop through four larval and one pupal aquatic stage to produce adult flying mosquitoes. Only the female mosquito bites, and it requires blood meal for the maturation of the eggs; the male feeds on plan and vegetable juices. Mating takes place only once soon after emergence of the adult female, the female stores the spermatozoa in a genital organ called spermatheca, from where they are released to fertilize successive egg batches (Najera *et al.,* 1996)

The aquatic stages commonly last between 7 and 14 days but this depends highly on temperature. The adult female may live from a few days to month or more; going through several cycles of blood feeds and egg laying (some 100-200 per batch), every 2-4 days; survival and egg development are mainly dependent on temperature and relative humidity. Under extreme climatic conditions mosquitoes may go into hibernation which allows the survival of the species through the winter in temperate climates, or long dry seasons in tropical arid regions (Najera *et al.,* 1996).

There are considerable variations in larval habitats. Different species will breed in water habitats ranging from permanent to transient collections, from fresh to brackish water, from standing waters to flowing canals and open streams, from large open marshes to the very small water collections between the leaves and plant axils, rock or crab holes, human and cattle foot prints or discarded artificial containers; car tyres, from open sun to deep shade, from very shallow pools to deep wells, from clean drinking water to water highly polluted with organic matter. The characteristics of breeding places are, therefore defined narrowly for every particular mosquito species, so that larval habitat characteristics can be used for mosquito species control.

Seasonal variation in the availability of specific breeding sites for specific species as well as the great influence of weather conditions on mosquito activity and survival are, to a large extent, responsible for the marked seasonality observed in mosquito densities and malaria transmission in most areas, especially in Africa. Mosquitoes also show some behavioral characters in host selection and resting. Mosquitoes choosing to feed on human or animals and frequency of their feeding are important factors that determine the chances of malaria transmission. Human habitations or domestic animal shelters, particularly those with thatched roofs, abundant cracks in wall surfaces and dark corners, provide good and, for some species, preferred resting places for mosquitoes to digest their blood meals and mature their eggs; as such, they favour mosquito development and survival (Najera *et al.,* 1996).

Of the four *Plasmodia* species that infect man: *P. vivax* causes benign tertian malaria, *P. malariae* causes quartan malaria, *P. ovale* causes ovale tertian malaria and *P. falciparum* causes malignant tertian malaria. However, the most important types of malaria are the benign tertian and malignant tertian cause by *P. vivax* and *P. falciparum* respectively. Vivax malaria is widely spread in tropics and sub-tropics and was also present in some temperate regions, especially in Europe. Falciparum malaria is found most commonly in warm moist regions- such as tropical Africa and Southeast Asia. It is found in parts of Middle East, Northern parts of South and Central America and in all these regions, it is the dominant form of malaria. Quartan malaria occurs through out the tropics. Ovale tertian malaria the, least common occurs in East and also in West Africa (WHO-UNICEF, 2005).

The global burden of malaria

In spite of all preventive and control methods available today, malaria still remains and may continue to be a major public health problem worldwide especially in tropical countries for the next decade or two. About 107 countries and regions are at risk of malaria transmission. The World Health Organization estimates that there are more than 1.1 million deaths due to malaria each year mostly among children less than five years (WHO, 2000). At least 85% of deaths from malaria occur in Africa, 8% in South East Asia, 0.1% in the Americas, 5% in Eastern Mediterranean and 1% in the western Pacific (Arrow *et al.*, 2004). A recent study put the number of cases from falciparum malaria at 515 million in 2002 (Snow *et al.,* 2005). Of all the malaria cases that occur worldwide annually, 75% are caused by *P. falciparum.* Malaria is also a major cause of anemia in children under five years and pregnant women, premature births, low birth weights for new born babies and increased infant mortality rates. In countries where malaria is endemic, it accounts for 25-35% of all outpatients department visits, 20-45% of all hospital admissions and 15-35% of all hospital deaths thereby posing great disease burden in already weaken health care systems.

The cost of malaria prevention and treatment is huge. There are great economic losses attached to malaria as a result of a decline in productivity due to hours lost each day from people suffering from the disease, and those people caring for them, and the loss is even higher as result of death due to the malaria. Malaria will inevitably affect trade and movement of people and foreign investment, for example tourist will not travel to regions or countries with high malaria risk (Gallup & Sachs, 2001). While an increase in the risk of malaria is expected in the near future, due to climate and environmental change (Martens 1998a), there has been indications that at least in some areas of Africa, general infant and malaria specific mortality may be declining, often due to specific interventions that are in line with social development and general education. Studies in Congo and Burkina Faso in the late 1970's indicated that malaria specific mortality could be lower than expected in areas where some decades ago malaria constituted a major cause of infant mortality. The authors (Carme *et al.*, 1984; Quinet *et al.*, 1987; Trape, Zoulani *et al.*, 1987; Vaise *et al*, 1981) attributed their findings to the widespread, even if indiscriminate, use of antimalarial drugs, often at doses which may not be adequate to ensure parasite clearance, but may achieve clinical cure and prevent death, even if collectively they will increase the drug pressure over the overall parasite population and may, therefore, be contributing to the selection of resistant parasites.

The resistance of the parasites to antimalarials has complicated the control of malaria worldwide, especially in Africa. One of the reasons for resurgence and increased burden of malaria is the development of resistance to traditional first line anti-malarial drugs for treatments such as Chloroquine and Sulfadoxine pyrimethamine (SP or Fansidar) by *P. falciparum*, the parasite that causes the severe form of malaria, and as such, this spread of drug resistant has raised the cost of treatment for the disease (White, 1998). Resistance to antimalarials has been responsible for increase in morbidity and mortality in many sub-Saharan countries (Fig 1.1; Snow *et al.,* 2001). Resistance level of Chloroquine (CQ), the first line recommended drug, of 17-30% have been reported (Wellems & Plowe, 2001). A recent study reported 60% resistance to CQ in Faladjie, in Mali, West Africa (Sangho *et al*., 2004).

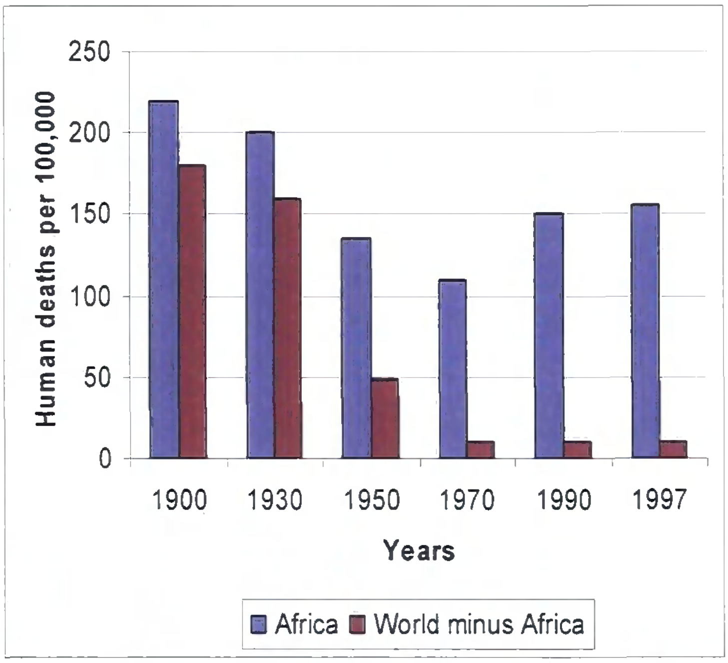

Fig 1.1: Human deaths due to malaria: Annual mortality rates since 1900 Source: (WHO-UNICEF 2005)

Roll back malaria

Malaria continues to be a pressing public health concern for populations and governments in sub-Saharan Africa (SSA) as stressed recently by several African leaders at the Arusha (Tanzania) meeting of the Global Funds for AIDS, TB and Malaria in November 2004 (Malaria consortium meeting 2004) and 3rd Pan-African conference on malaria of multilateral Initiative on malaria November 2002 in Arusha, Tanzania. The Abuja declaration on 25th April 2000(www.rbm/who/docs/abuja 17) and the Millennium Development Goals (www.mdgs.e4gr.org/index/html 12), have both increased the political commitment for malaria control and prevention. The need to increase coverage on the use of the most effective and available interventions to achieve high impact amongst the high-risk groups, especially pregnant women and children less than five years has been high on the agenda.

Recognition of the unacceptable mortality and morbidity from malaria and malaria-related illness in WHO Africa region, and the availability of a number of evidence based, cost-effective interventions, led to the formation, in 1998, of the Roll Back Malaria Initiative (www.rbm.who.int/wmr 1998). This initiative was created under the auspices of the WHO, United Nations Children's Funds (UNICEF), World Bank and other partners to support countries particularly in SSA in tackling the effects of malaria. The Initiative sets out clear goals and objectives to reduce the toll of malaria through public-private and sustainable actions towards strengthening country health system through the following interventions and service delivery areas:

- Supporting, promoting and ensuring access to correct, affordable and appropriate malaria treatments especially for young children within 24 hours of the onset of the disease
- Prevention and control of malaria in pregnancy for pregnant women through the support and promotion of preventive measures such as intermittent preventive treatment (IPT), especially those in their first pregnancies.
- Supporting and promoting access to a suitable combination of personal and community protection measures such as insecticide- treated nets (ITNs)
- Prediction and containment of malaria epidemic

Malaria burden in Africa

Malaria in SSA is a problem of dimensions unlike those seen anywhere else in the world today. Approximately 90% of all malaria cases and 90-95% of all malaria related deaths are in Africa (WHO, 2005). In some areas of SSA people receive about 200-300 infective bites per year (Beier *et al.*, 1990; Molineaux & Gramicia 1980). The population in Africa at risk of malaria infection is estimated at 66% and contributes 59% to the global malaria burden. In 2000, malaria was the major cause of 18% (803,000) deaths in children under 5 years in SSA (WHO-UNICEF, 2005). The economic burdens of malaria in Africa are huge. It has crippling effects on the continent's economic growth and perpetuates vicious cycles of poverty. The disease cost Africa US$10 to 12 billion each year in lost domestic product (Sachs and Malaney, 2002). At macroeconomic level, annual economic growth in malarious countries between 1965 and 1990 ranged between 0.4% of gross domestic product (GDP) per capita, compared with 2.3% in the rest of the world (Sachs and Malaney, 2002).

The economic burden of this disease on human populations in malarial regions is devastating. As Sachs & Malaney (2002) describe: "*The numbers are staggering: there are 300 to 500 million cases every year; and between one to three million deaths, mostly of children, attributed to this disease. Every 40 seconds a child dies of malaria, resulting in a daily loss of more than 2,000 young lives worldwide. These estimates render malaria the pre-eminent tropical parasitic disease and one of the top three killers among communicable diseases*"

Social scientists, especially economists, have studied malaria's social and economic impacts at several scales, peering inside families, looking across households and communities, and comparing entire nations and continents. What these researchers have found is remarkably consistent, that is malaria imposes substantial social and economic costs and impedes economic development through several channels, including quality of life, fertility, population growth, savings and investment, labour productivity, premature mortality, and medical costs (Sachs & Malaney, 2002). Economists have tried to establish a monetary value on this burden by measuring the impacts on households, health systems, and national economies.

At the household level, malaria imposes both direct and indirect costs. Direct costs include time lost from work as well as the cost of medical treatment (including transportation and medical care). Indirect costs, which are in many instances difficult to measure, include loss of work efficiency and time. For young children in particular, indirect costs also include nutritional deficiencies, cognitive and educational disabilities, and physical retardation. Pain and suffering are clearly substantial costs of malaria, but are perhaps most difficult to quantify and monetize. In general, long-term effects, such as child development and compromised immunity, are unknown (Hutubessy *et al.*, 2001).

These direct and indirect impacts can collectively impede economic development and growth. Malaria is estimated to decrease annual per capita GNP growth by 0.25-1.30% in tropical countries where malaria exists, after accounting for initial endowments, overall life expectancy, and geographic location (Sachs & Malaney, 2002). To the extent that slow economic growth limits malaria-control funds, there is a vicious cycle of poverty and this is why malaria is considered both disease of poor and cause of poverty as it diminish economic opportunities for huge numbers of people. Figures 1.2, 1.3 and 1.4 illustrate morbidity and mortality due to malaria in Africa.

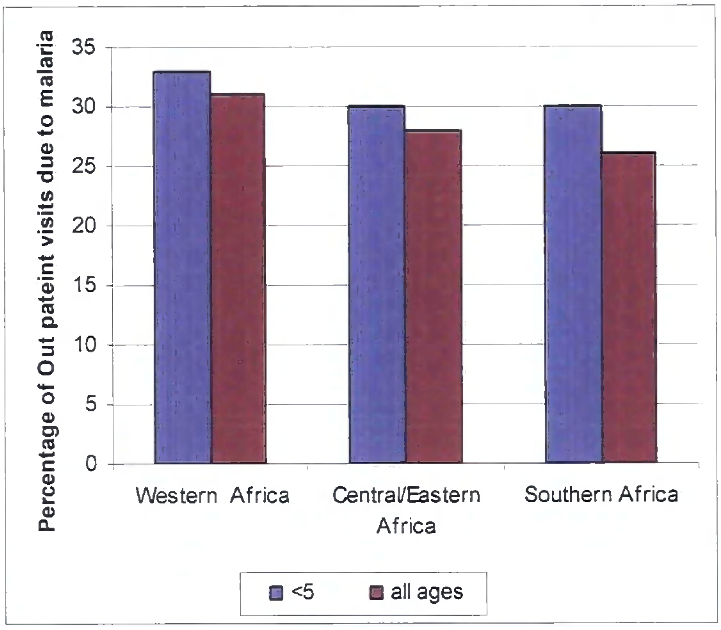

Fig 1.2: Percentage of outpatients visits due to malaria in Africa (WHO-UNICEF, 2005)

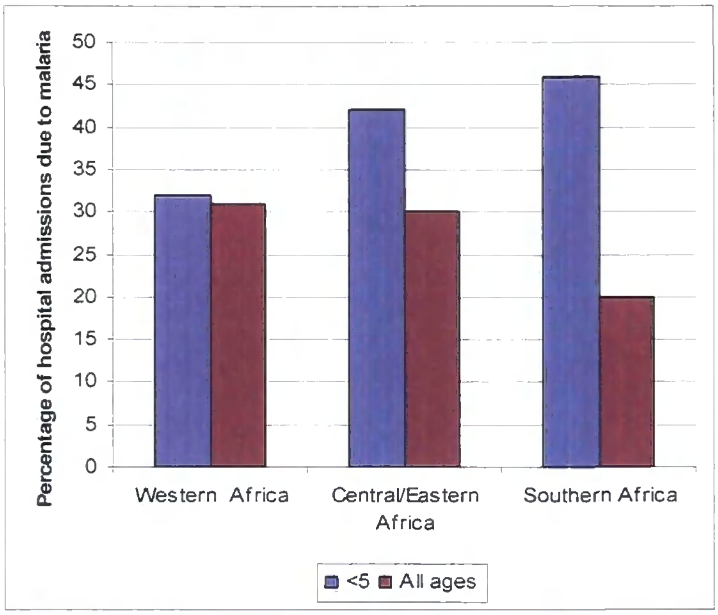

Fig 1.3: Percentage of hospital admissions due to malaria in Africa (Data from WHO-UNICEF, 2005)

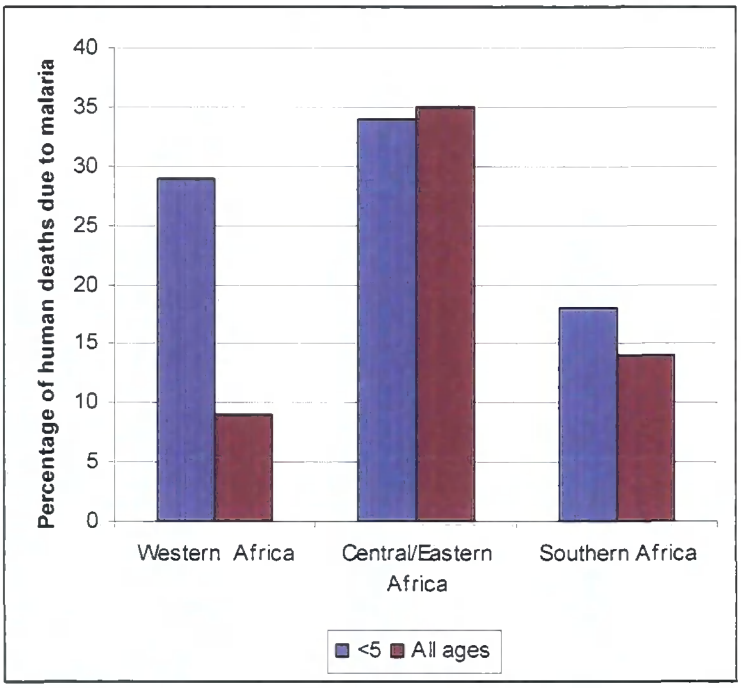

Fig 1.4: Percentage of human deaths due to malaria in African region (Data from WHO-UNICEF, 2005)

Malaria vectors in Africa

The main determinants of the dynamics of malaria transmission in Africa are the great efficiency of Africa's malaria vectors as compared to other regions. The vector population in SSA where the greatest burden of malaria on the continent is experienced are uniquely effective, with six species of the *An. gambiae* complex being the most efficient vectors of human malaria in the region and are often considered the most important in the world. *Anopheles arabiensis* is generally more zoophilic (feeding on animals) and exophilic (resting outdoors) than *An. gambiae* (Gillies & Coetzee, 1987). Nevertheless both are considered endophilic (resting indoors) and endophagic (biting humans indoors) (Mnzava *et al*., 1995; Faye 1997). However in the Sahel area of Senegal, the anthropophagic rate of *An. gambiae s.s.* and *An. arabiensis* were similar during the rainy season: 61.9% and 58.5% respectively. In addition 35.2% of *An. gambiae s.s.* and 28.1% of *An. arabiensis* fed on cattle. The vectors exploit the behaviour of the human population, that remain outside of houses during sunset and sunrise when the activities of mosquitoes increase the chance of man-vector contact and consequently malaria transmission. *Anopheles merus* is a salt water breeder but is limited to brackish lagoons and swamps along the coast of East Africa in Kenya, Mauritius, Mozambique, and Somalia. It is occasionally found in habitats in Zimbabwe and Tanzania in East and South Africa (Gillies and De Mellion, 1968). The biting behaviour of this species is similar to *An. gambiae s.s.* but is more zoophilic (feed mainly on animals) and exophilic (rest mainly outdoors) than its sister species *An. melas* (Coluzzi 1984). Both species should be considered potential vectors of malaria in their own rights. *Anopheles quadriannulatus* occurs in Ethiopia, Zimbabwe, Mozambique, Tanzania in East Africa and Southern Africa (Hunt *et al.,* 1998). It feeds mainly on cattle and this characteristic makes it a less efficient malaria vector in most parts of Africa, although there may be some exception. There is also a quadriannulatus type found in Ethiopia.

Anopheles gambiae s.s and *An. arabiensis* are widely distributed members of *An. gambiae* complex (Lindsay *et al.* 1998; Coetzee *et al.*, 2000). *Anopheles arabiensis* is more widespread (White, 1974; Coetzee *et al.*, 2000, Rogers *et al.,* 2002) and is endemic in drier Savannah areas. *Anopheles gambiae s.s.* on the other hand is more wide spread in humid Savannah and forest areas of Africa, but its distribution does extend into some dry savannah regions (Faye *et al.* 1997). In West Africa for example, *An.gambiae s.s.* occurs in a wide range of climatic zones, from the Sahel to forest areas (Petrarca *et al.,* 1987).

Although *An. gambiae* complex breed in different varieties of water bodies, the most important ones are shallow open water pools (Gillies & DeMeillon, 1968). The creation of such pools may range from borrow pits, drains, brick pits, car tracks and hoof-prints around ponds and water holes and those resulting from over flow of the river, pools left by receding rivers and rain water collecting in natural depressions. The stamp of human activities is clear in many of these sites, either directly or, by overstocking and subsequent loss of plant cover, erosion and scoring.

Anopheles gambiae s.s. breeds in rice fields (Mukiama and Mwangi, 1989; Mutero *et al.*, 2000; Ijumba *et al.*, 2002). It readily feeds on human hosts, indoors and outdoors, but rests mainly indoors. It also feed on animals where its preferred human host is not available. *Anopheles arabiensis* thrives best in open sunlit temporal pools such as rice fields and rice nurseries. It is widespread in Africa but often prefers drier savannah areas. It is more zoophilic (feed more on animals) and exophilic (rest mainly outdoors) than *An. gambiae s.s.*, but both species are endophilic (rest indoors) and bite humans indiscriminately indoors (endophagic) and outdoors (exophagic). *Anopheles melas* often colonizes highly saline areas. It is restricted in its distribution to the areas of brackish water conditions of mangrove swamps in West Africa (White, 1974). The population of this species also peak in the wet season when the flooding and rainfall combined with tidal water from the river to create ideal breeding sites. It is more dominant in and around domestic animal shelters than human houses (Bryan *et al.* 1987). This probably indicates its preference for feeding on domestic animals. However, in places where *An. melas* occur alone, it can be an important vector of malaria especially where humans are the only host available.

Rice cultivation in Africa

Africa's lands are too dry for rain-fed cultivation of crops to meet the growing demands from its population today. This is because rainfall in many parts of Africa is erratic and variable. Therefore as demand for food increases, land area for potential irrigation schemes are also expected to increase.

The African continent has the highest population growth rate in the world today (www.faostat.fao.org) WRI/UNEP/UNDP (1995). Population growth in Africa is estimated to be 18 million yearly (Anon 2000). The population of Africa is expected to grow from its current level of 700 million to 1.2 billion in 2030. One-third of Africa's population is now estimated to be living in big cities as population increases, (World Bank 1999). In such a situation it is unlikely that many people will have the means of food production by themselves as urbanization increases. Urbanization results in an increased demand for food and so many African countries spend a high proportion of the already struggling foreign exchange reserves on importing foods to meet demands from their growing populations. A report from WRI/UNEP/UNDP in 1995, put food importation by African countries at 90% of total food consumption in the last 20 years and this scenario is expected to increase from 9 million metric tons in 1990 to 29 million metric tons in 2020. However, despite this increase, malnutrition in African children is expected to increase by 14 million during the same period of time (Rosengrant & Perez, 2000). This pressure on many already weakened economies of African governments led to designing alternative solutions for improving and expanding agricultural productivity through large scale rice irrigation schemes. One way to increase food production is by irrigation.

Importance of rice to Africa

Rice originated in an area extending from central India to China and has now become the world's most important cereal. It is the staple food for nearly half of the world's population (2.7 billion), providing about 35-60% calories consumption (Guerra *et al.* 1998). Over 70% of the world's rice production is in irrigated schemes, mostly in Asia. The introduction of early maturing and high yields varieties in 1960s resulted in a marked increase in yields compared with traditional varieties. Out of the 700 million rice consumers world wide, 35 million (5%) are in Africa (Table 1.1) and the near East (Grist, 1996). In 1996, Africa consumed 11.6 Metric tonnes of milled rice per year, 33.6% of which was imported. Between 1989-1991 and 1995, milled rice import trends showed a small decline of 55,000 tonnes as paddy production increased by 2.3 Metric tonnes over the same period. The regional trends in rice imports show that the small decline in imports could be attributed to the decrease in imports in East Africa. West Africa and Southern Africa showed upward trends in rice imports. Demand for rice is particularly high in West Africa, and is increasing at an annual rate of 5.6% (WHO, 1997). It is the most important staple food in sub-Saharan countries, especially in The Gambia, Liberia, Sierra Leone, Mauritania, Guinea, Guinea-Bissau, Madagascar, and Senegal, where it is eaten everyday and sometimes three times a day. Studies revealed that in The Gambia, rice consumption was about 78 Kg/person /year (Kagbo, 1993). Similar findings in Burkina Faso showed that households obtained 33% of their cereal consumption from rice representing 45% of their annual income expenditure on cereals (WARDA/PEEM, 1993; WHO 1997).

Cultivation of rice is dispersed all over West Africa as swamp and upland rice where 60% is grown under rainfed or upland conditions support by irrigation schemes. Its production has steadily increased over the years since in 1970s reaching almost 7 million metric tons of milled rice by the end of last decade This increased production has been due to expansion in area of cultivation and 30% due to yield increased (Fagade, 2000). Estimated annual rice production is about 1.5 million tones while consumption is about 1.9 million tonnes. West Africa accounts for 51.5 percent of the total quantity of rice consumed in Africa. Some countries import more than 50 percent of their annual consumption, including Benin, Burkina Faso, the Gambia, Ghana, Liberia, Mali, Niger and Senegal. Nigeria is the biggest producer in the region and imports only 16.2 percent of its requirements.

The annual rice production in West Africa is not commensurate with the annual rice requirement for the population. This necessitates the annual importation of 400,000 tonnes which has had a negative impact on the already crippling economies of African countries, especially those in West Africa (www.warda.org).

Table 1.1: Rice production in Africa (2003) (Data from www.faostat.fao.org), Mt= Metric tonnes

Country	**Planted area of Rice (ha)**	**Rice Production (Mt)**	**Yield (Mt./ha)**
Algeria	200	300	1.5
Angola	20,000	16,000	0.8
Benin	30,000	66,000	2.2
Brunei Darussalam (Africa)	240	400	1.67
Burkina Faso	51,000	97,103	1.9
Burundi	19,500	63,000	2.23
Cameroon	24,842	78,678	3.17
Central African republic	14,000	27,000	1.96
Chad	80,000	112,000	1.4
Congo, Republic of	500	1,000	2
Congo, Dem.Republic	413,685	314,614	0.76
Cote d'Ivoire	510,000	818,000	1.6
Egypt	615,000	5,800,000	9.43
Ethiopia	8,350	15,500	1.86
Gambia	10,000	20,500	2.05
Ghana	150,000	316,000	2.11
Guinea	525,000	845,000	1.61
Guinea Bissau	65,000	97,000	1.49
Kenya	11,000	50,000	4.55
Liberia	120,000	112,000	0.92
Madagascar	1,219,400	2,800,000	2.3
Malawi	56,000	86,882	1.55
Mali	400,000	693,203	1.73
Mauritania	16,975	77,409	4.56
Morocco	2,300	8,100	3.52
Mozambique	178,992	200,439	1.12
Niger	27,800	76,500	2.75
Nigeria	4,900,000	5,952,000	1.01
Rwanda	7,607	27,891	3.67
Senegal	75,215	177,756	2.36
Sierra Leone	200,000	250,000	2.94
South Africa	1,400	3,200	2.29
Sudan	4,800	15,000	3.13
Swaziland	50	170	3.4
Tanzania	325,939	640,189	1.96
Togo	35,000	68,100	1.95
Uganda	80,000	120,000	1.5
Zambia	10,000	12,000	1.2
Zimbabwe	250	600	2.4

Imports of rice in West Africa stood at 2.6 million tonnes in 1990-1992 representing an estimated $750 million in share of foreign exchange from the region. Further projections from FAO put imports to increase to US$4 million tonnes by the year 2000, raising the annual foreign exchange outflow to more than US$1 billion. With increased commitment from governments in Africa, rice production has steadily increased but was not commensurate with demand from growing population. This also resulted in an increase in annual importation of rice (Fig 1.5).

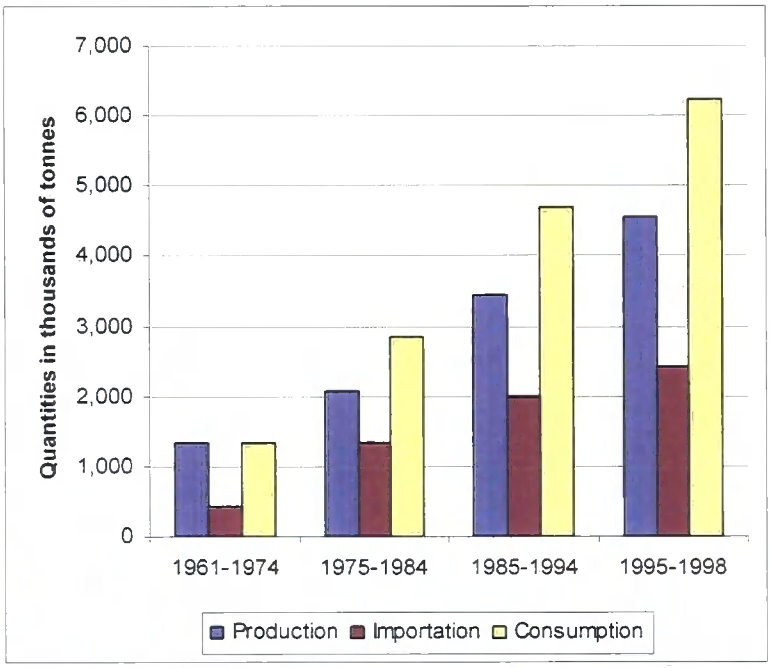

Fig: 1.5 Rice production, import and consumption in West Africa in tonnes (www.faostat.fao.org 2003).

Rice irrigation and malaria transmission in Africa

Water resource development for agriculture in Africa includes irrigation schemes on all levels. Irrigation for rice production in SSA has more than doubled in the last three decades and the potential to further develop rice harvest areas is considerable (Sissoko *et al.*, 2004) as growth in production achieved in the 1990s has been due solely to an expansion of cultivated area at an annual rate of 3.5% (Lancon and Erenstein, 2002). The rapid population growth rate of African countries has resulted in an increased demand for food and this prompted governments to developed programs to increase food production by initiating irrigation schemes (Ijumba *et al.*; 2001, Ijumba and Lindsay 2002).

Several studies have demonstrated that transformation of available lands into irrigated areas for rice production might create suitable habitats for large populations of disease vectors. Prominent among these are the anopheline mosquitoes responsible for malaria transmission (White, 1972, Carnevale *et al.,* 1999, Ijumba and Lindsay, 2002). We know for certain that introduction and expansion of rice cultivation through irrigation schemes produces major ecological impacts. There is an increase in surface breeding sites which may favour some species and often change their predominance. The result of such ecological changes may mark an increase or decrease in malaria transmission. In Africa however, many studies have shown different results on the effect of rice irrigation on malaria incidences. In the Benoue Valley of northern Cameroon for example, malaria incidence increased after large scale irrigation development following completion of the Lagdo dam (Robert *et al.,* 1992). The overall malaria incidence in Tigray region of Ethiopia was seven times higher in villages close to dams compared with control villages (Ghebreyesus *et al.,* 1999)

The Gezira irrigation scheme developed in Sudan to grow cotton increased malaria by providing breeding sites for *Anopheles* in its canals. In Mali in West Africa, surrounding areas have seasonal transmission (holoendemic malaria) but in areas within an irrigation scheme, malaria transmission was close to perennial because mosquito breeding occurred throughout the year (Dolo *et al.*; 2004). Rice growing through irrigation particularly in the semi-arid savannah zone of Africa can alter the malaria transmission pattern from seasonal to perennial (Dolo *et al.*, 2004, Sissoko *et al.*, 2004).

Although irrigation is often deemed to be followed by malaria outbreaks in other continents, in Africa however, this may not always be the case. Many irrigation schemes are constructed in holoendemic malaria areas which will increase mosquito populations and the biting nuisance but may have little effect on malaria transmission. One possible explanation for this is that introduction of irrigation for rice production results in creation of wealth for such communities (Robert *et al.,* 1985; Audibert *et al.,* 1990; Boudin *et al.,* 1992). Such wealth can help local communities to improve their living standards and improve affordability to use better protective measures such as insecticide treated bed nets against mosquito bites and access to health care services promptly. Rice cultivation has greatly increased *An. gambiae* populations in West Africa, yet the pattern of malaria transmission remains largely unaffected (Ijumba & Lindsay 2001). Recent studies have concluded that for most parts of SSA that are characterized by stable malaria transmission, the introduction of irrigated agriculture has no or only little impact on malaria transmission (Ijumba & Lindsay, 2001; Sissiko *et al.*, 2004). In Burkina Faso for example, where vector densities are 10 times higher in irrigated rice areas, the sporozoite rates are 10% of those in surrounding savannah areas, resulting in similar inoculation rates in both areas (Robert *et al.*, 1985, Favia *et al* 1997). In Burundi, at intermediate altitudes, rice cultivation has been linked to an increase in the population of *An. arabiensis* but malaria incidence has remained unchanged (Cooseman *et al.*, 1984).

Chapter 2

MALARIA TRANSMISSION IN THE GAMBIA

The Gambia is a small country in the Sahel region of West Africa with an area of about 11,480 km^2. Almost 10% of the land area is covered by the Gambia River and another 20% by swampy land and flood plains. The Gambia is bordered by Senegal on three sides except on the west coast bordering the Atlantic Ocean. The river stretches about 400 km eastwards and a narrow strip of land extends 15-30 km north and south of its banks. The country had a population of 1.36 million inhabitants in 2003 (Central Statistics Department, 2003). The climate is typical of the Sahel Savannah with one rainy season from June to October and a long dry season from November to May. Annual precipitation is in the range of 600-800mm. During the rainy season, due to the influence of the sea, temperatures are cooler in the western half of the country than the eastern parts. Temperatures are high during the dry season; mean monthly temperatures are 38.8-40°C. In general the country's mean temperature varies from 25 to 30°C in the western parts of the country to 35 to 40°C in the eastern parts of the country. Salt water intrusion occurs at the lower reaches of the river due to tidal influences. During the dry season, this intrusion can travel as far as 200km inland giving rise to large areas of salt marsh and mangrove forest immediately adjacent to the river. This situation is reversed during the rainy season as a result of high precipitation and flow of fresh water from the Futta Jallon highland that makes the river and adjacent flooded areas less saline (Sylla *et al.,* 1995).

Soils and Topography

The Gambia forms part of the tertiary continental plateau which is dissected by the River Gambia and its tributaries. To the east, the plateau forms extensive surfaces at an altitude of 40-50 meters and is dissected by rather narrow valleys. The River Gambia basin is subjected to a transitory tropical climate in the southern part (Guinea and to purely tropical climate in the northern part (Senegal). Two main types characterize the soil structure of The Gambia: sandstone covered with woodland savannah and farmland, and the alluvial deposits along the River Gambia (Trolldalen, 1991). In general, the soil in The Gambia is exposed to long dry conditions (November to May) causing it to lose its porosity. As rainfall creates water bodies such as pools in the floodplains that last longer, such conditions support the breeding of malaria vectors and hence maintain malaria transmission.

Anopheles gambiae complex in The Gambia

All the important malaria vectors in The Gambia belong to the *An. gambiae* complex which includes *An. gambiae s.s., An. arabiensis and An. melas* in The Gambia (Bryan, 1993, Lindsay *et al.,* 1993; Table 2.1). In most areas of The Gambia, *An. gambiae s.s* and *An. arabiensis* are the dominant species. *Anopheles arabiensis* is found in small numbers in the drier areas of the north bank of the river and has been found in the dry season in areas where rice cultivation is done by irrigation (Bryan *et al* 1987). Recent findings suggest that around 30% of the *An. gambiae* complex in Farafenni area (North Bank of The Gambia about 100km from the capital Banjul) is *An. arabiensis* (C. Green and M. Kirby unpublished data). In the western half of the country where the water is saline and where there are large mangrove swamps, *An. melas* is the dominant species and although it is not a principal malaria vector in The Gambia, it can play a crucial role in malaria transmissions (Bryan, 1983).

Relatively few studies have investigated larval breeding sites in The Gambia. Bertram *et al.* (1958) carried out a study on the coast and showed that freshwater *An. gambiae s.l* were breeding near a small stream and in puddles created by car tracks and *An. melas* in flooded areas beneath or near mangroves. More recently the major breeding sites of *An. gambiae* complex were found on the landward edge of flooded alluvial sediments near the river (Thomas and Lindsay, 2000). In 1996-1997, Bøgh *et al.* (2003) confirmed that the major mosquito breeding sites of the *gambiae* complex are flooded alluvial soil bordering the river, extending 1.5km into flooded areas (plains). Polymerase Chain Reaction analysis of mosquitoes collected along transects surveyed in that study revealed that *An. melas* was the dominant species (81.5%) followed by *An. gambiae s.s* (18%) and *An. arabiensis* (0.5%). The study also indicated that by sampling in specific habitat, *An. arabiensis* was breeding mainly in rain fed rice fields along the edge of alluvial soil. The breeding, feeding and resting behaviours of *An. gambiae* complex species in The Gambia are somewhat similar. *Anopheles gambiae s.s.* and *An. arabiensis*, the two principal malaria vectors breed well in open, sunlit fresh waters (pools, rice fields, flood plains, puddles, and in both human and animal foot prints). *Anophele gambiae s.s.* feed mainly on humans but like *An. arabiensis* also take blood meal from animals where preferred host is not available. Both species rest indoors and outdoors. *Anophele melas* on the other hand is a salt water breeder and colonize highly saline areas of the flooded plains bordering the river (White, 1974). It is usually restricted in brackish water conditions of mangrove swamps in West Africa where salinity can be up to 70% of seawater.

Table 2.1: Characteristics of malaria mosquitoes in The Gambia

***Anopheles* Species**	**Resting sites**	**Host preference**	**Breeding sites**	**Feeding Time**
An. gambiae s.s	Indoor, Outdoor	Mainly human & domestic animals	Sunlit temporary pools, ponds Rice fields, Flood plains, Hoof prints, human foot prints, puddles.	Mainly after midnight
An. arabiesnsis	Indoor, Outdoor	Mainly human & domestic animals	Sunlit temporary pools, Rice fields, Rice nurseries	Mainly after midnight
An. melas	Indoor, Outdoor	Mainly human & domestic animals	Salinity of up to 70% seawater Lagoons, Mangrove swamps	Mainly after midnight

Malaria transmission pattern in The Gambia

Malaria transmission in The Gambia, as in many other parts of the Sahel, is characterized by very focal and seasonal transmission. Breeding sites of mosquito larvae are often restricted by the low level of precipitation (Julvez *et al.*, 1992). As a result of this there are geographical variations in the distribution of malaria in The Gambia (Thomson *et al.*, 1996; Touré *et al.*, 1996; Julvez *et al.*, 1997). Malaria transmission is brief but often intense from July to the beginning of December when the mosquito populations decline rapidly (Lindsay *et al.*, 1993). Intensity of malaria transmission is greater in the eastern parts of the country, Central River Division (CRD) and Upper River Division (URD) (Malaria Situation Analysis Report (MAS), Department of State for Health, (DOSH) 2002). It is one of the leading causes of morbidity and mortality in The Gambia; especially among children less than five years. Twenty percent of antenatal consultations and 40% of under-five visits in Maternal and Child Health services are due to malaria (MSA, DOSH, 2002). Although the economic burden of malaria in The Gambia has not been quantified, malaria leads to lost days of productivity, absenteeism from school and increased household expenditure on health. It is the most frequent cause of death especially in the rural areas among children under five years. The Malaria Situational Analysis Report 2002 also indicated a mortality rate among children under five years old as high as 105 deaths/1000 and a cause of severe anemia (Hb< 5.0g/dl) in pregnancy. The actual proportion of anaemia cases that can be attributed to malaria is unknown. However, severe anaemia increases the risk of death among mothers and infants. From 1992 to 1995, this accounted for between 8-10.5% of all maternal deaths and was also estimated to be a contributory factor in 41.3% of all deaths in the Maternity Unit of the Royal Victoria Teaching Hospital, Clinical Records from Royal Victoria Hospital (1992-1995). Low birth weight (LBW) deliveries are also associated with malaria in pregnancy. Over the last 10 years the prevalence of LBW deliveries in primigravidae at health facilities in rural Gambia varied between 18-30%. The prevalence of placental malaria infection, an indication of maternal infection, was estimated at 26.4% between 1991-1992 in Basse Health Centre, (Clinical Records from Basse Health Center 1991-1992), and 51.1% during the rainy season in 1997 in Bansang Hospital areas in the most eastern part of the country, (Clinical Records from Bansang Hospital, 1997). However, 90% of all malaria cases seen in Gambian health facilities were diagnosed based on clinical manifestation of fever, making these data unreliable.

Rice cultivation and malaria transmission in The Gambia

The development of agricultural systems is the ultimate aim of any developing nation, including The Gambia. In recognition of rice as the stable food in The Gambia, technical assistance with rice cultivation from the Taiwan Agricultural Technical Mission commenced in 1996. Since then the team with Gambian experts have experimented with more than 800 varieties of rice and selected six varieties that are better adapted to the Gambia's lowland environment and to the rain-fed conditions (Lin, 2002). This introduction of high yield varieties of rice over the years has resulted in increased crop yield. However, its impact on malaria transmission is unknown. Compared to the local varieties of rice, the high yield varieties are stiff strawed, and early maturing. An increase in the amount of water and sunlight in fields favours long periods of breeding by freshwater members of the *An. gambiae* complex. Early maturing rice encourages multi cropping, making water available in the fields for longer periods and encouraging more mosquito generations. During the rains (June to October), which is the period for rice cultivation, rice fields bordering the river with saturated clay soil are flooded or partly flooded and temperatures are also higher during these periods (Bøgh *et al.*; 2003). These conditions present open sunlit surfaces that are more favourable for the breeding of members of the *Anopheles gambiae* complex.

Importance of rice to The Gambia

Agriculture is the mainstay of The Gambia's economy. It has the capacity to both generate and save foreign exchange. It is the major source for food and income for the majority of the population (60%). The major food crops in The Gambia are rice, millet, sorghum, maize and horticultural crops. Rice is generally grown on lowland swamps but its production in The Gambia is mainly rainfall dependent. However, it is by far the crop with the highest yield potential of all the crops grown in The Gambia. Of the 558,000 ha available to agriculture annually, 180,000 hectares is planted with groundnut and coarse grains on uplands constituting 32% of the total land areas available to agriculture. Rice cultivation alone constitutes 20% (Marong, 2000).

Rice is the staple food in The Gambia, the cultivation of which started before the time of colonial government. Its cultivation has been encouraged to increase domestic food production in order to reduce costly food imports and to meet the ever-growing population demand. The Gambia has the highest per capita rice consumption (117.33Kg) among Sahelian countries and the third largest in West Africa (WARDA 1993). Statistics from FAO put Gambia's total rice production in 2003 at 20,500 metric tonnes (Table 2.1) while data from WARDA in 2004 put it at 100,000 metric tonnes in 2003. In 1999, total rice production in The Gambia was about 18,992 metric tonnes, 12% of the total rice requirements (Department of Planning 1999), while in 2000, total rice requirements was estimated to be 157,616 metric tonnes. This huge deficit is made up with costly imports which are a big drain on the scarce foreign reserves of the country. Figures from the Central Statistics Department show that in 2000 a sum of 195.6 million dalasi, equivalent to 7 million USD was spent to import 93,931 metric tonnes of rice. It is for this reason that rice has such a prominent role in The Gambia's economy and dietary needs. The development of rice production has been undertaken by the Government of The Gambia and a steady increase in rice production has been registered since 1991 (Fig 2.1).

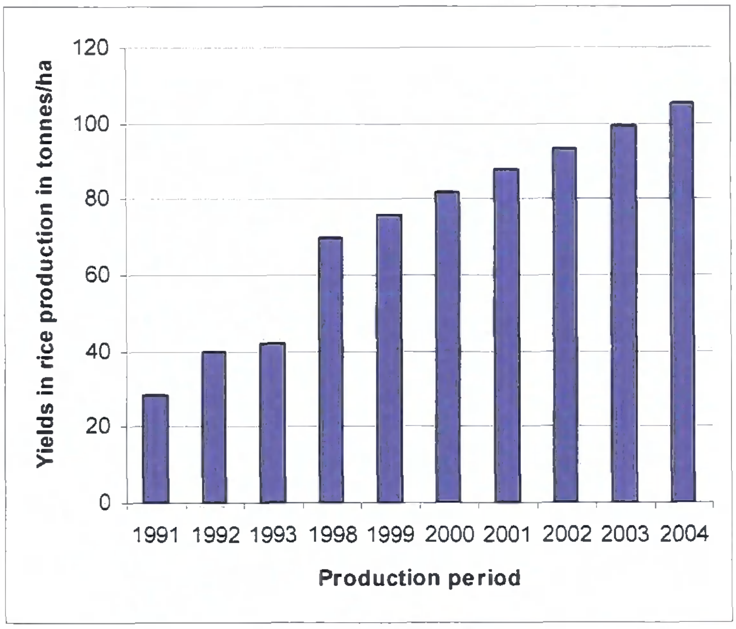

Fig 2.1: Yields in Rice production in The Gambia 1991-2004, Source: WARDA (2004)

Previous studies on rice cultivation and malaria in The Gambia

Rice fields provide a wide range of potential breeding sites for members of the *An. gambiae* complex. Rice production in The Gambia is mainly rain dependent in the wet season, but its production during the dry season by irrigation has been initiated in The Gambia in early 1980s in central parts of the country. Studies since then have shown that dry season cultivation was associated with large populations of malaria vectors (Snow, 1983; Lindsay *et al.*, 1991). For children, increased exposure to malaria infections was associated with living adjacent to breeding sites, thus in both the dry and wet seasons, compounds close to large areas of stagnant water were likely to have larger numbers of *An. gambiae s.1* than those further away (Lindsay *et al.*, 1995).

Study Rationale

A large intervention trial of larviciding against malaria vectors is presently underway in The Gambia. Preliminary findings from this study showed that one of the major habitats for mosquitoes are the rice fields bordering the River Gambia. The aim of this study was to establish precisely where in the rice fields most breeding of the *An. gambiae* complex occurs, at what time of the season and what conditions are required to generate most adult mosquitoes. This information will help direct larval control activities to specific parts of the rice fields at certain times of the year, thereby helping to reduce costs of running such programmes and interventions.

Hypothesis

My primary hypothesis was that the freshwater contained in rice fields provided ideal breeding conditions for *An. gambiae s.s.*, the principal vector of malaria in The Gambia. Moreover, mosquito numbers may be greater in the rice fields because these sites are protected from fish and they may receive fertilizer treatment.

Goal

To determine and describe the preferred breeding habitats and the ecology of mosquito larvae in swamp rice fields in The Gambia.

Specific objectives

1. To determine what species of the *An. gambiae* complex occur in rice fields at different times of the year and in relation to the height of rice;
2. To find out whether mosquitoes are more common in paddies closest to human settlements compared to those more distant;
3. To describe the distribution of mosquito larvae within individual paddies;
4. Determine the biotic and abiotic characteristics of the paddies during the rainy season;
5. Assess the number of mosquitoes being produced in different parts of the rice fields from the nearest settlement;
6. Find out whether artificial and natural fertilisers affect the production of mosquitoes from rice fields.

Chapter 3

UTILIZATION OF SWAMP RICE FIELDS BY MEMBERS OF THE *ANOPHELES GAMBIAE* COMPLEX IN THE GAMBIA

Fig 3.1: Rice fields at the beginning of the rainy season, May/June 2006.

SUMMARY

Background

Rice fields are major producers of malaria mosquitoes in Africa. To date most studies have investigated the impact of irrigated rice on malaria transmission. Here I explore how swamp rice cultivated in the floodplains of the River Gambia is important for the production of *An. gambiae* mosquitoes, the principal vectors of malaria in The Gambia.

Methods

Larvae of mosquitoes were sampled along three transects each 500 metres long, running from the landward edge of the floodplains to the River Gambia, close to Tamba Koto village. Larvae and other invertebrates were sampled using area samplers along each transect running from village to river once a week at distance classes of 0, 25, 50, 75, 100, 200, 300, 400, and 500m. In each paddy, larval sampling was done at three points; near edge, centre and far edge. In paddies where there were no field boundaries sampling points were at least 25m apart.

Results

A total of 375 anopheline larvae were sampled during the study. Of these 80 were members of the *An. gambiae* complex (36 *An. arabiensis*, 23 *An. gambiae s.s.* and 21 *An. melas*). The remainder were assumed to be other anopheline species as they could not be identified by DNA and PRC analysis after 3 tests. Three hundred and fifty-one (94 %) were found in the first half of the rice growing season (August -October). Larvae were more concentrated in paddies within the first 350m sampling zones from the landward edge (93%) compared to paddies that were further away (7 %), and were highest at the edges of paddies compared to centres. There were more larvae collected in small water bodies in rice fields than large ones. Anopheline larvae were associated with culicine larvae and both were found in areas where other invertebrates were also collected. Eighty percent of cow dung was found within 350m sampling zones from the landward edge of transects.

Discussion

Most aquatic invertebrates were concentrated in paddies close to the land. Those closer to the river were subject to tides and fish predation, conditions not suitable for aquatic insects. The high number of anophelines larvae found in paddies closest to the village may be a result of ovipositing mosquitoes laying their eggs in the nearest water body, the more nutritious water bodies found there, the lack of fish predators and the presence of still water. These sites should be particularly targeted during larval control operations.

INTRODUCTION

Rice has been the staple food in The Gambia for several centuries. The demand for rice consumption has explosively increased over the last two decades as the population growth rate has dramatically increased. With a population growth rate of 2.8%, The Gambia Population and Housing Census (Census report 2003), the influx of refugees and immigrants from the sub-region, the per capita rice consumption in The Gambia was the highest among Sahelian countries and third in West Africa (117.33kg/person/year) (Western African Rice Development Association, WARDA 2000).

Rice fields bordering the River Gambia are known as swamp rice and are the most frequently cultivated fields in The Gambia. These are cultivated during the rainy season, which lasts from June to November. Cultivation of rice starts each year in June or July with ploughing of fields and the preparation of rice nurseries. Rice nurseries are small raised beds on or close to the flood plains where rice plants are raised temporally for a period of one to two months before been transferred to the main paddy rice fields. This activity is largely carried out by women, with men helping to transplant rice plants from the nurseries to the main rice field (flooded or partly flooded close to the river) from late August to October.

There have been a number of studies conducted on malaria mosquito production in rice fields in Africa (Ijumba & Lindsay, 2001. These have shown that rice fields are often excellent breeding sites for mosquitoes that are capable of transmitting malaria. Increases in the abundance of *An. gambiae* complex usually correspond with rice cultivation either by irrigation during the dry season or rainfed during the wet season (Lindsay *et al.* 1991). Flooded or partly flooded rice fields present many different water bodies that are capable of acting as breeding sites for *An. gambiae* complex. However the rice fields are most productive when they are first flooded and the height of the rice is short (Muirhead-Thomson, 1951; Holstein, 1954; Surtees, 1970; Snow, 1983; Lindsay *et al.,* 1991). Later when the rice is fully grown and shades the water surface, larval breeding will progressively decline as adult females are deterred from laying their eggs (Snow, 1983; Lindsay *et al.,* 1991)

Recent studies showed that rice fields along the river were an important source of *An. gambiae* complex mosquitoes (S. Majambere, unpublished information). Here I examine the distribution and phenology of these mosquitoes in a traditional rice growing area and attempt to identify the factors governing their presence and absence. This information will provide fundamental information on the importance of rice fields in the ecology of malaria and help direct larviciding activities to specific parts of the rice fields at certain times of the year, thereby helping to reduce costs of running such programmes and interventions.

MATERIAL AND METHODS

Study area

The study was conducted in swamp rice fields of Tamba Koto Village (N: 13° 31.561', W: 15° 31.150'). The study village was situated in the North Bank Region of The Gambia, 11km east of Farafenni town, close to the River Gambia. The population of the village at the time of this study was 315 inhabitants of which 20% were children less than five years of age (The Gambia Population and Housing Census Report, 2003). The area is generally flat, open farmland and woodland savanna which is typical of Sahel savannah. The major agricultural crops cultivated there were groundnuts, rice, millet and sorghum and vegetable gardening is carried out by women. The community is predominantly Mandinka and people live in compounds as extended family members. The area has a short rainy season from June to October followed by a long dry season from November to May as in the rest of the country.

Community sensitization

Village meetings were held on the 26th and 29th June, 2006, with village elders and women groups respectively to explain the purpose of this study. This was done to inform the study population that the study would look at mosquito production in the local rice fields. They were informed about the potential benefits that could be derived from the study and the importance of getting their cooperation and participation for the successful implementation of the study. Farmers whose fields were surveyed during the study were likely to have part of their crops damage by repeated larval mosquito sampling but were recompensed with a bag of rice at the end of the study.

Larval surveys

Three parallel transects, starting 200-240m away from the village, each approximately 500m in length, 20m wide, and 200m apart were sampled at weekly intervals from June 2006 to January 2007, during the rainy season of 2006. Transects were on the alluvial plains of the River Gambia and ran from the landward edge near the village to the river. Larvae were sampled along the transects at the following distances: 0m, 25, 50m,75m, 100m, 200m, 300m, 400m and 500m (Fig 3.2). The sampling distances were dictated by paddy distribution. The transect started at 0m, at the first rice paddy and ended at 500m, close to the river.

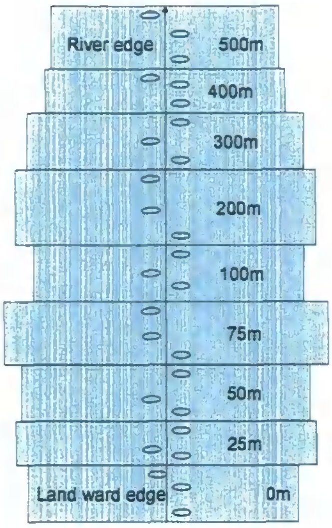

Fig 3.2: Schematic diagram of the sampling frame used along each transect.

Each distance was marked with wooden pegs (Fig 3.3) and geographical coordinates located using (a hand held global positioning system; GPS 12 XL GARMIN, AQUAPAC) at the beginning of the study. At each distance three samples were made with an area sampler (AS): at the edge of the paddy, at the centre and the far edge of the paddy. On each visit sampling was done in different sites of the paddies. This procedure was repeated along each transect weekly.

Fig 3.3: Wooden peg with red flag in one of the study plots

The area sampler (AS) was an open tube made from aluminium with serrated teeth around the bottom lip which allowed it to be buried into the substrate. It measured 39.5cm long with an upper diameter of 47 cm and a lower diameter of 40 cm (Fig 3.4). Area samplers were randomly inserted into water bodies and left for 30 seconds to allow water to settle and for the mosquito larvae to come to the surface. A standard dipper (350 ml) was used to take out all water and organisms that were trapped in the AS and transferred into a white plastic bowl of 35cm in diameter to aid identification. Mosquito larvae were collected and identified as anophelines or culicines. Other free swimming insects trapped in area sampler were also collected, placed in field bottles in 100% ethanol and transported to the laboratory for identification.

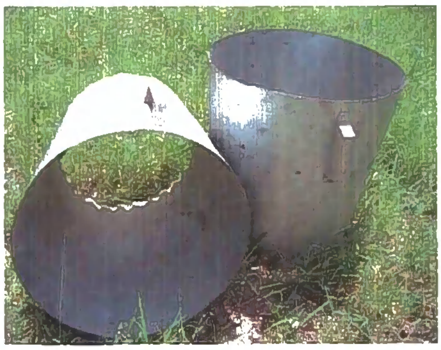

Fig 3.4: Area samplers used for collecting aquatic mosquito life stage

Polymerase Chain Reaction

Species identification of members of the *An. gambiae* complex was done using polymerase chain reaction analysis (PCR; Scott *et al* 1993). PCR was carried out by extraction of DNA from larvae by grinding tissues with a sterile eppendorf micro pestle. The mixtures were incubated at 56°C for 45mins and then increased the temperature to 94°C for 10mins. Samples were centrifuged at 14,000 revolutions per minute (rpm).The top layer of the DNA in solution were removed and placed in a clean 1.5ml eppendorf. These procedures were carried out with 2.5 μl of the DNA extracted in a volume of 25 μl containing 1x PCR buffer (Bio-labs) and 200 uM of each dNTP, and 10pmol/μl primers UN, AR, GA, ME and 0.5 U Taq (Bio-labs). The primers used to identify the mosquitoes from the *An. gambiae*-complex were those of Fanello *et al.* (2002). Thermocycling was carried out at 94°C for 5 minutes, followed by 30 cycles of 94°C for 1 minute, 53°C for 1 minute, 72°C for 1 minute, with a final extension of 72°C for 5 minutes. PCR products were visualized by electrophoresis through a 1.5% agarose gel, at 100V for 45 mins followed by examination under UV light.

Physical measurements

At each sampling point a variety of water parameters were measured. Water depth and height of rice were measured with the use of a rule placed directly into water for depth and against rice plant for height. Water pH, turbidity, temperature and salinity were measured with a water meter (MTW, Germany; Fig 3.5) inserted just under the water surface.

Fig 3.5: Water meter used in the study

Cow dung was measured by counting turds along each transect at the same sampling points where larvae were surveyed.

Statistical analysis

Data were statistically analyzed by using SPSS version 15.0. Regression analysis, T test and non-parametric tests were used to see if differences between variables were statistically significant. Distribution of variables which were non-normal were log transformed to normalize the distributions. Non-parametric methods were used to analyze variables without assuming that they were normally distributed. Results were reported as significant if P values were less than 0.05 ($P < 0.05$). Backward conditional binary logistic regression was used for exploring what parameters were associated with the presence and absence of mosquito larvae. Other invertebrates sampled were recorded as the total number of different taxonomic groups collected during the surveys i.e. Odonata; (sub-order Anisoptera) Odonata; (sub-order Zygoptera) Coleoptera, Ephemeroptera, Hydrometridae, Notonectidae, Corixidae, Nepidae, Gerridae, Noucoridae, Pleidae, and Veliidae. Multivariate analysis was carried out using GLIM.

Results

Transects description

Transect 1 Started at 200m from the village, which corresponded with 0m, the start of transect. Rice was grown on nursery beds here for re-transplanting into the main paddies. The soil here was sandy. The actual wet areas on this transect started at 240m from the village. From 396m to the river, were the main paddies which are tidal dependant from the River Gambia (Table 3.1).

Transect 2 Rice fields began at 119m from the village, followed by nurseries at 200m. At the beginning of transect the soil was clay, whilst at 200m it was sandy. The main paddies began at 317m, and continued to the end 750m to the river (Table 3.2).

Transect 3 Rice nurseries began at 240m from the village. This was followed by grassland at 270m through to 455m while the main paddies began at 455m to the river (Table 3.3).

Table 3.1: Features of transect 1

Distance from village	Feature	Vegetation	Characteristics	GPS coordinates
Transect 1 0-86m	Upland grassland	Grass and sparse trees	Porous Sand soil Domestic animals (goat, sheep, donkey) are kept here	N:13° 31.533' W:15° 31.030'
86-200m	Upland Orchard garden	Mango, Cashew, Neem, Oil palm, maliana and Baobab trees	Loam soil. Fire wood collection	N:13° 31.485' W:15° 31.063'
200-240m	Upland agriculture (Rice Nurseries)	Rice nurseries with sparse trees, Oil palm, Cashew	Porous Sand soil with grass.	N:13° 31.469' W:15° 31.078'
240-323m	Upland rice fields (Wet areas on this T. starts here) (50m sampling point)	Rice plants, transferred from nurseries, sparse tress (Oil palm tress)	Saturated clay soil, these areas are not affected by tidal water from the river	N: 13° 31.431' W:15° 31.103'
323- 396m	Barren flood plains	Seapurslane and grass	Saturated clay soil, no rice cultivation pathway for domestic animals coming from river or grazing	N: 13° 31.399' W:15° 31.126'
396-850m	Main rice field	Mangrove, grass, rice plants	Clay soil	N: 13° 31.210' W: 15° 31.273'

Table: 3.2 Features of transect 2

Distance	**Feature**	**Vegetation**	**Characteristics**	**GPS coordinates**
Transect 2 0-199m	Upland grass land	Grass, sparse trees (Mahoney trees)	Animal rearing	N:13° 31.562’ W:15° 31.115’
200-225m	Upland rice fields	Rice plants	Clay soil	N: 13° 31.555’ W:15° 31.123’
225-250m	Upland rice nurseries	Rice plants, grass	sandy soil	N:13° 31.538’ W:15° 31.136’
250- 317m	Upland rice fields	Rice plants, grass	Clay soil	N:13° 31.510’ W:15° 31.161’
317-425m	Barren flood plains	Seapurslane, Grass, path way for animals	Clay soil	N: 13° 31.465’ W:15° 31.202’
425-750m	Main rice field	Mangrove, grass, rice plants	Clay soil	N: 13° 31.323’ W: 15° 31.346’

Table: 3.3 Features of transect 3

Distance	**Feature**	**Vegetation**	**Characteristics**	**GPS coordinates**
Transect 3 0-240m	Upland agriculture	Open savanna, sparse trees. Trees types: Mahogany, Neem trees, subsistence farming. Groundnut, Maize, Millet, Sorghum	Porous Sand soil with grass. Domestic animals (goat, sheep donkey) reared here	N:13° 31.636’ W:15° 31.191’
240-270m	Rice Nurseries fields	Rice plants, grass, sparse trees	Saturated clay soil	N: 13° 31.629’ W: 15° 31.203’
270- 400m	Upland grass land	Grass, spares trees	Saturated clay soil	N: 13° 31.600’ W: 15° 31.272’
400-455m	Barren flood plains	Seapurslane, Grass	Clay soil, Path way for domestic animals	N:13° 31.591’ W:15° 31.296’
455-860m	Main rice field	Mangrove, rice plants	Clay soil	N: 13° 31.518’ W: 15° 31.483’

Rainfall records

Total rainfall during the 2006 rainy season was 807.9mm from Farafenni Metrological Station as compared to 858.3mm recorded in 2005 from the same station. Rain started in the first week of June and then ceased until the second week through to the end of July when it fell on intermittent scale. Heavy rainfalls were registered in August (361.2mm) and September (158.6mm) respectively (Fig 3.6). It stopped all together in the second week of October.

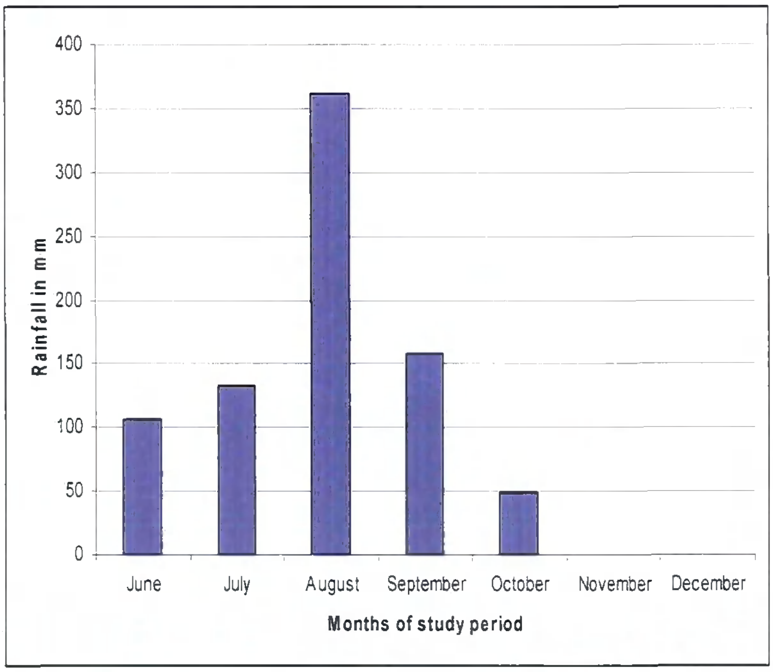

Fig 3.6: Rainfalls recorded during the study in 2006

Rice cultivation

Rice cultivation generally starts in June each year when farmers plough fields with a long hoe used by women locally called "*dabajango*" and raise nurseries (Fig 3.7). Rice seeds were sown on raised beds, approximately 10m x 5m in area; although some can be up to 10m in area. This is usually in June. The seedlings were transferred to the main paddies closest to the village by end of August and later part of September to the fields closest to the river. Synthetic fertilizer (Urea) was applied to fields by farmers in September when transplanting rice plants. Rice is a cereal that droops at maturity and is harvested by cutting the pinnacles at the base with a knife and then tied into bundles. The crops were transported on the head or by domestic animals to be dried usually on roof tops or on other platforms at homes. Rice was harvested from December to January.

Fig 3.7: Rice nursery raised in July 2006 at the start of transect one

There were both rainfed and tidal dependant rice fields observed during the study. All paddies within the first 350m distance from the village, which marked the first four sampling points, were rainfed dependant. These are shallow and lie between upland fields and mangrove swamps and are unaffected by tidal water from the river. Soil types here are sandy to alluvial sandy-clay and can hold water for longer periods than those closer to the river. These fields were divided into portions by small bunds (embankments) which prevents outflow of water. As the rain fall lasts for only five months in The Gambia, early maturing varieties of rice are usually grown in these fields. Tidal-dependant fields were those that occurred close to the river and received a water supply from the river as tidal floods in addition to rainfall. These fields are characterized by heavy alluvial clay soil. They are affected by salt water intrusion from the river during the dry season and early wet season and, as a result of this, these fields are planted with rice at the latter part of the rice growing season, usually late September.

Flooding patterns in fields

Although the rainy season started in June, the fields did not flood immediately because of the porosity of the soil. Rice paddies were flooded from August to January (Figs 3.8, 3.9, 3.10, 3.4, 3.5, 3.6). Paddies close to the land were flooded first (August), but later paddies close to the river became flooded, and these remained flooded until January, although water levels fluctuated greatly even at these points due to the tidal nature of this area. Water depth changes from village edge of flooded area to river edge. One important observation made during the period was that within an individual paddy, one sampling point could be wet while another was dry. Paddies close to the village were completely dry by the beginning of October, but those nearer the river continued to have tidal water from the river. This pattern was similar in all three transects.

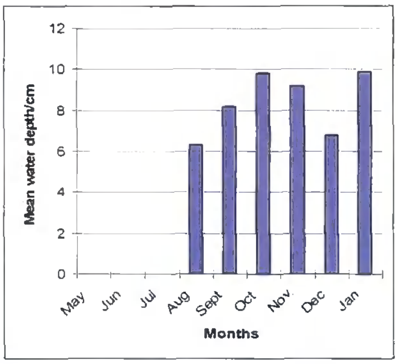

Fig 3.8: Mean water depth in wet paddies during the study period, transect 1 in 2006

Table 3.4: Presence of water in paddies, transect one. Blue rectangles represents flooded sites and purple rectangles no data were collected.

T1	Week									
Date	10.07.06	31.07.06	07.08.06	14.08.06	21.08.06	28.08.06	04.09.06	11.09.06	18.09.06	25.09.06
Sampling	1	4	5	6	7	8	9	10	11	12
0										
25										
50						🟦		🟦	🟦	🟦
75				🟦	🟦	🟦	🟦	🟦	🟦	🟦
100				🟦		🟦		🟦	🟦	🟦
200										🟦
300										🟦
400										🟦
500										🟦
Date	02.10.06	9.10.06	16.10.06	23.10.06	30.10.06	06.11.06	13.11.06	20.11.06	27.11.06	4.12.06
Sampling	13	14	15	16	17	18	19	20	21	22
0				🟥						
25				🟥						
50				🟥						
75		🟦	🟦	🟥		🟦				
100		🟦		🟥						
200	🟦	🟦	🟦	🟥	🟦	🟦	🟦	🟦	🟦	🟦
300	🟦	🟦	🟦	🟥	🟦	🟦	🟦		🟦	🟦
400	🟦	🟦	🟦	🟥	🟦		🟦			🟦
500	🟦	🟦	🟦	🟥	🟦		🟦			
Date	11.12.06	18.12.06	25.12.06	01.01.07	08.01.07	15.01.07	22.01.07	29.01.07		
Sampling	23	24	25	26	27	28	29	30		
0										
25										
50										
75										
100										
200										
300	🟦	🟦	🟦							
400	🟦	🟦	🟦							
500	🟦	🟦	🟦	🟦						

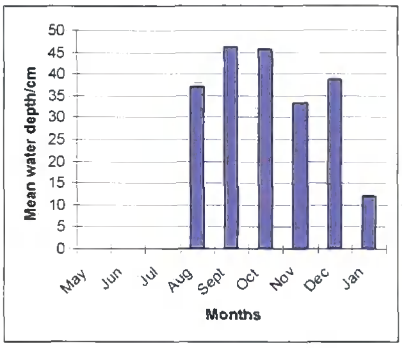

Fig 3.9: Mean water depth in wet paddies along transect 2 in 2006

Table 3.5: Presence of water in paddies along transect two at various sampling points

T 2	Week									
Date	11.07.06	25.07.06	01.08.06	08.08.06	15.08.06	22.08.06	29.08.06	05.09.06	12.09.06	19.09.06
Sampling	1	3	4	5	6	7	8	9	10	11
0					🟦			🟦	🟦	🟦
25										
50										
75					🟦	🟦	🟦	🟦	🟦	🟦
100										
200								🟦		
300								🟦		
400								🟦		
500								🟦		
Date	26.09.06	03.10.06	10.10.06	17.10.06	24.10.06	31.10.06	07.11.06	14.11.06	21.11.06	28.11.06
Sampling	12	13	14	15	16	17	18	19	20	21
0	🟦				🟥					
25					🟥					
50					🟥					
75	🟦	🟦	🟦	🟦	🟥		🟦			
100		🟦	🟦		🟥					
200		🟦	🟦	🟦	🟥	🟦	🟦	🟦	🟦	🟦
300		🟦	🟦	🟦	🟥	🟦	🟦	🟦		🟦
400		🟦		🟦	🟥	🟦		🟦		
500		🟦		🟦	🟥	🟦		🟦		
Date	05.12.06	12.12.06	19.12.06	26.12.07	02.01.07	9.01.07	16.01.07	23.01.07	30.01.07	
Sampling	22	23	24	25	26	27	28	29	30	
0										
25										
50										
75										
100										
200										
300				🟦						
400	🟦	🟦	🟦	🟦						
500	🟦	🟦	🟦	🟦	🟦					

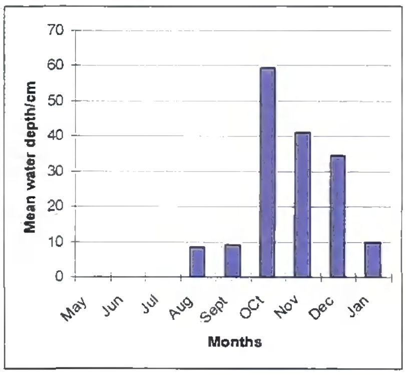

Figure 3.10: Mean water depth in wet paddies along transect 3 in 2006

Table 3.6: Presence of water in paddies, transect three

T 3	Week									
Date	12.07.06	26.07.06	02.08.06	09.08.06	16.08.06	23.08.06	30.08.06	06.09.06	13.09.06	20.09.06
Sampling	1	3	4	5	6	7	8	9	10	11
0					🟦	🟦	🟦	🟦	🟦	🟦
25					🟦	🟦	🟦	🟦	🟦	🟦
50					🟦	🟦	🟦	🟦		
75						🟦	🟦	🟦		
100										
200									🟦	🟦
300									🟦	🟦
400									🟦	🟦
500									🟦	🟦
Date	27.09.06	04.10.06	11.10.06	18.10.06	25.10.06	01.11.06	08.11.06	15.11.06	22.11.06	29.11.06
Sampling	12	13	14	15	16	17	18	19	20	21
0	🟦	🟦	🟦							
25	🟦	🟦	🟦							
50	🟦	🟦	🟦							
75	🟦		🟦							
100			🟦							
200	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦
300	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦	🟦
400	🟦			🟦	🟦	🟦	🟦	🟦		🟦
500	🟦			🟦	🟦	🟦	🟦	🟦		🟦
Date	06.12.06	13.12.06	20.12.06	27.12.07	03.01.07	10.01.07	17.01.07	24.01.07	31.01.07	
Sampling	22	23	24	25	26	27	28	29	30	
0										
25										
50										
75										
100										
200										
300		🟦								
400	🟦	🟦	🟦	🟦						
500	🟦		🟦	🟦	🟦	🟦				

Larval collection against height of rice plant

A total of 375 anopheline and 442 culicine larvae were sampled during the study period. Highest numbers of mosquito larvae were collected in August, September and October 285 (76%). However, the number of anopheline larvae caught declined in November and none were found in December and January as most fields have already dried up. As the height of rice progressed, number of larvae collection declined (Figs 3.11, 3.12, 3.13).

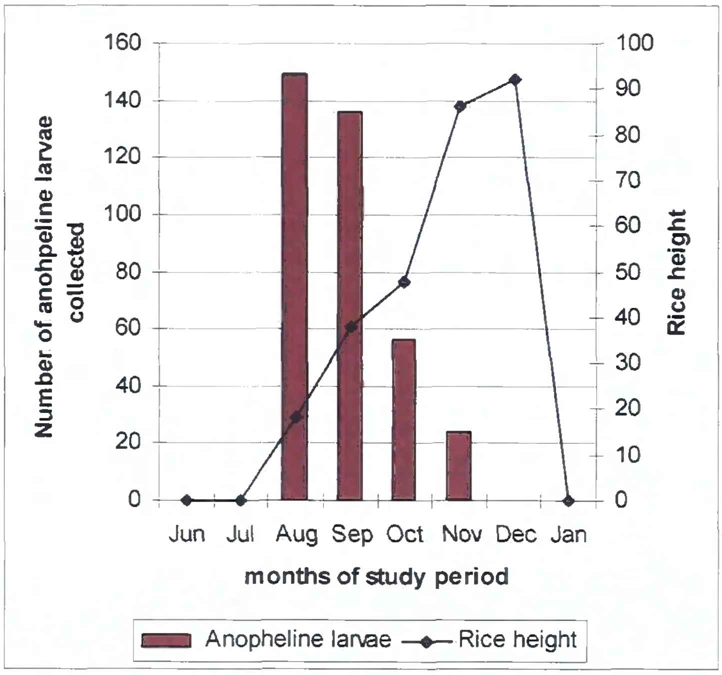

Fig 3.11: Combined anopheline larval collections for all 3 transects against growth of rice plants (cm).

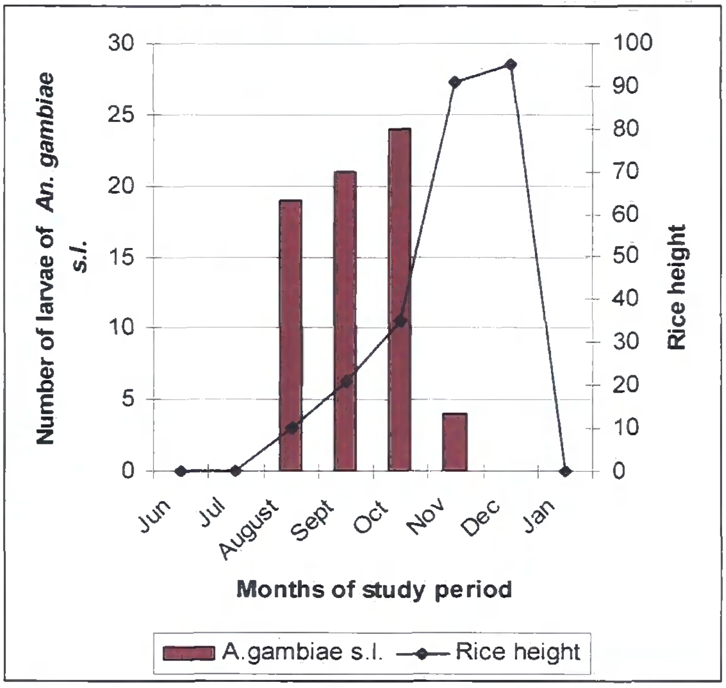

Fig 3.12: *An. gambiae* complex larval collections against rice height (cm).

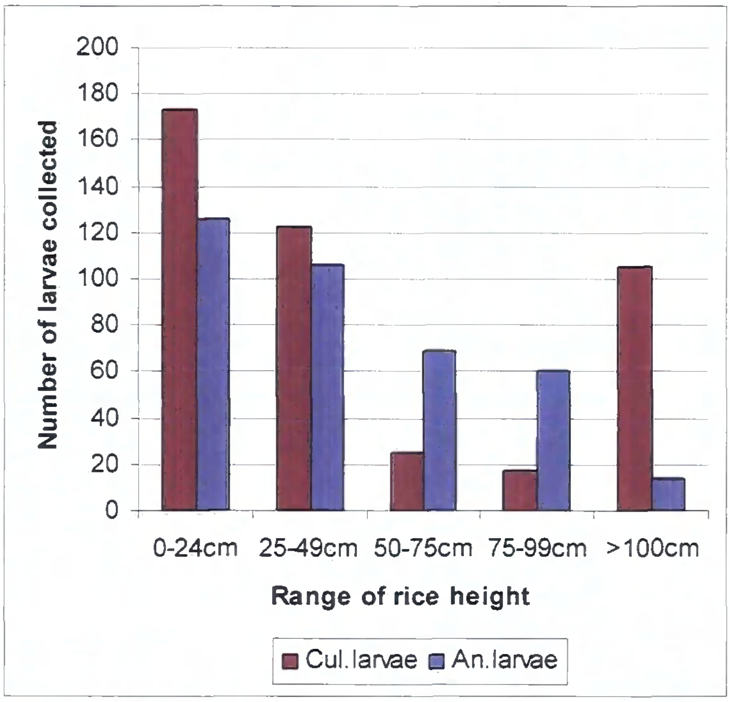

Fig 3.13: Combined anopheline and culicine collections for three transects in relation to height of rice.

Larval collection along the three transects

Mosquito larvae were sampled at three points, near edge, middle and far edge of each paddy along three transects. There were more anopheline larvae found on the near edge of each paddy compared to the middle of the same paddy (Wilcoxon Signed Ranked Test, Z= -2.14, *P*<0.032), there were also more anopheline larvae found on the far edge of each paddy compared to middle of the same paddy (Z= -2.93, *P*<0.003), but there was no significant difference between the near edge and far edge of paddies within 350m sampling zones (Z= -0.177, *P*>0.860). Ninety-three percent of anopheline larvae (349/375) and 91% of culicines (403/442) were sampled within the first 350m sampling zones from the village. More anopheline were sampled along transect 1 (174/375; Fig 3.14), while more culicines were sampled along transect 2 (253/442) (Fig 3.15, Table 3.8).

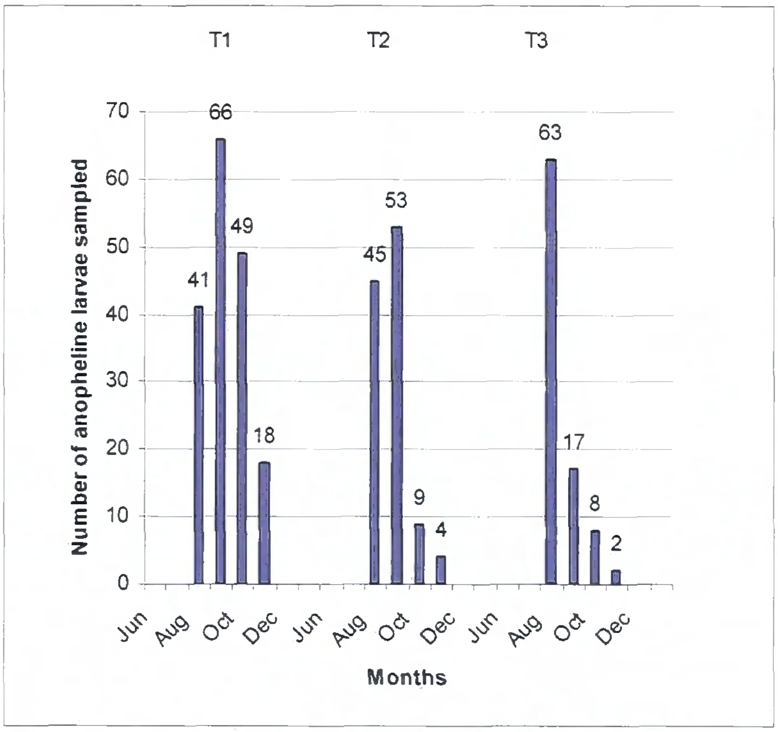

Fig 3.14: Anopheline larvae collected along combined transects over the study period.

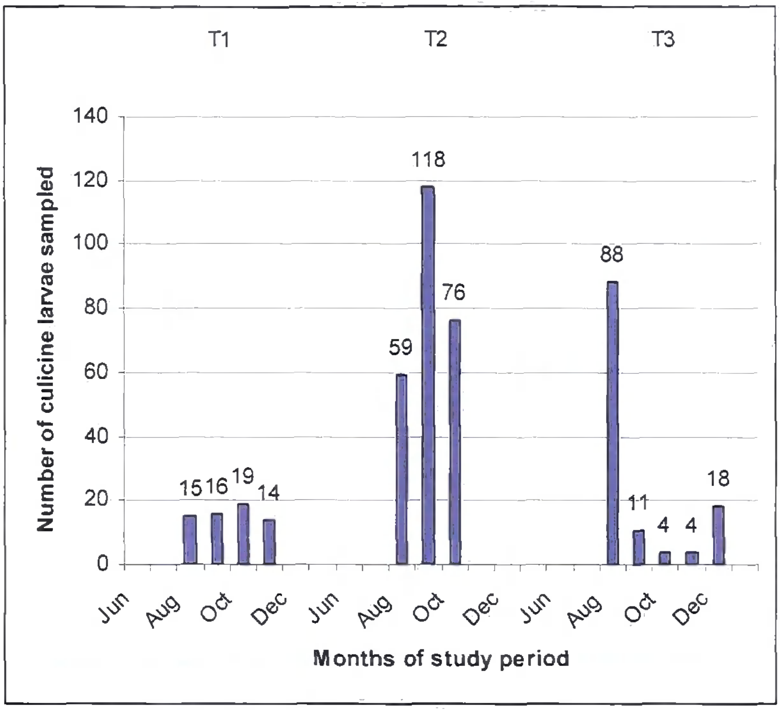

Fig 3.15: Culicine larvae sampled along combined transects over the study period

Larval collection at various distances from the village

Table 3.7: Number of larvae sampled at various distances for three transects

Distance from village (m)	Number and percentage of anopheline larvae	Number and percentage of culicine larvae
200	65 (17.3%)	123 (27.8%)
225	27 (7.2%)	42 (9.5%)
275	28 (7.4%)	26 (10.4%)
350	229 (61.1%)	212 (47.9%)
450	0	0
550	26 (7.0%)	0
650	0	0
750	0	0
860	0	19 (4.2)
Total	375 (100%)	442 (100%)

DNA was extracted from 80 *An. gambiae* mosquitoes. Of these, 36 were *An. arabiensis* (45%), 23 were *An. gambiae s.s.* (29%), and 21 *An. melas* (26%). Four were either lost during DNA extraction or the extraction had failed. *An. gambiae s.s.* appeared soon after the rains while *An. arabiensis* appeared later and continued to the end of the wet season.

The rest of the samples (291) could not be identified as member of *An. gambiae* complex by PCR and so were considered other anopheline species. All three members of the *An. gambiae* complex were found together, close to the village (Table 3.8).

Table 3.8: Numbers of *An. gambiae s.l* sampled at various distances from the village

Distance from village (m)	*Anopheles gambiae s.s.*	*An. melas*	*An. arabiensis*
200	2	0	6
225	4	7	12
275	1	4	3
350	8	10	9
450	0	0	0
550	8	0	6
650	0	0	0
750	0	0	0
860	0	0	0
Total	23 (29%)	21 (26%)	36 (45%)

Sampling of other invertebrates

All insects, 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). Invertebrates caught in traps were counted and identified. A total of 1376 invertebrates were collected during the study. Of these, 862 (63%) were collected within the first 350m of each transect whilst 514 (37%) came from beyond 350 meters (Fig 3.16, Table 3.9). Mosquito larvae and other invertebrates occurred at the same time soon after the rice fields were flooded. Concentrations of invertebrates were higher along transects one and two (Paired Sample Test, T= 32.618, P<0.001) where 285 (76%) of total larvae were also collected. 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.* (OR=52.32, 95% CI =8.39- 326.29, *P*=<0.001), culicine larvae (OR=5.05, 95% CI=1.65- 15.46, *P*= 0.005).

There was also a positive relationship between the abundance of *An. gambiae s.l.* larvae and other aquatic insects ($r^2 = 0.19$, F = 123.5, P<0.001).

Table 3.9: Number of other invertebrates collected along each transects

Taxonomic divisions	Transect 1		Transect 2		Transect 3	
	0-350m	<350m	0- 350m	<350m	0- 350m	<350m
Odonata	50	7	47	8	0	0
Coleoptera (larvae)	13	3	24	4	0	0
Coleoptera (adults)	2	2	15	2	0	0
Ephemeroptera larvae	2	0	0	0	0	0
Hydrometridae	48	27	45	29	16	29
Notonectidae	56	32	67	24	11	12
Corixidae	18	4	13	3	0	1
Nepidae	58	24	7	17	4	3
Gerridae	48	30	75	36	22	23
Noucoridae	52	47	83	44	32	38
Pleidae	8	16	57	31	3	11
Veliidae	4	6	32	21	0	3
Others (Red ants & Spider)	0	13	0	8	7	25
Total invertebrates	**359**	**150**	**408**	**219**	**95**	**145**

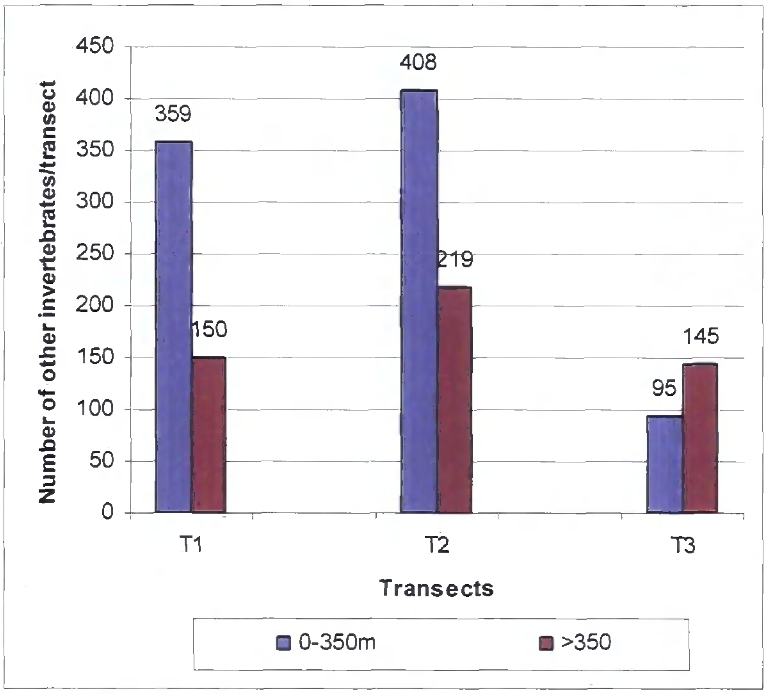

Fig 3.16: Collection of other invertebrates at various distances along the three transect

Physical measurements along the three transects

Water depth, pH, and temperature were similar in the first 350m along each transect compared with parts nearer the river (Table 3.10). A total of 165 samples of cow dung was counted along the three transects. Of these 132 (80%) were found in the 350m sampling area near the village as did larvae (Table 3.11).

Table 3.10: Characteristics of water parameters, distribution of larvae and count of dung piles sampled along three transects (95%CI).

Variables	Transect 1		Transect 2		Transect 3	
	0-350m	>350m	0-350m	>350m	0-350m	>350m
Water depth (cm)^a	7.3 (6.5-8.1)	10 (9.4-10.5)	13.9 (11.2-7.3)	14.2 (12.4-16)	8.7 (8.1-9.3)	12.9 (12-14)
Water Turbidity (ntu) ^{b}	22.3 (18.5-23.5)	13.4 (12.7-14.1)	11.1 (8.9-13.04)	13.6 (12.8-14.4)	12.4 (11.0-14.0)	10.7 (10.3-11.2)
Water pH^c	6.4 (6.0-6.8)	7.4 (7.2-7.5)	6.9 (6.5-7.2)	7.6 (7.5-7.8)	7.3 (7.0-7.0)	7.6 (7.5-7.8)
Water conductivity (mS/cm)^b	0.8 (0.7-1.4)	1.2 (0.7-1.5)	0.9 (0.5- 1.3)	1.1 (0.4-1.5)	0.8 (0.3-1.2)	1.1 (0.7-1.4)
Water Temperature (°C)^a	30.1 (29.3-30.8)	28.9 (28.3-29.4)	30.3 (30.0-31.1)	28.5 (29.9-29.0)	30.2 (29.6-30.7)	28.6 (28.2-28.9)
Dung piles	49	14	47	8	36	11
Anopheline larvae	167	7	109	2	73	17
Culicine larvae	67	0	246	0	110	19
Anopheles gambiae s.s.	8	1	1	0	6	7
Anopheles arabiensis	9	1	1	0	20	5
Anopheles melas	9	0	1	0	11	0
Other Anophelines	141	4	106	2	36	5

Where ^{a}= mean (95% CI), ^{b}= geometric mean (95% CI), and c= mean of squared numbers (95% CI).

Statistical modelling

Backward stepwise binary logistic regression was used to determine the most important factors associated with the presence or absence of anopheline larvae in the rice fields. The best model predicted 96% of all sites without larvae (449/467), but only 59% with larvae (41/69) (Table 3.11).

Table 3.11: Factors associated with the presence or absence of anopheline larvae.

Variable	Wald	Odds Ratio (OR)	95% OR	*P*
Sampling point				
Landward edge		1.00		
Centre	7.20	0.274	0.106-0.705	0.007
Riverside edge	6.58	0.296	0.117-0.750	0.010
Size of water body				
1-9m perimeter		1.00		
≥10m perimeter	6.42	0.256	0.089-0.734	0.011
Distance				
	6.00	0.995	0.991-999	0.014
Rice				
Absent		1.00		
Present	18.00	52.32	8.39-326.29	<0.001
Culicine larvae				
Absent		1.00		
Present	8.05	5.05	1.65-15.46	0.005
Other invertebrate taxa				
	5.16	1.20	1.026-1.412	0.023
Week				
Week 1		1.00		
Week 7	4.48	0.091	0.010-0.837	0.034
Weeks 2-6, 8-22				ns

Binary logistic regression showed that anopheline larvae were more likely to be found close to the landward edge of the paddies nearest the village (Odds Ratio, OR = 0.995, 95% CIs = 0.991-0.999, *P*=0.014) collected on the landward edge of the paddies compared with the centre (OR = 0.274, 95% CI= 0.102-0.705, *P*= 0.007), or far edge of paddies (OR=0.296, 95% CI= 0.117-0.750, *P*=0.010). Mosquitoes were most likely to be found in small water bodies where rice was grown compared to larger bodies (OR=0.256, 95% CI=0.089-0.734, *P*=0.011). The analysis also showed that anophelines were strongly associated with rice plants (OR=52.32, 95% Cl =8.39- 326.29, P=<0.001) culicines larvae (OR=5.05, 95% CI=1.65- 15.46, P= 0.005) and habitats rich in different types of insects (OR = 1.20, 95%CIs= 1.026-1.412, P=0.023). The likelihood of finding anophelines larvae varied only between week 1 and 7.

Discussion

Irrigation projects especially for rice cultivation world-wide have been associated with negative impacts on human health, particularly with respect to vector-borne diseases. However, evidence for a direct link between irrigation development and increased malaria transmission varies from region to region and is inconsistent (Harrison & Scanlon, 1975; Oomen *et al.*, 1995; Ijumba & Lindsay, 2001), with increased transmission in some situations (Coosemans, 1985; Goonasekere & Amerasinghe, 1988; Robert *et al.*, 1992), but not others (Robert *et al.*, 1988; Boudin *et al.*, 1992).

The relationship between irrigated rice cultivation and malaria transmission seems to vary spatially and temporally. For example, studies in Madagascar (Marama *et al.*, 2004), Ghana (Klinkenberg *et al.*, 2005, and Côte d'Ivoire (Dossou *et al.*, 1994) suggested that irrigation enhances malaria transmission. In contrast in Tanzania (Ijumba *et al.*, 2002), Senegal (Faye *et al.*, 1993) and Côte d'Ivoire (Dossou *et al.*, 1998) irrigation diminished malaria transmission. One potential reason for variation in malaria transmission in rice cultivation areas could be due to the type of water management and drainage system (Klinkenberg *et al.*, 2003).

This study is one of the few to have documented mosquito production in association with traditional rice growing. Overall 91% of *An. gambiae* s.l. larvae were found in rice fields within the first 350m from the landward edge, nearest the village. This area was not only rich in malaria vectors it was also where culicines were common and the area was also rich in other invertebrates.

This area of open water close to land represents the shortest flight distance for ovipositing females leaving their indoor resting places to lay their eggs in breeding sites. Paddies close to the land were rain-fed and separated from the tidal water from the river. Thus tidal water may be unsuitable for mosquito larvae and other invertebrates, for a number of factors. Firstly, predators, like fish believed to predate on larvae in rice fields (Meisch 1985, Lacey & Lacey 1990, Wu *et al.* 1991, Victor *et al,* 1994), were not found in paddies close to the land in this study as the paddies were demarcated by raised embankments to prevent salt water intrusion which could have also prevented fish from getting into these paddies. Whilst tropical rice fields are known to harbour a wide range of indigenous fishes (Fernando, 1956a; Ardiwinata, 1957; Heckman, 1974, 1979), the extent to which mosquitoes are controlled by predators (Riviere *et al.*, 1987; Marten, 1989) is still a poorly explored subject. Previous studies suggested that fish are generalist predators (Hess & Tarzwell, 1942) that feed on a range of invertebrates (Bence, 1982 and Bence, 1988). Secondly, small invertebrates will find it difficult or impossible to maintain their position in the water column in areas of tidal water and may get flushed away. Thirdly, cow dung was far more common near the landward edge of the floodplain, suggesting that this might improve mosquito productivity in these sites, by providing water rich in nutrients.

A key factor that is likely to exacerbate malaria transmission in rural Gambia is the close proximity of settlements to breeding sites, especially in rice cultivation areas. Previous studies carried out in urban settings of Senegal and Uganda demonstrated that clinical malaria cases were strongly associated with close proximity to breeding places, acting as the main malaria transmission sites (Trape *et al.* 1992; Staedke *et al.* 2003; Girardin *et al.,* 2004).

Most anopheline larvae were aggregated at the edges of rice paddies, as has been found in other studies (Hocking, 1953; Minakawa *et al.,* 1999). This finding could be partly explained by ovipositing females laying their eggs at the first few patches of water they encounter since we found that larvae were more common on the landward edge of the paddies. In addition, it is the edges where floating debris collects, thus the propensity to find larvae at the edges may be also due to rain or wind carrying them there.

Malaria transmission in The Gambia peaks in September and October, towards the end of wet season. Similarly highest numbers of *An. gambiae s.l.* were also found at this time, which is typical of the rural savannah areas of West Africa. The best explanation for this observation was that mosquito breeding tends to follow rice cultivation. Highest catches of larvae were in August and September when rains have steadied and soon after rice plants were transplanted, as there was little shade over the water. In October their numbers steadily declined when the rice plants were fully grown and shaded the surface (Klinkenburg *et al.*, 2003). No further catches were made in the following three months, since most of the rice plots had dried up.

The main limitation of this study is the small area of rice fields where it was conducted. However, conditions that prevailed in these sites were representative of many other areas in the middle reaches of The Gambia where most swamp rice is grown.

Conclusion

Swamp rice fields are an important habitat for mosquito larvae in rural The Gambia. Larvae of both anophelines and culicines were found to be higher along edges of rice fields close to the study human settlements. Larval control efforts should be programmed in the early stages of rice cultivation season (May/June) when field preparations take place and in August when rice seedlings are transplanted to paddies and should be directed to such areas near village as priority sites. The good thing about this is that it will help to reduce the cost of larval control.

Mosquito larval habitat ecology is important for determining the densities of larvae and their species, which in effect influences malaria transmission pattern in a given area. Better understanding of the relationship between rice cultivation, be it on traditional systems or modern irrigation systems, occurrence, abundance and distribution of mosquito density can provide useful data that can be relevant for designing malaria vector control programs (Faye *et al.*, 1993).

Since rice is a staple food in The Gambia, its cultivation is seen as salvaging the rural communities from food shortage. However, where rice cultivation is undertaken, personal protective measures such as sleeping under ITNs, should be strongly promoted as the strategy has been proven very effective in reducing malaria-related morbidity and mortality (Lengeler, 2004).

CHAPTER 4

EMERGENCE OF ADULT MOSQUITOES FROM NATURAL RICE FIELDS IN THE GAMBIA

Fig 4.1: Emergence net trap being emptied in a rice field

SUMMARY

Background

Malaria vectors are often found breeding in high numbers in rice fields. Most studies to date have investigated the impact of irrigated rice cultivation on mosquito production. Here I conducted a study to determine the productivity of mosquitoes in natural rice cultivation in swamps bordering the River Gambia.

Methods

Adults were sampled using emergence trap (Fig 4.1) along three transects in rice fields from the landward edge of the flooded alluvial plains bordering the River Gambia to the river. Samples were taken in three zones along each transect: 200- 350m from the

landward edge of the rice fields, 450-550m and 650-850m. Sampling was repeated weekly for the entire rice growing period in the wet season, from month to month.

Results

All members of the *An. gambiae* complex, most other anophelines and culicine mosquitoes were found within 350m from the landward edge of the wetlands. 263 adult mosquitoes were collected, of which 68 was *An. gambiae s.l.*, 139 were *Culex* spp., 30 were *Aedes* spp. and 26 were other anophelines. Ninty percent of *An. gambiae* complex collected were *An. arabiensis,* 6% were *An. gambiae s.s.* and 1.4% were *An. melas.* More adult anophelines were collected from transect 1 than transect 2 (Odds Ratio (OR) = 0.035, 95% CI=0.005-0.235, *P*=0.001) and transect 3 (OR= 0.036, 95% CI= 0.005-0.270, *P*=0.001). Adults were more likely to be collected on the edge of the paddies compared to the centre (OR= 0.148, 95% CI=0.040-0.554, *P*= 0.005). Similarly more culicines and other invertebrates were also collected in the first 350m from the landward edge where most larvae were also collected.

Discussion

Anopheline mosquitoes were most likely to be found in small, non-tidal water bodies, where rice was grown and close to the landward edge. These sites were also those most heavily colonised by culicine mosquitoes and other aquatic invertebrates. Natural rice production is associated with increased production of malaria vectors in paddies close to human settlements.

INTRODUCTION

Despite significant imports of cereal, the number of malnourished children living in SSA increased by more than 75% in the last three decades; and in 1997 it was estimated that 32.7 million children under 5 years of age were malnourished (Rosegrant & Meijer, 2002). To remedy this situation, thousands of small irrigation and multi-purpose dams have been constructed in Africa, and the irrigated land surface has been extended concurrently (Amersinghe, 2003; Keiser *et al.*, 2004). In Côte d'Ivoire, for example, an estimated 500 small dams have been built in the last three decades (Aka *et al.*, 2000). It is well known that rice fields in many instances provide the ecological requirements of malaria vectors (Ijumba & Lindsay 2001). In spite of these fears, rice cultivation has been on the increase in the African region in order to provide food for the increasing populations. However, although rice fields provide suitable breeding places for *Anopheles* mosquitoes and rice cultivation leads to an increase in the biting rates, the species which are adapted to these sites are not the same in all parts of Africa. Several examples illustrate this phenomenon: *An. funestus* is common in the rice fields of Madagascar, *An. pharoensis* in saline water rice fields in the delta of the Senegal river, *An. arabiensis* in northern Cameroon and Burundi, *An. gambiae* Mopti form in the Kou Valley (Burkina Faso); and *An. gambiae* Savanna form in the rice fields of Kafine near Bouake (Cote d'Ivoire; Carnevale *et al.*, 1999). These examples also indicated that the vectorial capacities of these species are not the same and malaria inoculation rates are not necessarily increased in rice land agro ecosystems. Whilst there have been many studies on the impact of rice production on malaria transmission in the literature, these are almost invariably focused on the cultivation of irrigated rice. Most of the studies on this subject were in Asia in the first half of the 20th century (Takken *et al.,* 1990). In Asia, anopheline vectors responsible for malaria transmission differ from those in Africa and so this will dictate the type of vector control methods, especially water management strategies. Few studies have reported how rice grown using traditional methods will affect malaria transmission. The distribution and abundance of mosquito larvae reflect the oviposition preferences of adult females and the ability of immature stages to tolerate the conditions that prevail in specific aquatic habitats (Reisen *et al.,* 1981).

In The Gambia there have been a number of studies that have investigated the affect of irrigated rice cultivation on malaria transmission. In Bansang, *An. gambiae s.l.*, *An. rufipes* and *Culex neavei* were found in association with rice cultivation reaching their peak numbers 4 weeks after full-scale irrigation began and then declined in abundance (Snow, 1983). *An. pharoensis* was most common around the middle of the rice-growing cycle 6-13 weeks after the start of full irrigation and showed more extended peaks of abundance. *Anopheles ziemanni* alone reached maximum numbers as the rice crop neared maturity. Whilst in Saruja, it was shown that large-scale irrigation resulted in two peaks of mosquito abundance each year, in the dry and wet season (Lindsay *et al.*, 1995). In The Gambia where malaria transmission is brief, seasonal and often intense, it is possible that extending the rice growing may have serious consequences. Deaths from malaria are typically confined to the end of the transmission season (Greenwood *et al.*, 1987; Alonso *et al.*, 1991) and so extending the season may increase mortality, as has been found in other parts of the world (Bouma and Vanderkaay, 1994). In villages without irrigation, as is common throughout rural Gambia, most food comes from upland family fields. These fields are usually managed by men where the whole family grows maize, sorghum, peanuts, and cotton. Crops harvested from the upland fields are stored in family granaries, part of which is sold to raise money to buy clothing, pay for health expenses, maintain the house and acquire farm equipment. Lowland fields, on the other hand, which cultivate rice, are customarily considered to be women's personal fields. Women grow rice during the rainy season and vegetables during the dry season. They store these crops, which they use to provide food for their families and for special occasions.

Here I studied the production of mosquitoes from naturally cultivated rice fields bordering the River Gambia. This investigation is important since recent studies investigating the applicability of larval control in The Gambia have identified rice fields as being important sources of anophelines larvae (S. Majambere, unpublished). This study aims to determine and describe when and where most adult mosquitoes emerge. Since earlier studies have indicated that mosquito production varied according to the distance from the River Gambia (Bøgh *et al.* 1999; Thomas and Lindsay, 2000). I studied adult emergence along three transects in the flood plain, running inland towards the river. It is essential to determine exactly where the adult mosquitoes emerge from as this would help direct larval control measures that can save scarce resources.

MATERIALS AND METHODS

Study area

Surveys were conducted in rice fields in Tamba Kota Village, 11km east of Farafenni town. Three transects each 500m in length; 20m wide and 200m apart were sampled at weekly intervals from June to December, during the rainy season 2006.

Adult Sampling

Adults mosquitoes were sampled using emergence traps in rice fields (Fig 4.2) in three zones along each transect: 200-350m from the landward edge of the rice fields marked the start of first sampling points, 450-550m the second sampling points and 650-850m the third sampling points. Traps were made from a conical metal frame and nylon netting and measured 1m in height and 1m in diameter at the base. They were positioned within 4m either side of each transects over water. In each zone samples were made within 1m from the edge of the paddy nearest the village, in the centre of the same paddy, and the third sample was made in the same paddy near the edge furthest from the village. This procedure was repeated in all three zones along each transect. Paddies near the land are separated from those further by barren floodplain covered by sea purselane (Fig 4.3).

Fig 4.2: Emergence trap in position in rice field

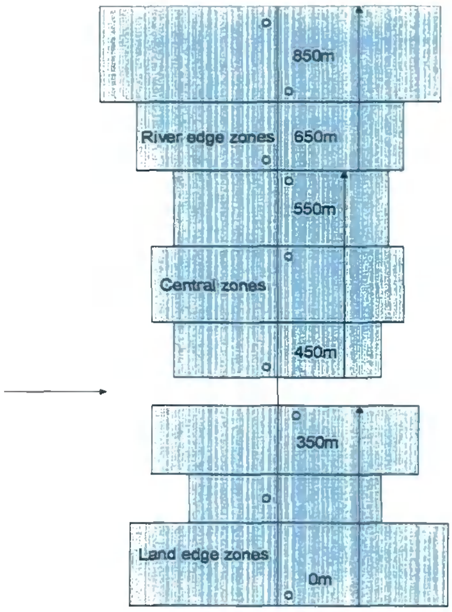

Fig 4.3: Diagrammatic sketch of traps in paddies, circles representing traps. Within each trap station the traps were positioned at the centre of a paddy, as well as the far and near edge of the paddy. Arrow indicates sea purselane.

The frame was kept afloat on the water surface but was prevented from moving laterally by fixing bottles filled with sand to the bottom of the frame. There is an opening at the top of each trap which connects to a light transparent container where the mosquitoes and other flying insects emerging from the water were trapped. These containers were filled with 250 ml of 60% glycol in order to kill and preserve adult mosquitoes. The netting sleeves on the side of each trap made it easy to remove trapped mosquitoes and other insects.

All invertebrates caught in the sampling container were removed using a small brush and transferred to a collection bottle. Care was taken to ensure that the traps were neither damaged nor stuck between vegetation and surface debris. They were gently moved to allow them to float freely. After collecting insects, the traps were always moved to new sampling sites where larvae were recently identified. Adult mosquitoes emerged and caught in traps were examined microscopically to distinguish *An. gambiae s.l.* from other adult species using identification keys provided by Gillies and Coetzee (1987). Individual species from the *An. gambiae* complex were identified using rDNA-PCR analysis. Each specimen was tested and those that failed to amplify after two tests were recorded as other species see chapter 3. Invertebrates were counted and identified using taxonomic keys in the laboratory.

Validation of emergence trap collections was done in Farafenni field station. Fifty holes were dug in which bowls filled with water to serve as artificial breeding sites were placed. One week after larvae were observed netting traps were placed over these and emptied weekly as done in the field. This was done with different depths of water in bowls. Mosquitoes and other invertebrates collected were identified and recorded on field forms.

Physical measurements

At each site, where sampling was done, water depth and height of rice plant were measured. This was done with a rule inserted into the water body for depth and against rice plant for the height.

Fish Sampling

Mosquito dippers were inefficient at catching the fast moving fish that inhabited the floodplains, fish were therefore sampled using a cast net (diameter: 230 cm, mesh size: 10 mm) and a hand net (25 x 17 cm, mesh: 2 mm) at 50m 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 10m 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.

Statistical analysis

Data was analyzed by using SPSS version 15.0. The association of particular species with site and distances was investigated using Binary logistic regression analysis (SPSS 15.0 for Windows). Adult mosquito densities were reported as the total number collected. Because other vertebrates were identified with a variable precision, the number of invertebrate taxa was recorded as a score of 1-12.

RESULTS

Adult mosquitoes sampled

A total of 263 adult mosquitoes were collected over the study period from 279 samples made with emergence traps. There were 94 anopheline, 139 culicine and 30 *Aedes vittatus* adults (Table 4.1). Sixty-eight mosquitoes were identified as members of the *An. gambiae* complex. Of these PCR analysis showed that 61 (90%) were *An. arabiensis*, 4 (6%) *An. gambiae s.s.* and 1 (1.4%) was *An. melas* (Table 4.2). Two (3%) specimens were either lost during identification process or DNA extraction had failed. Twenty- six anopheline species that could not be identified by PCR were classified as other anopheline species. More *An. gambiae* complex mosquitoes were collected along transects 1 and 2 (29 and 27 respectively) than transect 3, (11, Table 4.1, 4.2). Adult mosquitoes were sampled in the second week of August rising and remained high through to October before declining in November (Table 4.3).

Table 4.1: Numbers of adult mosquito collected from each transects

Transects	Adult mosquitoes	*Anopheles gambiae* complex	Other anopheline	Culicines	*Aedes*
1	105	29	6	56	14
2	116	27	14	59	16
3	42	12	6	24	0
Total	263	68	26	139	30

Adults of the *An. gambiae* complex were collected when the rice fields were first flooded in August, but the last adult was collected in early November coincident with the drying out of the fields close to the landward edge and increased height of rice. Of the 68 *An. gambiae* complex collected, 19 (28%) were collected in August while 45 (66%) were sampled in September and October combined. Larvae were established soon after rice plants were first transplanted in August, the number steadily decreased over time through to October.

Table 4.2: Breakdown of *An. gambiae* complex for the three transects

Transect	Species of the *Anopheles gambiae* complex			
	An. arabiensis	*An. gambiae s.s*	*An. melas*	Unknown
1	28	0	0	1
2	22	3	1	1
3	11	1	0	0
Total	61	4	1	2

Table 4.3: Adult mosquito collection per month of study period

Month	Number of adult mosquitoes collected
August	103
September	79
October	60
November	11
December	10
January	0
February	0

As the rice plants became fully grown and established in November, numbers of adult mosquitoes and *An. gambiae s.l.* declined while none were found in the last 2 months of the study period (Table 4.3, Fig 4.4, 4.5).

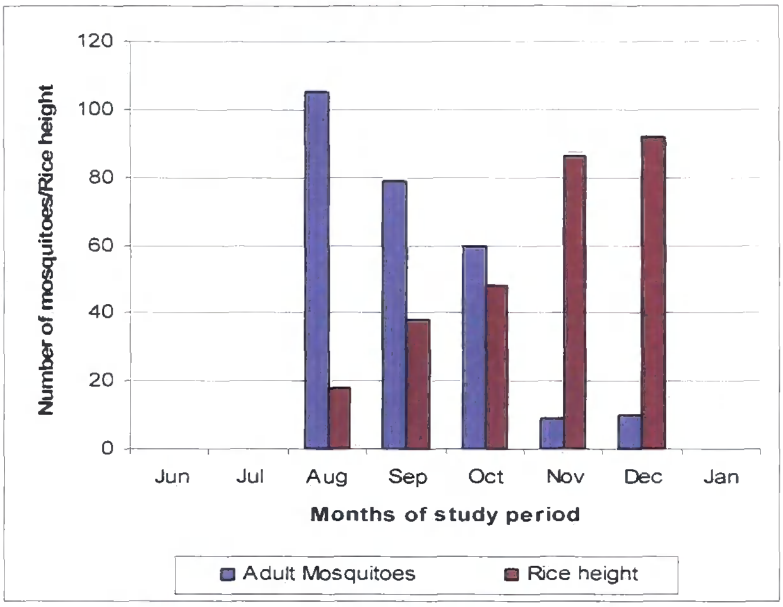

Fig 4.4: Adult mosquito collection against growth of rice plant (cm) over study period

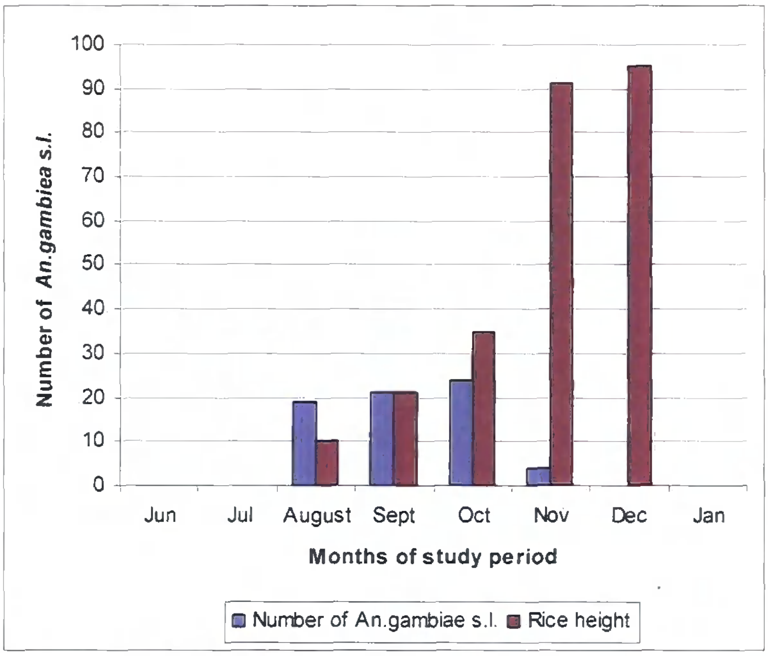

Fig 4.5: Collection of *An. gambiae* complex along three transects against growth of rice (cm) over the study period.

Spatial distribution of mosquitoes along the three transects

The greatest concentration of malaria vectors were found in the first 350m of each transects. Of the 263 adult mosquito collected, 245 (93.1%) were collected from these sampling points of rice fields whilst 18 (6.8%) were collected at greater distances. Adults of *Anopheles gambiae* complex adults were also more common in fields within the first 350m sampling areas (64/68).

Rice plants were first transplanted in fields within the 350m sampling points because these areas got flooded before the fields further away. Size of water bodies within paddies in the first 350m sampling areas were smaller compared to those within beyond, as a results of this, more mosquito collections were within the smaller water bodies in the first 350m sampling areas from the village (Table 4.4).

Table 4.4: Water depth and invertebrates collected along the three transects.

Variables	Transect 1		Transect 2		Transect 3	
	0-350m	>350m	0-350m	>350m	0-350m	>350m
Water depth	6cm	10.5cm	8.3cm	11.2cm	7.2cm	12.2cm
Adult mosquitoes	87	18	116	0	42	0
Anopheles gambiae s.l	25	4	27	0	12	0
Anopheles gambiae s.s	0	0	3	0	1	0
Anopheles arabiensis	24	4	22	0	11	0
Anopheles melas	0	0	1	0	0	0
Culicines	50	6	59	0	24	0
Coleoptera (adults)	56	13	31	3	34	7
Odonata	34	15	31	1	6	2
Hydrometridae	18	3	11	1	0	0
Notonectidae	7	7	19	1	0	0
Corixidae	7	4	9	0	0	0
Nepidae	7	15	0	0	0	0
Gerridae	21	7	8	0	0	0
Noucoridae	10	14	9	3	0	0
Pleidae	11	4	2	1	0	0
Veliidae	18	12	3	5	0	10
Others	65	88	12	87	36	27
Total	**225**	**166**	**135**	**96**	**75**	**36**

There were more adult *An. gambiae s.l* collected at the landward edge of paddies than in the centre (Table 4.5; Paired Sample Test, T= -2.7174, *P*<0.031). Of the 61 *An. arabiensis* collected, 57 were from the landward edge whilst 4 were from center. All 4 *An. gambiae s.s.* and the 1 *An. melas* were sampled from the landward edges. Overall, 94% of all species of *An. gambiae* complex (64/68) were collected less than 350m from the landward edge. Most aquatic invertebrates (60%) were collected in the first 350m of landward edge of the alluvial floodplains of each transect compared with sites more distant, even though more than twice as many samples were made more than 350m from the landward edge, compared with the near zone alone (i.e. 188 *vs* 91). Only invertebrates that were caught in emergence traps were sampled and classified.

Table 4.5: Collection of *An. gambiae* complex by sampling points within individual paddies

	Sampling Points			
Species of *Anopheles gambiae s.l*	Edge	Centre	River edge	Lost
An. gambiae s.s	4	0	0	0
An. arabiensis	57	4	0	0
An. melas	1	0	0	0
Lost Species	0	0	0	2
Total	62 (91%)	4 (6%)	0	2 (3%)

Fish species sampled

Four species of fish were sampled in the study along three transects: 17 *Periophthalmus barbarus,* 12 *Tilapia guineensis*, 1 *Epiplatys spilargyreius* and 1 *Porogobius schlegelli.* No fish were collected within 350m zones near the landward edge of each transect. Fish were more likely to be sampled between 450-850m from the start of transect than 0-350m (Relative risk = 14.0, X^2_{M-H} allowing for variation between transects = 11.7, $P < 0.001$). Anophelines were associated with culicines (Liner Regression, F=2641.172, $r^2 = 0.903$ $P<0.001$) and with other species (Linear Regression, F =248, $r^2 = 0.98$, $P<0.001$ Fig 4.6).

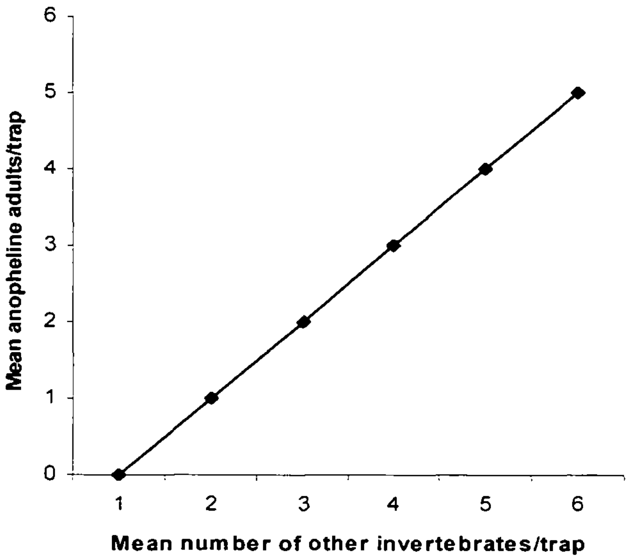

Fig 4.6: Presence of anopheline adults and other invertebrates in the same sampling point

Statistical modelling

The presence or absence of culicine adults overwhelming predicted the presence or the absence of anophelines adults (binary logistic regression; $P < 0.001$). This simple model correctly predicted 89% of sites with anophelines adults (54/61) and almost 100% without (217/218). Excluding culicine numbers from the model, we examined the relationship between the presence and absence of anophelines adults and other variables (Table 4.6). This model correctly predicted 83% of sites with anophelines adults (48/58) and 95% without (196/217).

Table 4.6: Factors associated with the presence or absence of anophelines adults.

Variable	Wald	Odds Ratio (OR)	95% OR	*P*
Transect				
Transect 1		1.00		
Transect 2	11.81	0.035	0.005-0.235	0.001
Transect 3	10.44	0.036	0.005-0.270	0.001
Sampling point				
Edge of rice fields		1.00		
Centre of rice fields	8.06	0.148	0.040-0.554	0.005
Size of water body				
1-9m perimeter		1.00		
≥10m perimeter	6.42	0.256	0.089-0.734	0.011
Rice				
Absent		1.00		
Present	7.24	517.45	5.46-49031.24	0.007
Water movement				
Tidal		1.00		
Non-tidal	5.26	13.92	1.47-132.09	0.022
Other insect taxa				
	9.67	2.57	1.42-4.67	0.002
Week				
Week 1		1.00		
Week 20	7.44	291.41	4.94-17,197.57	0.006
Weeks 2-19				ns

Adult anophelines collections varied along the different transects with Transect 1 collecting more adults than transect 2 (Odds Ratio (OR) = 0.035, 95% CI=0.005-0.235, *P*=0.001), and 3 OR= 0.036, 95% CI= 0.005-0.270, *P*=0.001). Adults were more likely to be collected on the edge of the paddies compared to the centre OR= 0.148, 95% CI=0.040-0.554, *P*= 0.005. They were most likely to be found in small, non-tidal water bodies where rice was grown (OR=13.92, 95% CI = 1.47-132.09, *P*=0.022). The likelihood of finding anopheline larvae varied weekly. Adults of *An. gambiae s.l.* were associated with higher invertebrates in general (OR=2.57, 95% CI = 1.42-4.67, *P*= 0.022). This relationship can also be seen in Fig 4.6 (r^2 = 0.43, F = 62.2, *P*<0.001).

Discussion

Understanding the breeding habitats of mosquitoes and why they prefer certain water bodies and sites over others is very important for the planning of sound mosquito control strategies and is particularly important in areas where both natural or traditional rice cultivation is practiced (Birley, 1995; Ijumba & Lindsay, 2001). Rice developments projects all over the world have been linked to negative impacts on human health, particularly with respect to vector borne diseases. Colizzi, 1992 emphasized the effect of human activities, particularly irrigation, on development of anopheline vectors and species composition in West Africa. Therefore, it has been suggested that agriculture was one of the reasons for rapid speciation of the *An. gambiae* complex in Mali (Favia *et al.*, 1997). Evidence for a direct relationship between irrigation development and increased malaria transmission is inconsistent (Harrison & Schanlon; 1975; Oomen *et al.*, 1994; Ijumba & Lindsay; 2001), with increases transmission in some conditions (Coosemans, 1985, Robert *et al.*, 1992) but not in others (Robert *et al.*, 1988; Boudin *et al.*, 1992).

This study has shown a wide diversity of mosquito species being produced in natural rice growing swamps bordering the River Gambia. By order of abundance, *An. arabiensis* was the dominant species of *An. gambiae* complex, 61/68 (90%) but *An. gambiae s.s.* and *An. melas* were also identified. Elsewhere in Mali (Toure *et al.*, 1994) and in neighbouring Bukina Faso in particular during the dry season, *An. arabiensis* was the dominant species (Costantini *et al.*, 1996). However despite 285 collections over 8 months during the rainy season only 263 adult mosquitoes were sampled. *Anopheles arabiensis* is frequently associated with rice fields, as has been described in The Gambia (Bøgh *et al.*, 2000, Thomas & Lindsay 2000), Tanzania (Charlwood & Edoh 1996), Kenya (Minakawa *et al.*, 1999; Gimnig *et al.*, 2001) and in Mali (Edillo *et al.* 2002). This outcome was consistent with the preliminary findings from the larval control trial currently underway in the same region in The Gambia where *An. arabiensis* predominates (S. Majambereh, unpublished). Mosquito density was higher in near edge of paddies closer to village than far edge of paddies more distance. All adult mosquitoes were collected in the first 350m sampling area, closest to the study village. This agrees with findings from the larval surveys described in the previous chapter. Adult numbers were greater along transects 1 and 2, than 3. The explanations for this finding was that transects 1 and 2 were closer to the village and had more rice fields within the first 350 meters from the village compared with transect 3. In contrast, there were more grassland areas within the first 350 meters along transect 3, and whilst this condition supported breeding of *An. gambiae*, their concentration was lower here. This finding is supported by several studies. Shidrawi (1972) concluded that more than 90% of anophelines adults were found in houses less than 300 meters from larval breeding habitats. Similarly in 1996, Charlwood and Edoh (1996) discovered that higher densities of larvae were found close to homes with high numbers of adult mosquitoes. Minakawa *et al.* (2002) also reported that anopheline adult densities were greater in areas where larval breeding habitats were close to houses.

In the adult survey, more adult mosquitoes were found on the landward edge of rice fields compared with centre of fields (91%). A similar result was found sampling larvae where densities were higher on landward edge of rice fields. One explanation for this finding was that these areas are close to the study village. Gravid female mosquitoes leaving these houses presumably oviposit in the first water bodies they find. Other studies have also found mosquitoes in rice fields were more common on the edges of rice fields (Trape *et al.*, 1992; Minakawa *et al.*, 1999; Staedke *et al.*, 2003). It is also likely that the non-tidal paddies close to the village are also unlikely to have fish, which are known to be important predators of mosquitoes (Aniedu *et al.*, 1993, Service 1973, 1977).

Rice fields are colonized by different species of mosquitoes (Lacey and Lacey 1990). Mosquito larvae were observed in appreciable numbers in the second week of August (Chapter 3) soon after rainfall had started to fall regularly. Adult mosquitoes were also collected in the second week of August rising steadily through to October and declining in November. Most *An. gambiae* complex were collected in September and in October (66%) towards the end of the rainy season, the typical period of peak malaria transmission. When rice plants were first transplanted in August, the number of adults mosquitoes collected was low but increased over time through to October. But as the rice plants became fully grown and established, the number of mosquitoes declined. This was probably because mosquitoes had reduced access to the water surface for oviposition

(Rafatjah 1988). This decline in mosquito abundance with increasing height of rice has also been described previously by numerous workers (e.g. Snow, 1983; Lindsay *et al.*, 1991, Ijumba *et al.;* 1997; Rafatjah, 1988). These findings clearly indicate that mosquito production was seasonal and highly associated with rice cultivation. Interestingly *An. gambiae s.l.* was found in association with other aquatic insects, and anophelines were strongly correlated with the increasing numbers of culicines. Biological (biotic) factors in and around habitats where mosquitoes larvae survive, such as presence of predators (Aniedu *et al.*, 1993, Service 1973, 1977), parasites and pathogens (Service 1973, 1977), availability and quality of food (Gimnig *et al.*, 2002) greatly affect the development and survival of *An. gambiae s.l.* Physical factors (abiotic) such as temperature (Lyimo *et al.*, 1992) and alkalinity (Le Sueur & Sharp, 1988) also affect larval distribution, although this was not apparent in this study. Rice fields contain varieties of insect predators of mosquito larvae (Service 1977), such as Hemiptera, Zygoptera, Ephemeroptera, as well as larval and adult Coleoptera. In Kenya for example, about 30 species of other invertebrates were confirmed to feed on mosquito larvae in laboratories. Despite the predators of numerous invertebrates, anopheline adults still emerged from these sites.

Conclusion

The greatest number of adult mosquito production occurred in the first 350m of rice fields close to the human settlement. Although rice cultivation provides a good source of income and food, it also exposes local communities to the risk of mosquito vectors. In other to promote agricultural productivities and also protect its negative impacts on public health there is need for closer collaboration between health and other sectors in planning and implementation especially in irrigations schemes so as to reduce the vector densities. As results from this study suggested that traditional rice growing supports mosquito production, there is potential for increase in malaria mosquito density and consequently malaria transmission in such local communities. As many governments in Africa today including The Gambia, have planned or are planning to improve rice growing because of economic benefits and self sufficiency in food production to meet demands form the growing population, effective health care programs that are geared towards malaria control and prevention should be designed and made available to help local rice growing communities. These results suggest that for large-scale application of larvicidies, it would be important to ensure that rice fields close to human settlements are particularly well covered with larvicides for significant source reduction.

CHAPTER 5

EFFECTS OF WATER QUALITY ON MOSQUITO PRODUCTION UNDER SEMI FIELD CONDITIONS IN RURAL GAMBIA

Fig 5.1: Semi-field ponds

SUMMARY

Background

It is well known by farmers that application of nitrogenous fertilizers and manure increases rice yields. Here I examine the effect of urea, cow dung and filamentous algae on mosquito production under semi-field conditions in Rural Gambia.

Methods

Four trials were conducted over a four month period. In each trial, 16 bowls were treated with four treatments types: cow dung, algae, fertilizer and water served as a control. The study was conducted at Medical Research Council field station in Farafenni in rural Gambia

Results

A total of 17,467 larvae were sampled over 4 month periods in 16 bowls. Of these 6,233 (36%) were anophelines and 11,234 (64%) culicines. Early instars (75%) were more common than late instars (25%). Population densities of anopheline larvae were

significantly greater in ponds treated with cow dung than those treated with just water (controls).Greater numbers of culicines were found in association with cow dung and algae than control bowls.

Discussion

The results indicated that population densities of anophelines were significantly greater in ponds treated with cow dung and that greater numbers of culicines were also found in ponds treated with cow dung and algae than control ponds. The results suggest that rice fields that are fertilized by dung would increase vector mosquito densities and thus increase malaria transmission.

INTRODUCTION

Mosquitoes are known to exploit an enormous range of water bodies. For example, the sibling species *An. gambiae s.s* and *An. arabiensis* may be found in rice fields, borrow bits, temporal pools, water collections in human foot prints, animal hoof prints, and water collecting vessels (Service and Townson, 2002). Whilst there is a considerable literature on the type of breeding sites colonized by different mosquito species, little is known about how water quality affects mosquito production.

The importance of the organic content of water has been studied by various authors (Hancock, 1934; 1938; Swellengrebel *et al.*, 1952; Holstein, 1954). However, few firm conclusions can be made beyond the fact that it is variable. Hopkins (1933) showed that *An. gambiae* larvae can be controlled by adding cut grass to larval breeding site that pollutes the water. Hancock (1934) achieved the same results by adding town refuse to larval breeding sites. Aquatic vegetation, such as algae, is associated with high larval densities of some malaria vectors, including *An. pseudopunctipenni* Grassi in South America (Rejmankova *et.al.*, 1993). Bond *et al* (2004) also in a controlled field that manually algal removed from breeding pools along a river in Southern Mexico study significantly reduced both larval and adult densities of *An. pseudopunctipenni* for up to 6 weeks. Similar results were achieved in a community based- program where algae were removed *An. subpictus* coastal breeding sites in South-east India (Rajagopalan *et. al.*, 1991). Recent studies have shown that synthetic nitrogenous fertilizers have been found to be responsible for a significant increase in anopheline and culicine larvae population in Indian rice fields (Victor and Reuben, 2000). In Kenya greater numbers of *An. arabiensis* larvae were found in ponds with ammonium sulphate compared with untreated ones (Mutero *et al.*, 2000). In this case the fertilizer was thought to reduce turbidity, which implies that part or all of this effect may have been due to the greater sampling efficiency of collecting larvae in less turbid treated ponds than the more turbid control ones.

Water bodies within a rice field represent a heterogeneous landscape with regard to biotic and abiotic characteristics. The physiochemical properties of any water body from which adult mosquitoes successfully emerge from is important since larval control interventions could be targeted at water bodies best suited to producing mosquitoes. In this study we compared three different water treatments that were commonly found under field conditions in Gambian rice fields. Filamentous algae naturally grow in water bodies, including rice fields close to the River Gambia. In these fields cows graze on the rice stubble during the long dry season and their dried dung litters the ground. On the onset of the rains, the cow dung dissolves to fertilise the fields. Sometimes dung is also collected by farmers and applied to their fields. Urea is the most common and highest concentration of nitrogenous synthetic fertilizer in solid form used by farmers in The Gambia. It contains about 46% of nitrogen (Addai, 1997).

In this study, I investigated whether the presence of algae (biofertilizer), cow dung (organic fertilizer) and urea (synthetic fertilizer) in water bodies colonized by mosquitoes will affect the productivity of breeding sites under semi-field conditions. The study will therefore be useful since it may help understand what impact nutrients will have on the production of mosquitoes in the rice fields in The Gambia.

MATERIALS AND METHODS

Sampling Frame

Sixteen plastic bowls filled with 20L of tap water served as artificial breeding sites for mosquitoes. The bowls were arranged in a grid of four parallel rows and were spaced approximately 1m apart (Figs 5.1 & 5.2). Each bowl had a surface area of $0.21m^2$. Bowls had 2 small holes, 1cm in diameter at the top covered with netting materials to prevent rain water washing out larvae from the bowls. The lip of the bowl was 5cm above the ground. Approximately 5g of alluvial soil from the flood plain of the River Gambia was added to each bowl in order to provide the biotic and abiotic conditions suitable for mosquitoes. Each bowl had one of the following treatments: cow dung, algae, synthetic fertilizer and clear water as controls. The amount of cow dung, algae and synthetic fertilizer used was roughly approximate to conditions seen in the field. Cow dung was collected from the field, homogenised and weighed. Thus in total, I used 200g of dry weight of cow dung, synthetic fertilizer (Urea) and wet weight of algae in each pond. Filamentous algae for this exercise were collected from those that grew around hand pump wells in Farafenni. Treatments were applied using a 4 x 4 Latin square design (Fig 5.2). For each of the four trials, each treatment was randomly allocated a different bowl number (Table 5.1).

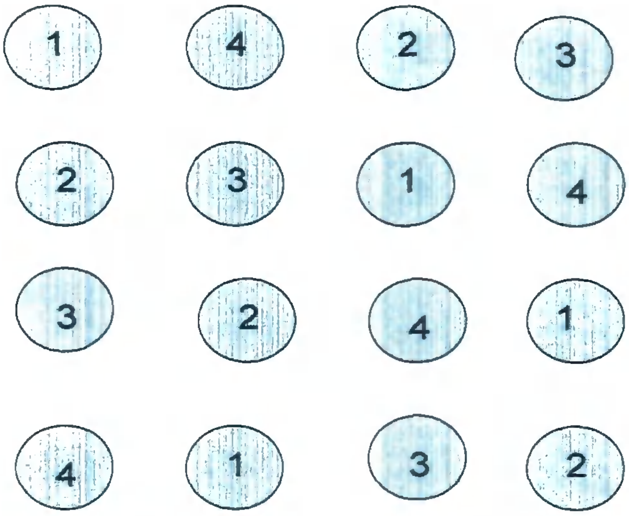

Fig 5.2: Position of bowls with numbers representing specific treatment arranged in a Latin square design

Table 5.1: Treatment schedules during the trial

Trial	Cow dung	Synthetic Fertilizer	Algae	Clean water
1	1	3	4	2
2	2	1	3	4
3	3	4	2	1
4	4	2	1	3

Bowls were covered for two days 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, from edges and centre. This was done everyday for 14 days over a 12 week period. Larvae were counted, identified as anopheline and culicine, and grouped and recorded according to their instars stage, 1st and 2nd stage as early instars and 3rd and 4th stage as late instars and then larvae returned to bowls. Pupae collected were removed from the bowls, counted and transferred into cages as per treatment where adult mosquitoes emerged. Adult mosquitoes were identified to species level with the aid of microscope and morphological key in the laboratory. *An. gambiae* complex mosquitoes from each treatment bowl were identified to species level by PCR (Scott *et al.*, 1993) (described in chapter 2).

Statistical analysis

Generalized Estimate Equations were used to examine whether there were significant differences between treatments, adjusting for repeated measures and allowing for the variation between bowls and trials. Statistical analysis used SPSS version 15.0.

RESULTS

Anopheline larvae sampled

A total of 17,467 immature stages of both anopheline and culicine were sampled during the study. Of these 13,185 (75.4%) were early instars and 4,282 (24.5%) late instars. Out of 17,467 larvae of both species sampled, 6,233 (35.7%) were anopheline larvae whilst 11,234 (64.3%) were culicines. Of the anopheline larvae sampled, 5164 (83%) were early instars while 1069 (17%) were late instars. There were 53% more anophelines larvae in bowls with cow dung compared with the untreated control, after adjusting for repeated measurements and differences between trials and bowls (Table 5.2, 5.3 & Fig 5.3). However there was no difference in the number collected in bowls treated with algae or urea compared with the control. Mean early stage (1&2) anopheline larvae collected was 1293 while late stage (3&4) was 238.

Table 5.2: Summary of anopheline larvae sampled in different treatments.

Treatments	Fresh water		Urea		Cow dung		Algae	
Anophelines Trial	Early instars	Late instars	Early instars	Late instars	Early instars	Late instars	Early instars	Late instars
1	35	41	34	28	42	35	68	65
2	580	138	413	93	777	167	922	194
3	422	19	434	6	803	79	435	56
4	27	9	48	5	96	115	28	19
Total	**1064**	**207**	**929**	**12**	**1718**	**396**	**1453**	**334**

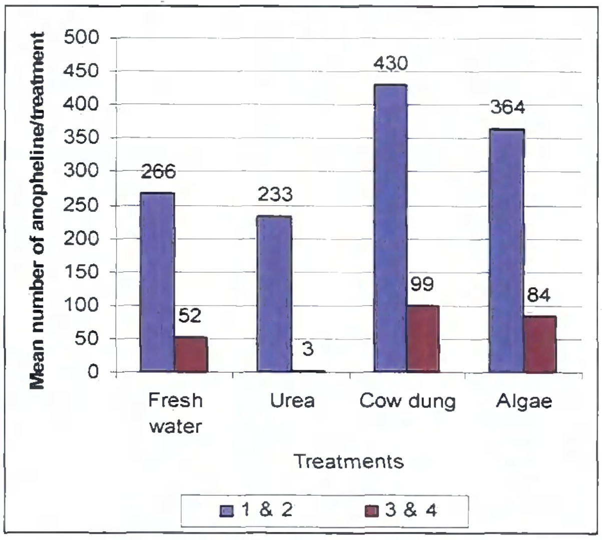

Fig 5.3: Mean anopheline larvae (1st & 2nd instars vs 3rd & 4th instars) sampled monthly by treatment over four months of trial period.

Table 5.3: Geometric mean number of early and late anophelines in different treatments

Treatment	Mean no. *Anopheles*/bowl (95% CIs)	Odds Ratio (95% CIs)	P (Significance)
Fresh Water	5.67 (4.32-7.02)	1.0	-
Urea	4.74 (3.47-6.01)	0.79 (0.48-1.31)	0.364
Cow dung	9.44 (7.62-11.27)	1.53 (1.13-2.08)	0.007
Algae	7.98 (6.12-9.84)	1.43 (0.94-2.17)	0.099

Culicine larvae sampled

A total of 11,234 culicine larvae were sampled during the study. Of these 8021 (71%) were early stages and 3213 late stages (29%). Production of immature stages of early culicines was greater than late stages in all treatments. Mean early stage (1&2) culicines larvae collected was 2026 while late stage (3 & 4) was 805.

Table 5.4: Summary of culicines larvae sampled in different treatments.

Trial	Fresh water		Urea		Cow dung		Algae	
Culicines	Early instars	Late instars	Early instars	Late instars	Early instars	Late instars	Early instars	Late instars
1	199	96	108	177	218	169	367	469
2	532	170	328	222	464	85	707	272
3	377	44	583	91	1379	270	1175	250
4	424	42	241	13	614	767	305	96
Total	**1532**	**332**	**1260**	**503**	**2675**	**1295**	**2554**	**1087**

There were 143% more culicine mosquitoes in bowls treated with cow dung and 111% more in bowls with algae than the control bowls (Table 5.5, 5.6, Fig 5.4). However, the number of mosquitoes collected in bowls treated with urea was similar to the controls.

Table 5.5: Culicines Geometric mean no. of early, late and pupae of culicines/treatment.

Treatment	Mean no. *Culicines*/bowl (95% CIs)	Odds Ratio (95% CIs)	*P* (significance)
Fresh Water	8.32 (6.36-9.95)	1.0	-
Urea	7.87 (6.36-9.38)	1.03 (0.61-1.75)	0.902
Cow dung	17.71 (14.50-20.92)	2.43 (1.66-3.56)	<0.001
Algae	16.25 (13.50-19.05)	2.11 (1.42-3.13)	<0.001

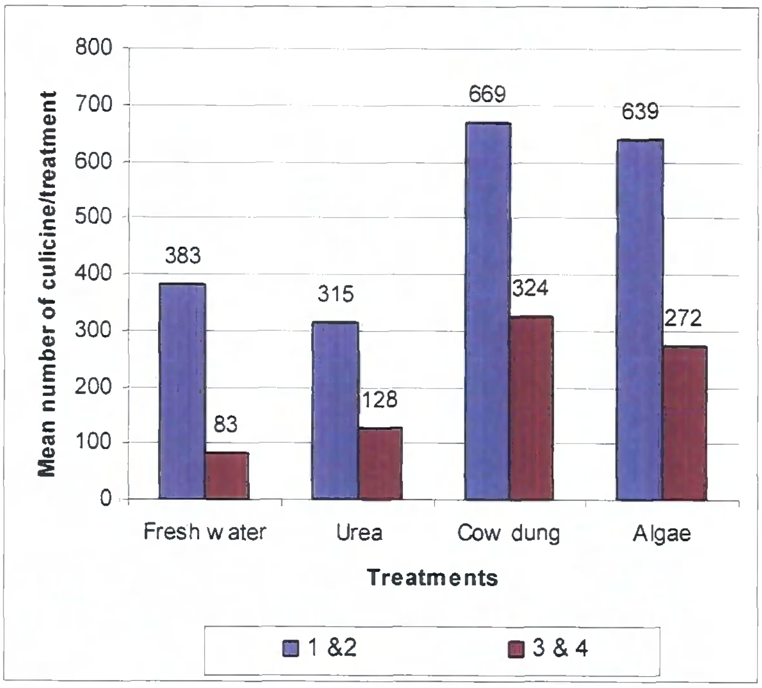

Fig 5.4: Mean culicine larvae (early and late stages) sampled monthly by treatment over four months of trial period

An. gambiae s.l. identification

Of 138 *An. gambiae s.l* identified most were *An. arabiensis* (54%) and *An. gambiae s.s.* (43%; Table 5.6). Again more larvae were found in bowls with cow dung (44%) and algae (36%) than the freshwater control and urea treated bowls (9%) respectively. It was interesting to note that the proportion of *An. gambiae s.s.* was greater in this trial than in larval survey. Sixty-eight out of 74 *An. arabiensis* and 52 out of 59 *An. gambiae s.s* were sampled in late wet season. Densities of *An. gambiae s.l.* were greatest in September, but declined slightly in October and November (Fig 5.5), which is typically the intense malaria transmission period in The Gambia. The presence of *An. melas*, which is a salt water breeder, was very low in this trial since the bowls contained fresh water.

Table 5.6: Species of *Anopheles gambiae* complex identified from each treatment

Treatment	Species of the complex			
	An. gambiae s.s	*An. arabiensis*	*An. melas*	Lost Species
Cow dung	24	35	1	1
Control (Water)	8	4	0	0
Urea (Fertilizer)	6	7	1	2
Algae	21	28	0	0
Total	**59**	**74**	**2**	**3**

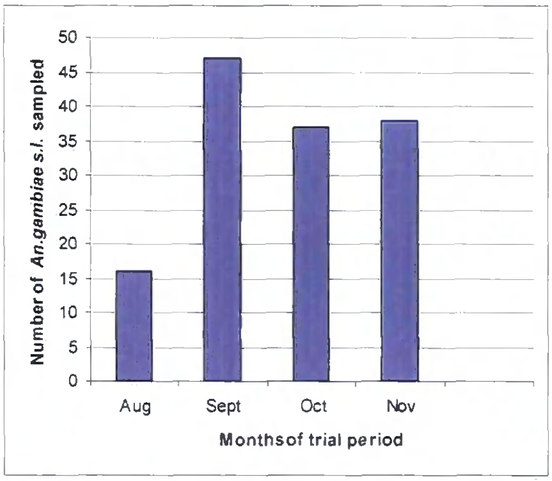

Fig 5.5: *Anopheles gambiae* complex identified per month of the trial period

Discussion

Improvements in agricultural productivity has stimulated and encouraged many African countries to improve agricultural productivity through irrigation schemes (Dalrymple 1986; Grist 1986; Juliano, 1993). In the last two decades rice cultivation practices has changed, the period has witnessed introduction of more irrigation schemes and increased areas for rice cultivation. The introduction of highly yielding varieties (HYVs) necessitates frequent use of farm inputs of fertilizer, insecticide and other agrochemical (Roy *et al.,* 1980). However the role of both organic and inorganic fertilizers in enhancing malaria vector population in rice habitats has not been well documented in SSA (Mogi *et al.,* 1984; Lacey & Lacey, 1990). Here I show that more anopheline mosquitoes were produced in bowls containing cow dung compared with freshwater controls.

Cow dung is an excellent source of bacteria and rotifers on which mosquito larvae feed, and is probably a key factor that supported their survival. This could be one explanation why most anopheline larvae are found in rice fields close to the landward edge of the floodplains. In these fields cows graze on the rice stubble during the long dry season and their dried dung litters the ground. At the onset of the rains, nutrients from cow dung dissolve to fertilise the fields. In addition cow dung is also collected by farmers and applied to their fields. Thus by fertilising their fields with cow dung, they inadvertently may increase malaria mosquito numbers. Filamentous alga (*Spyrogyra* spp) naturally grows in water bodies, including rice fields, close to the River Gambia were also found to have increased the production of culicines. Presumably mosquito larvae feeding on the algae gain nutrients from this source.

Urea is the most common synthetic fertilizer used by farmers in The Gambia on rice fields to provide nutrients for their crops. In my study the application of urea did not increase mosquito numbers. These findings were in contrary to that by Simpson and Roger (1991) who found a greater population of mosquito larvae in rice plots where nitrogenous fertilizer (urea) was used than those without. The differences between these findings however are unclear. However, it is important to note that, this study was conducted under semi field conditions, whilst that of Simpson and Roger (1991) was done in actual rice fields. Applying urea in fields will have a different effect on larval survival and development as many other micro-organisms, including bacteria and algae are already present. Rice fields are larger, have more open water bodies that could have been readily colonised by malaria mosquitoes whereas in this study, artificial ponds were much smaller.

Adult mosquito identification from the study showed a great diversity of species. *An. gambiae s.l.*, *Culex* and *Aedes* were all identified from the same treatment bowls, confirming the earlier suggestions that these species often share the same habitats. *Anopheles arabiensis* was the dominant species accounting for 53.6% while 42.7% were *An. gambiae s.s.*, the two most important malaria vectors in the study area of The Gambia. Similar findings have been reported in other parts of West Africa. In Mali (Toure *et al.*, 1994) and Burkina Faso during the dry season, *An. arabiensis* was the dominant species (Costantini *et al.*, 1996). *Anopheles melas* was also identified, although they were fewer in number compared to the other two members of the complex. This might have been because this particular species is a salt water breeder and while the study was conducted in fresh water conditions, its number tend to be much lower than the other species.

Conclusion

No studies have investigated the effects of organic and inorganic fertilizer on larval production in natural rice fields in Africa. It should be noted that treatments used in this study were not meant to provide the same quantity of nutrients, but reflect what can be found in Gambian rice fields. Bowls treated with urea are likely to produce more nitrogenous compounds than cow dung or filamentous algae. However, cow dung and algae will generate bacteria and rotifers on which mosquito larvae will feed, while cow dung may also release soluble nutrients suitable for larval food. It was found that immature stages of both anophelines and culicine mosquitoes were greatest in ponds treated with cow dung compared to those without. By extension rice fields fertilized by dung could support production of mosquitoes that are capable of transmitting malaria.

Therefore, both farmers and malaria experts should direct their control measures to natural fields fertilized by dung.

Chapter 6

GENERAL DISCUSSION

Rice is the staple food in many African countries and constitutes a major part of the diet in many others. During the last three decades, the continent has seen a steady increase in demand that outpaced its production. This makes its cultivation more important in the strategic planning for food security for the continent (Andrade *et al.*, 1984). In 1996, Africa consumed a total of 11.6 million tonnes (Mt) of milled rice per year, of which 3.3 Mt representing (33.6%) were imported (Tshibaka and Klevor, 2002). As many as 21 of the 39 rice-producing countries in Africa, imported between 50 to 99% of their rice requirement. The Gambia also registered a steady increase in rice production over the last 10 years, but this does not commensurate with demand from the growing population. Rice cultivation has long been blamed for increasing in mosquito populations. We do know for certain that rice fields in many instances provide ecological requirements of vectors of malaria (IRRI, 1987, Ijumba & Lindsay 2001). In spite of these fears, rice cultivation has been on the increase in the African region in order to provide food for the increasing populations. However, despite its ecological suitability for multiplication of anopheline vectors, the epidemiological impact of rice cultivation varies according to the local malaria situation (Carnevale *et al.* (1999). It can be associated with an increase in malaria and morbidity, as in Burundi (Coosemans (1985) and the uplands in Madagascar (Laventure *et al.* 1996), or decrease as in northern Cameroon (Audibert *et al.* 1990), in the Senegal River valley (Faye *et al.* 1993, 1995, in the Kou valley in Burkina Faso (Boudin *et al.*1992) and in The Gambia River valley (Lindsay *et al.* 1991). The phenology of adult mosquitoes in relation to rice production has been well documented (Chandler and Highton 1975; Snow 1983; Klinkenburg *et al.* 2003), but fewer studies have described the dynamics of larval populations (Mwangangi, Shililu *et al.*, 2006; Muturi, Mwangangi *et al.*, 2007). Here we describe the dynamic process of 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 study is one of the few to have documented mosquito production in association with traditional rice growing and confirms our earlier conclusions that rice fields are a major site for malaria vectors in the middle reaches of The Gambia (Majambere, Fillinger *et al.* in prep). In this study larvae colonized 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). These findings clearly indicated that mosquito production was seasonal and highly associated with rice cultivation.

Overall 93% of anopheline larvae and 91% of culcines were found in rice fields within the first 350m from the landward edge and nearest the village. Ninty-one percent of *An. gambiae* complex were found aggregated at the edges of rice paddies, as has been found in other studies (Hocking, 1953; Minakawa *et al.*, 1999). More adult mosquitoes were sampled on the edge of the paddies compared to center. This area of open water close to land was rich in culicines and other invertebrates and represents the shortest flight distance for ovipositing females leaving their indoor resting places to lay their eggs in breeding sites, a key factor that is likely to exacerbate malaria transmission in rural Gambia. Previous studies conducted in urban settings of Senegal and Uganda also demonstrated that clinical malaria cases were strongly associated with close proximity to breeding places, acting as the main malaria transmission sites (Trape *et al.* 1992; Staedke *et al.* 2003; Girardin *et al.*, 2004).

Paddies close to the land in this study were rain fed, demarcated by raised embankments to prevent salt water intrusion the river. This made these fields permanent breeding sites during the rains as compared to paddies closer to the river which were tidal dependant. Thus tidal water may be unsuitable for mosquito larvae and other invertebrates as it will be difficult or impossible to maintain their position in the water column in areas of tidal water as may get flushed away.

The adult emergence study was designed to assess what species and number of mosquitoes is produced in different parts of rice fields. It provided an opportunity to further our understanding of the effect of traditional rice cultivation and production of mosquitoes in rice fields close to human habitations. Our data confirm that *An. gambiae s.s, An. arabiensis* and *An. melas* are the principal malaria vectors in this part of The Gambia. Results from this study further confirmed that density of *An. arabiensis* was higher (89.7%) than other members of the complex. Our data showed that anthropophilic mosquito species mainly consist of *Culex spp., An. gambiae s.l.*, and *Aedes spp.* Similar findings were reported from rural parts of Kenya (Surtees, 1970; Chandler & Highton, 1975), where irrigated rice cultivation was practiced, although our study looked at traditional swamp rice cultivation. Hence it was concluded in Kenya that members of the *An. gambiae* complex and *Mansonia* spp. were the predominant rice field-breeding mosquitoes. More recent studies carried out in Burkina Faso and Côte d'Ivoire came to the same conclusions (Robert *et al.,* 1988; Doannio *et al.,* 2002). Conclusions from this investigation is important, since recent studies investigating the applicability of larval control in The Gambia have identified rice fields as being important source of anophelines larvae (S. Majambere, unpublished). This finding is supported by others that indicated that the landward edge of the floodplains, where rice is often grown, is an important source of anophelines (Bøgh *et al.* 1999; Thomas and Lindsay, 2000).

Achievements in rice production especially in Asia have encouraged many African countries to improve agricultural productivity through irrigation schemes and high yielding varieties of rice (HYVs; Dalrymple 1986; Grist 1986; Juliano, 1993). The introduction of HYVs necessitates frequent use of farm inputs of fertilizer, insecticide and other agrochemical (Roy *et al.,* 1980). However, the role of both organic and inorganic fertilizers in enhancing malaria vector population in rice habitats has not been well documented in SSA (Mogi *et al.,* 1984; Lacey & Lacey, 1990). However, findings from this investigation showed that water found in rice fields close to land was relatively rich in nutrients with large amounts of cattle dung. Cow dung is an excellent source of bacteria and rotifers on which mosquito larvae feed. Cows graze on the rice stubble and their dung litters the ground. On the onset of the rains, dissolved dung fertilise the fields. Dung is also collected by farmers and applied to their fields. Thus by fertilising their fields with cow dung, they inadvertently may increase malaria mosquito numbers. 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 and presumably they provide the nutrients for the organisms upon which mosquito larvae feed. 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, with large numbers being herded to the Casamance further south. Unlike an earlier study in Kenya (Mutero *et al.* 2004) nitrogenous fertilizer did not increase larval numbers.

There was a higher proportion of *An. gambiae s.s.* (43%) sampled in this trial compared with 29% from the larval survey and only 6% from the adult survey in the field. The high percentage of *An. gambiae s.s.* in the semi field sites could be due to the preference of this mosquito for small open breeding sites, as well as a reflection of it's more anthrophilic nature, attracting it to Farafenni town, where the semi field trials were conducted. Results from the semi field trial showed that more anopheline mosquitoes were produced in treatments containing cow dung compared with freshwater controls. Interestingly counts of dung in the field showed that it was far more common near the landward edge nearest the village (80%), suggesting that this might improve mosquito productivity in these sites, by providing water rich in nutrients. This probably was a key factor that supported their survival and could be one explanation why most anopheline larvae are found in rice fields close to the landward edge of the floodplains. Filamentous alga (*Spyrogyra* spp) that grows naturally in water bodies, including rice fields close to the River Gambia, were found to increase both anopheline and culicine production.

Swamp rice fields are an important habitat for mosquito larvae in rural Gambia. This is of major significance to the future health of people living in the middle reaches of The Gambia where swamp rice is cultivated, especially if rice cultivation is increased in the future as demand increases. Since this method of rice cultivation is practiced widely in different parts of West Africa (Agyen- Sampong, 1994), our findings are of general interest. The greatest amount of adult mosquito production occurred in the first 350m of rice fields close to the human settlement. The results further suggest that in areas of extensive swamp rice cultivation malaria control efforts could be supplemented by mosquito larval control measures which would be targeted in time for approximately 3 months a year and in space at the most productive areas. Findings from this study showed that rice cultivation was strongly associated with increased production of different mosquito species and other invertebrates in paddies close to human settlement compared to those near the river, and that cow dung and filamentous algae were found to support both anopheline and culicines production.

In conclusion, traditional rice cultivation has increased mosquito population, but precisely how that affects malaria transmission is uncertain. We do know that access to health services in our study area was good with a high percentage of people sleeping under ITNs, a key intervention that has a proven track record of reducing malaria-related morbidity and mortality (Lengeler & Snow, 1996; Takken, 2002; Lengeler, 2004). Thus in areas with traditional rice cultivation it is essential that the local community is protected against anopheline mosquitoes.

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