STUDIES ON THE PRODUCTIVITY OF ANOPHELES BREEDING SITES IN RELATION TO ADULT MOSQUITO DENSITY

by

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DEDICATION

This thesis is dedicated to
Constance my wife,
Mujuni, Kemilembe and
Mulokozi our children,
for their kind support and
perseverance

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L.S.H.T.M (United Kingdom)

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TANZANIA

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ABSTRACT

Entomological studies were conducted in Tanga region, Tanzania, over a two year period, to assess the effect of various environmental features and larval population density on the productivity of *An. gambiae s.l.* breeding sites for adult mosquitoes.

These studies included:

(i) Nineteen rounds of systematic dipping for larvae in about 150 mapped potential breeding sites around two villages. During this time records were kept for each potential breeding site of the dominant plants, suspended mud, odour, shade, depth, speed of water-flow, nature of margin, surface particles, and other features. The insects suspected of feeding on mosquito larvae were recorded. These included members of the family Notonectidae, *Culex tigripes*, and dragon- and damsel-fly larvae.

(ii) Field experiments to assess the effect of different larval densities on the emergence of adult *Anopheles gambiae s.l.*

(iii) Quantitative estimation of productivity of adult *An. gambiae s.l.* per unit area of water.

(iv) Field monitoring of the relationship of water depth and the production of first instars of *An. gambiae s.l.*

(v) Investigation of the impact of larviciding with temephos and *Bacillus thuringiensis israelensis* around one village on the larvae and on the density and parous rate of the adult female mosquito population in comparison with untreated controls. For these studies adult mosquitoes were trapped weekly using CDC light traps hung beside untreated bednets.

(vi) Mark-release-recapture to quantify immigration of *An. gambiae s.l.* from other villages into the one in which larviciding was carried out.

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CHAPTER 1

GENERAL INTRODUCTION

1.1 Malaria transmission in Tanzania

Tanzania generally experiences malaria transmission throughout the year. The intensity of transmission of the disease varies from place to place depending on the rainfall distribution and altitude, but on the average, over 75% of the country is hyperendemic to holoendemic for malaria (Clyde, 1967). There have been some changes in the picture of malaria distribution in the country over the years. This is partly attributed to increased population movements and changing pattern of agricultural activities which have led to higher levels of malaria transmission even in areas which were considered to be malaria free (Clyde, 1967; Matola, *et al.*, 1987). Likewise, the appearance of resistance in *P. falciparum* to antimalarial drugs has also contributed to the worsening malaria situation in Tanzania. It is now evident that chemotherapy can no longer be relied upon as an effective control measure against malaria (Kihamia, 1986). This has led the government to reconsider the possibility of using mosquito control as one of the main strategies against malaria in the country (WHO, 1983; Kihamia, 1986; Sepeku, 1986).

1.2 Malaria control by larviciding and adulticiding in Tanzania

Since malaria vector control activities were introduced in Tanzania by the German colonial government at the end of the 19th century, they have remained confined to urban and periurban areas and to a few centres of economic activity (Clyde,1967; Kilama, 1986). Malaria vector control activities include drainage and filling-in works in most towns, and routine application of larvicides as a back-up measure. Kerosene was widely used as a larvicide and later replaced by Malariol-HS (oil with a spreading agent containing 5% DDT) (Clyde, 1967). Adulticiding using pyrethrum, HCH,

Dieldrin or DDT has been used in agricultural and mining estates and on the fringes of towns to supplement larval control activities, in the Pare-Taveta scheme in the 1950s (Bradley, 1991) and in the whole of Zanzibar and Pemba islands in the 1960's (Clyde, 1967 Kilama, 1986). In most towns however, most of these activities have currently been abandoned or are at an abysmally low level of activity owing to lack of funds and trained manpower (Kilama, 1986). The only urban centres where malaria control activities are currently taking place are Dar es Salaam and Tanga. Here malaria control activities, conducted under the aegis of Tanzanian-Japanese Malaria Control Project, have been using fenitrothion for indoor residual spraying, although refusals of householders to entry of spraymen is increasingly becoming a problem (M.P. Membe, presonal comm.).

In the rural areas, malaria control activities almost solely rely upon the treatment of the sick and use of prophylaxis in the vulnerable groups (Clyde,1967; Kihamia, 1986). Virtually no mosquito control activities have ever been undertaken in the rural areas of Tanzania (Kilama, 1986). The exclusion of mosquito control from rural malaria control activities was apparently based on the following considerations:

(a) very high operational costs of running vector control programmes;

(b) anti-larval measures were considered impossible because of the ubiquity of breeding sites and scattered homesteads;

(c) chemotherapy and chemoprophylaxis were thought to be more cost effective (Clyde, 1967; Kilama, 1986; Wernsdorfer, 1988).

In recent years however, *P. falciparum* has developed a considerable level of resistance to chloroquine and certain other antimalarial drugs (Kouznetsov, *et al.*, 1980; Kihamia and Gill, 1982; Kilimali and Mkufya, 1985a and b; Mutabingwa *et al.*, 1985; Kilimali, *et al.*, 1989), integrated vector control has been advocated (WHO, 1982, 1983), community participation encouraged and mosquito control in the rural areas deserves fresh assessment.

Rural Tanzanian communities have undergone rapid changes in the past 20 years. Formerly scattered homesteads were resettled into well defined villages with a comprehensive structure of local government during the 1974 "Ujamaa" villagization campaign to make possible the provision of basic social facilities such as schools, health centres and dispensaries. It therefore seems more likely that one could now define the breeding sites supplying malaria vectors to a given village and that there would be adequate manpower and leadership available to carry out larval control on a sustained basis. Malaria has been controlled in some parts of India by involving the community (Sharma, 1987).

However, the following questions need to be answered before antilarval measures can be taken as part of mosquito control activates in the rural areas:

First: What evidence is there that antilarval measures are practically and economically possible in rural areas?

Second: After having applied adult control measures, for example by the use of pyrethroid impregnated bednets, what contribution could antilarval measures make to enhance the control of the adult mosquito population?

Third: What is the relative productivity of different breeding sites for adult anopheline mosquitoes and can attention be concentrated on the most productive? Is it the sites which are difficult to discover that are more productive or the more obvious rice fields, footprints, puddles, pools, ponds, streams and swamps?

Fourth: What environmental features can be used as markers for particular species so that the most productive breeding sites could be identified without specialist advice?

Fifth: With some sustained effort by members of the community could supplementary larval control be applied in specific villages which also use insecticide impregnated bednets for adult mosquito control?

Sixth: How can one, in practice, evaluate antilarval measures quantitatively?

These questions formed the basis of this project.

CHAPTER 2

LITERATURE REVIEW

2.1 Anti-larval measures: Mainstay of early mosquito control activities

Malaria is known to have afflicted people for many centuries but its cause remained a mystery for most of the time (Boyd, 1949; Russell, *et al.* 1963). Despite lack of proper knowledge as to what malaria really was, Romans and Greeks were able to carry out drainage works as early as 600 BC, which increased the area of arable land and the health of the people by greatly reducing malaria (Celli, 1933; Boyd, 1949; Russell, 1955). Similarly, the recession of malaria in Europe and the United States of America in the 19th century was primarily due to environmental changes involving drainage works (Russell, 1955; Kitron and Spielman, 1989).

Following the incrimination of *Anopheles* as vectors of malaria by Ross in 1897 (Bruce-Chwatt, 1985) the practical importance anti-larval measures began to be more realised. Drainage and oiling soon began to be applied in many places around the world for the control of malaria and other mosquitoes. Among the early successful projects in the control of malaria, principally by anti-larval measures, were those by Gorgas and Le Prince in Havana, Cuba, and Panama (Gorgas, 1903; Russell, 1955; Bruce-Chwatt, 1985). Other projects were carried out in Ismailia; Staten Island, New York, Malaya and in some towns in west Africa (Ross, 1909). Most of these early anti-anopheline activities were conducted in towns where breeding sites were thought to be localised (Ross, 1901), although some of these towns may have been quite comparable to the present day rural villages.

Large scale eradications of *An. gambiae s.l* from rural areas were carried out in northern Brazil (Soper and Wilson, 1943) and in Egypt (Shousha, 1948) where this mosquito had been introduced accidentally. The principal method of control was larviciding with paris-green (Copper-aceto-arsenite) and oil. Occasionally, small quantities of pyrethrum and DDT were

used against adult mosquitoes. It is argued that the elimination of *An. gambiae s.l* from both Brazil and Egypt was made possible by the exotic nature of this species in these countries where it was not well established (Logan, 1953). This could be true, but the fact remains, that concerted antilarval measures together with some adulticiding were able to eradicate *An. gambiae s.l* in rural areas (Logan, 1953). Later, the Sardinian Project in the Mediterranean showed that although an indigenous species, *An. labranchiae,* was difficult to eradicate it could be brought under control and, with some extra input, malaria was eradicated (Logan, 1953). Watson (1953) described attempts at source reduction and larviciding to control malaria vectors around copper mines in Zambia. However, the information on the control of *An. gambiae s.l* in African rural areas using anti-larval measures as one of the control methods is generally, rather scanty. This is attributed to the reliance on residual spraying as the main tool for anopheline mosquito control, the greater part of which was basically a series of insecticide trials (Zahar, 1985). It can therefore be concluded that there is no concrete evidence against the use of antilarval measures for the control of *An. gambiae s.l* in the rural areas of Africa.

It may be worth noting here that seasonal and geographical variations of larval and adult mosquitoes have in most instances not been incorporated into models for malaria transmission and control (Kitron and Spielman, 1989). These models have not put much emphasis on the heterogeneity which exists in nature (Molineaux, 1985). However, such variations are critical in determining the epidemiology of malaria in local situations (Kitron and Spielman, 1989). There is therefore, a need for a more pragmatic approach in the control of malaria since it is increasingly becoming evident that malaria varies from place to place (Kitron and Spielman, 1989).

2.2 Impregnated Mosquito bednets and antilarval measures for anopheline control in rural areas.

Recently, a number of trials have been conducted in various parts of the world to assess the effect of pyrethroid impregnated bednets on malaria transmission as an alternative to spraying of houses with residual insecticides (Rozendaal, 1989, Curtis, *et al.* 1990). The advantages of using pyrethroid impregnated bednets over conventional residual spraying for

malaria control have been outlined by Curtis *et al.* (1990) and Njunwa *et al.*, (1991). Briefly, these include the following: (i) In impregnated bednets the residual insecticide forms a barrier around the sleeping person making it more likely for the mosquito to pick-up a lethal dose of insecticide while attempting to take a blood meal. (ii) The nets can be impregnated at a low cost, although in some countries like Tanzania, purchase of nets may require a government subsidy in the same way as for drugs. (iii) The process of impregnation and re-impregnation of bednets is simple and safe for the community to adopt without close supervision. (iv) The additional effect of bednets killing other bloodsucking insects like bedbugs serves as an incentive for using nets regularly and creates enthusiasm during reimpregnation.

As an attempt to study the effect of permethrin impregnated bednets on malaria transmission in a holoendemic area a trial was conducted in Tanzania for a period of 3½ years (Curtis, 1990; Lyimo *et al*, 1991; Magesa *et al*, 1991; Njunwa *et al* , 1991). The entomological results showed that the introduction of impregnated bednets caused a significant reduction in the densities, sporozoite inoculation rates and survival rates of *An. gambiae s.l* (Magesa *et al*, 1991). Despite this significant decline in the vectorial capacity of *An. gambiae s.l*, the numbers of this species remained large enough to maintain malaria prevalence in the human population at levels not significantly different from those before intervention (*Magesa et al*, 1991; Lyimo *et al*, 1991) in the traditional villages where the houses were made of wattle and daub rather than brick and iron roofs found in the former sisal estate camps where some reduction in endemicity was achieved. Curtis (1992) and Bermejo and Veeken (1992) have reviewed the effect of impregnated bednets on malaria in different endemicity levels. They have shown that in areas of low to moderate malaria transmission, impregnated bednets reduce malaria related mortality and morbidity. However, in areas of high malaria transmission, the impact was less encouraging.

From these and other observations (Molineaux and Gramiccia, 1980) it can be deduced that impregnated bednets alone may not be sufficient to make marked impact on malaria parasite rates in the rural areas where malaria is holoendemic. In such circumstances, larval control might be a useful supplement to the overall attempt at controlling malaria in these areas.

2.3 Categories of breeding sites

A breeding site can be defined as "a collection of water in which eggs, larvae or pupae of mosquitoes are found; larval habitat", (WHO, 1963). It includes permanent breeding sites (with water staying long enough for vegetation to grow), temporary breeding sites (sites tending to dry-up before vegetation has grown), and potential breeding sites (water bodies normally containing no immature stages) (Bates, 1949; W.H.O, 1963).

A number of classifications of mosquito breeding sites in general have been given by several workers based on various criteria (Boyd,1930; Shannon, 1931; Hopkins, 1952; Bates, 1949 and Laird, 1988). While each of these classifications has its own limitations, it still serves the purpose in particular locations (Laird, 1988). However, the classification systems which put emphasis on the factors limiting the ecological distribution of mosquito larvae, like that of Bates (1949) and Laird (1988) are valuable since those factors form the basis for the choice of larval control measures (Wernsdorfer, 1988). In practice, it is important to develop a classification system of mosquito breeding sites in a particular region in order to carry out rational control measures (Bates, 1949). Such on-the-spot classifications have been found to be extremely practical (Bates, 1949). For a classification system to be useful it should be short and comprehensive for easy use by field staff (Laird, 1988).

Looking at some of these classification systems of breeding sites e.g. Bates, (1949) and Laird, (1988), it is clear that some terminologies are used interchangeably and the definitions are not given. For example, the criteria for calling a water body a pond, pool or puddle; also at times the exact meaning of marsh or swamp are not clear. There is need for defining terminologies in order to avoid errors when recording breeding sites (Hopkins, 1952).

2.4. Breeding site preference for *An. gambiae s.l* vand *An. funestus*

2.4.1 Choice of a breeding sites

The breeding site preference of mosquitoes has long been a matter of debate. Initially, it was thought that adult female mosquitoes did not select breeding sites for oviposition but laid their eggs indiscriminately, and larvae survived and developed in only a few appropriate sites (Buxton and Hopkins, 1925; Lamborn, 1922). However, it is increasingly becoming evident that the differences observed in the distribution of larvae of different species may be a result of selective behaviour of the adult female mosquito for particular breeding sites prior to oviposition (Bates, 1940; Thomson, 1940; Webb, 1987).

In spite of these observations, little has been done on this subject both in the field and in the laboratory using *An. gambiae s.l* (Causey *et al*, 1943; Muirhead Thomson, 1945; Hocking and McInnes, 1948; McCrae, 1984; Webb,1987) and the results have been rather inconclusive and/or conflicting. It seems therefore, that there are many questions to be answered before our knowledge about oviposition behaviour can effectively be put into practice. Hence, the remaining possible criteria for use as a guide in determining breeding sites preferred by *An. gambiae s.l* and *An. funestus* are the association of particular environmental features with the presence or absence of breeding without attempting to explain this in terms of oviposition behaviour (Bates, 1949).

In the following two subsections (2.4.2 and 2.4.3) breeding sites of *An. gambiae s.l* and *An. funestus* will be discussed. In subsection 2.5 the environmental features of these breeding sites will be discussed together.

2.4.2 *An. gambiae s.l* breeding sites.

Work on the breeding sites of *An. gambiae s.l* has been comprehensively reviewed by Gillies and De Meillon, (1968); and Zahar, (1985). The sites where this species has been found

breeding are diverse (Gillies and De Meillon, 1968). These sites include:

(i) shallow open sun-lit water collections. In this category there are borrow-pits, ruts, car-trucks, and hoofprints. Most breeding is said to be in this first category of sites

(ii) Rice fields - considered to be most productive when they have been recently flooded and planted. This does not, of course, include the cultivation of hillside-varieties which are grown on relatively dry land. It is only the irrigated method of rice growing which is associated with *An. gambiae s.l* breeding.

(iii) Other places include, edges of swamps, vegetated stream margins, irrigation and drainage channels, and ponds.

While these breeding sites are generally productive of this mosquito species, their distribution in different times of the year and geographical locations vary greatly (Gillies and De Meillon, 1968; Christie, 1959; Service, 1973). For example, Christie, (1959) found that, during the dry season, larvae of *An. gambiae s.l* survived in wells and swamps which were not their preferred habitats. Also, Goma, (1960) showed that Ugandan swamps were not a major source of malaria mosquitoes despite their large expanse, unless they were exploited for agricultural purposes. These observations suggest that simple generalizations about mosquito breeding sites can be misleading. Therefore, studies to identify sites which are most important in specific situations are necessary. Unfortunately, the existence of numerous different types of *An. gambiae s.l* breeding sites seems to have discouraged attempts at controlling larvae of this species in the rural areas. The validity of this pessimistic view needs to be critically examined.

2.4.3 *An. funestus* breeding sites

This mosquito species is known to breed in sites with clear and relatively permanent water (Gillies and De Meillon, 1968). Such sites include edges of swamps, streams, rivers, lakes, ponds, with vegetation along the margin; borrow pits and ditches (Gillies and De Meillon, 1968). Although the breeding sites of this mosquito species seem to be more defined than those of *An. gambiae s.l*, there are instances where *An. funestus* breeds in unusual sites. This tends to happen in the absence of alternative sites. For example it has been found breeding in deep shade, middle of swamps, in open expanses of water including large rivers and in

wells and domestic water-containers (Ingram and De Meillon, 1929; Swellengrebel *et al*, 1931; Symes 1936; Garnham, 1938; Garnham, 1948). It may be useful to determine the importance of these alternative sites in mosquito production since it is a common observation that *An. funestus* virtually disappears in the dry season or when anti-adult measures are carried out and for it to reappear when they are removed (Smith, 1959). It is anticipated that by sampling all accessible sites in a village during all the seasons of the year it may be possible to identify the important remaining breeding sites of this species when most breeding sites have dry-up. These sites could then be targeted for control during that particular season. However, the ability of these species to reach high densities rapidily on the onset of the rainy season, in spite of having been naturally reduced to almost undetectably low densities in the previous dry season, testifies to their very high reproductive capacity, and casts doubt on the prospects for such "dry season attacks" unless carried out in such a way as to cause large scale eradication.

2.5 Effect of environmental features on breeding of *An. gambiae s.l* and *An. funestus.*

For rapid identification by relatively untrained staff of sites requiring control it would be very helpful if easily described environmental features of breeding sites could be used as markers for the presence or absence of *An. gambiae s.l* and *An. funestus*. Initially it would be convenient to categorize the environmental features so as to deal with each of them separately, though in nature there could be interactions between environmental features required by each species (Krebs, 1978).

Bates (1949), classifies the features of larval environment into physicochemical and biological categories. Physicochemical features include: temperature, light/shade, water movement, depth, suspended mud, a sloping muddy margin, surface characters, hydrogen-ion concentration (pH), nitrates, organic materials, and finally inorganic salts. Biological features include microorganisms, vegetation, parasites, predators and competitors.

2.5.1 Temperature

A number of studies have shown that there is a wide temperature range in the habitats where

An. gambiae s.l and *An. funestus* breed (Gillies and De Meillon, 1968). In the laboratory *An. gambiae s.l* was found to tolerate higher temperatures than *An. funestus* (De Meillon, 1934). However, the temperatures of water in the breeding sites do not normally reach such extremes as 45^0 C tested by De Meillon (1934). Nevertheless, it can be said that *An. gambiae s.l* is more likely to be found in warm water, its death point being 42^0C (Haddow, 1943). Although *An. funestus* prefers cooler water it can tolerate temperatures as high as 39^0 C (Haddow, 1943). Available information indicates that temperature would not be a reliable feature as an indicator of likely presence or absence of a particular mosquito species in a given breeding site, and in any case it would be too inconvenient to measure water temperature during larval control activities. Diurnal variations in temperature are wide, especially in sun-lit sites.

2.5.2 Light/shade

Sunlight has been shown to exert some influence on the distribution of larvae of a number of mosquito species and the degree to which a breeding site is shaded may be a very useful guide for locating breeding sites in a control programme (Russell, 1963). However, very little is known on the role of sunlight in determining *An. gambiae s.l* distribution and to a lesser extent this is true of *An. funestus* also (Gillies and De Meillon, 1968). While it is generally said that *An. gambiae s.l* breeds in shallow, sunlit water bodies, and *An. funestus* in shaded places it is difficult to tell which features have direct influence particularly when vegetation, sunlight and presence/absence of mud vary markedly between breeding sites of these mosquito species. At this stage it may be important to take account of confounding effects by any of these factors in order to highlight the features which are directly influencing the distribution of larvae. It appears that this kind of approach has not previously been adopted in this type of study, but will be attempted in the present project.

2.5.3 Water Movement

An. gambiae s.l is said to prefer stagnant water and *An. funestus* flowing water (Gillies and De Meillon, 1968). However, it is questionable whether water movement is important in itself, or whether it has an indirect influence through vegetation and there are in fact many *An. funestus* breeding sites which do not contain flowing water (Gillies and De Meillon, 1968).

Studies involving Asian mosquito species such as *An. minimus* , *An. aconitus*, and *An. maculatus*, which are said to be stream-breaders, showed that females tended to oviposit more in still than in running water (Thomson, 1940). If the presence of vegetation is what matters then a clear definition of the relevant vegetation types would be more useful in guiding mosquito control activities. It should be noted that it is very common to find a body of water with apparently stagnant water surface but with measurable flow below the surface.

2.5.4 Depth

Although anopheline larvae spend most of the time at the surface of water (Bates 1949), the depth of a breeding site may have some influence on them in a number of ways including the presence or absence of predators and of vegetation that grows from the bottom. In most studies of mosquito larval ecology, the sites have been referred to only as "shallow" or "deep" (Boyd, 1930). Obviously, what may be considered shallow in one place could be regarded deep in another. So the statement that *An. gambiae s.l* and *An. funestus* breed in shallow water and at the edges of deep water respectively (Gillies and De Meillon, 1968) needs to be more accurately qualified and there is need for having consistency in the usage of these words, by defining the range of each category of site. In this project depth was the main criteria used for distinguishing sites described as "puddles", "pools" or "ponds" from each other.

2.5.5 Suspended mud

While *An. gambiae s.l* is known to be found in water which is muddy, and exposed to sunlight (Gillies and De Meillon, 1968) it seemed worthwhile to re-assess field observations using a logistic model to see which of these features exert independent effect and which may be usable for control purposes.

2.5.6 Flocculated red scum and oil-like layer

Slowly running water surrounded by vegetation sometimes tends to accumulate a flocculating scum of precipitated iron salts with colour ranging from brown to red. Usually

this is not suspended. Also, an apparently oily layer is formed in the same places which diffracts light and creates a rainbow effect. A series of observations (by Lines, personal comm) created an impression that this scum and oily layer were dis-favoured by anophelines as well as culicines for breeding. It was therefore felt necessary to include these in the list of features that were being evaluated for their importance in serving as visual cues (without chemical analysis) for identifying the breeding sites of *An. gambiae s.l* and *An. funestus*.

2.5.7 Surface dust particles

Lines (personal comm.) noted that presence of dust or particles on the surface of water is very commonly seen in the breeding sites. In his observations he noted that these particles were more frequently found in recent water collections, in which *An. gambiae s.l* were likely to be found. Could such a feature be generally associated with anopheline breeding? Very little is known about the surface characters and their relation to mosquito breeding (Bates, 1949). Nevertheless, while surface scum is known to kill larvae in laboratory larval colonies, Eckstein (1936) found that unlike *An. messeae*, *An. atroparvus* could tolerate the presence of talc and pollen on the water surface.

2.5.8 Sloping muddy margin

Another impression that was considered worth testing was the observation that *An. gambiae s.l* breeding sites always had a sloping muddy margin (A. McCrae, personal comm). If that observation is tested and found to be valid then making the walls of certain water collections such as wells, vertical might be a useful supplementary method in the control of this mosquito species. Other mosquito species have been controlled by similar method (Herms and Gray, 1944).

2.5.9 Foul odour and organic pollution

Foul odour from breeding sites is usually considered to be a sign of organic pollution disfavoured by anopheline mosquitoes for breeding (Hopkins, 1952). It was therefore considered necessary to establish, which proportion of each type of sites in the fields away

from homesteads and pit latrines are foul, as indicated by odour, inhibit breeding by malaria vectors.

2.5.10 Chemical factors: Hydrogen ion concentration (pH) and salinity

Although the effect of pH on the breeding of *An. gambiae s.l* and *An. funestus* has not been intensively studied, available information suggests that no usable conclusions could be reached owing to the wide range occupied by a particular mosquito species (Hancock, 1930; Kirkpatrick, 1925; Pomeroy, 1931; Symes, 1932). In Muheza, Tanzania, Ragoonanansigh *et al*, 1992 found that the pH ranged between 5.1 and 10.1 for water occupied by culicines, and 7.0 to 9.2 for anophelines. Fortunately, these levels of pH did not seem to interfere with the effect of and *B. sphaericus* on mosquito larvae (Rangoonanansigh, *et al*, 1992). So, no further work on pH was carried out in the present project.

Salinity affects the distribution of anophelines in general (Hopkins, 1952; Gillies and De Meillon, 1968). In Tanzania, studies on the distribution of the members of the *An. gambiae* complex showed that *An. merus* is confined to the coastal strip where the water is highly saline, while *An. gambiae s.s* and *An. arabiensis* breed in inland fresh waters (Mnzava and Kilama, 1986). *An. funestus* generally is said to breed in water with low salinity levels (Gillies and De Meillon, 1968). Rangoonanansigh *et al* (1992), while working in Muheza, found that the breeding sites of *An. funestus* had salinity ranging from 0.023 g/l to 0.181 g/l sodium chloride. This is consistent with Jepson *et al*, (1947) who reported that the usual breeding of *An. funestus* in Mauritius had a concentration of less than 2 g/litre (0.2%) of sodium chloride. *B. sphaericus* applied against larvae in Muheza worked well in this level of salinity (Ragoonanansigh, *et al*, 1992) and so in the present project measurements of this feature were not taken.

2.5.11 Vegetation and predators

Among the biological features only vegetation and predators will be considered here since they can be easily seen with the naked eye for possible use as a guide to the likely presence of anopheline breeding.

2.5.11.1 Vegetation

Larvae of most mosquito species are known to be associated with particular types of vegetation (Bates, 1949; Hess and Hall, 1945; Gillies and De Meillon, 1968). In the past attempts to find some correlation between species of plants and specific mosquito larvae have yielded unsatisfactory results (Bates, 1949). Therefore, in the present project attempts were made to group plants into categories, and to assess individual plant species for their independent effect on mosquito larvae. Independent association with certain types of vegetation may be important in certain types of breeding sites such as swamps which in some places may be extensive but unimportant in anopheline breeding (Goma, 1960).

2.5.11.2 Predators

Predators have been shown to be the major cause of the 97-99% mortality observed in rice field breeding mosquitoes in the Philipines (Mogi *et al*, 1984). Likewise, Christie (1958) in his study at Muheza considered notonectids to be the most important enemies of *An. gambiae s.l*, but this was not extended to longitudinal studies in a variety of breeding sites. Service (1977) and Service and Highton (1980) while working in Kenya in irrigated rice fields reported that there were numerous dead insects in plots of rice field where an organophosphate insecticide had been sprayed against rice stem borers. Fourteen days later, the rice plots were found to produce more *An. gambiae s.l* than those which had not been sprayed presumably due to absence of mature predators. Also, it was noted that *An. gambiae s.l* re-established themselves faster than predators in previously sprayed rice fields resulting in higher output of adult mosquitoes. On the other hand Ragoonanansingh *et al.* (1992) while working in Muheza, Tanzania, found that unlike Abate (temephos), the application of a bacterial insecticide, *Bacillus sphaericus*, to a puddle did not affect the Notonectid population. Now that the use of integrated control methods are given more emphasis, an attempt was made to re-assess the significance of these predators and to test further their response to chemical and biological insecticides in various types of breeding site.

2.6. Variations in adult mosquito densities and productivity of local breeding sites

In a number of studies conducted in Muheza, Tanzania, including the recent one on the evaluation of the effect of permethrin impregnated bednets, it was noted that there were marked seasonal and between-village variations in adult mosquito densities (Gillies 1954, 1955; Draper and Smith, 1957; Magesa, *et al*, 1991). With regard to attempts to carry out effective larval control such variations raise a number of important questions including the following:

(i) Can these differences be explained in terms of productivity of breeding sites in and near each village?
(ii) Does the nature of the breeding sites change radically between the wet and the dry seasons or are the large seasonal differences in the mosquito populations simply due to there being more available sites in the wet seasons?

The backgroung to question (i) will be discussed in subsection 2.6.1 and that to question (ii) in 2.6.2.

2.6.1 Estimation of productivity of *Anopheles* breeding sites for adult mosquitoes.

Productivity of a breeding site for anopheline mosquitoes can be defined as the total number of adult mosquitoes emerging from that site, or from a unit area of that site, per night. One of the most important questions that needs to be answered is: what is the relative importance of different types of breeding sites in terms of producing adult mosquitoes? As already mentioned, the mere size of a breeding site does not always imply extensive breeding (Goma, 1960). However, in most malaria control operations the most commonly used measure of larval breeding is the positivity index (percentage of breeding sites found positive per sampling occasion), and in a few studies larval density index (number of larvae per dip) (Zahar, 1985). Both of these estimates have deficiencies (Zahar, 1985; Christie, 1954) and on their own cannot give an estimate of total productivity for the following reasons:

Firstly, the positivity index only indicates that breeding is still going on, which may be sufficient for the aim to eradicate breeding (Zahar, 1985); however, it does not give an impression of the degree of success or failure of less ambitious control activities. The larval density index gives some measure of the density trend following control measures, but this measure may be misleading because it does not take into account the size and number of breeding sites (Zahar, 1985). In his conclusion Zahar, (1985) stated that "the increasing interest in anti-larval measures would call for developing larval sampling procedures that should take into account the extent of various types of breeding places and the clumped distribution of larvae". Service, (1971), proposed that it should be possible to find a relationship between a few absolute population estimates and a simple comparative index such as larvae per dip for different categories of breeding sites. Although this approach does not seem to be very precise, it does give estimates that are within acceptable limits of accuracy (Service, 1971).

Cairncross *et al* (1988), was able to identify the most productive breeding sites for the adults of *Cx. pipiens* group, in Pondicherry, India, by using the "Productivity Index" (mean number of pupae per square meter of breeding site multiplied by the estimated total area in which pupae were found). The results of this study showed that, although soakage pits (i.e shallow open pit collecting domestic sullage water, excluding sewage) constituted only 3% of the total surface area of breeding site, they produced 32% of mosquitoes in that area. This estimate of relative productivity of soakage pits, was 60 times greater than of wells with a relative surface area of 5%, (a little larger than that of soakage pits). In their conclusion it was stated that: " The most significant mosquito breeding sites in a town are not necessarily the most obvious, the most extensive, or those intuitively most likely".

In the present project, attempts were made to establish calibration factors (to be explained later) in order to relate the number of larvae per dip to the number per unit area of breeding site as previously attempted by Cambournac (1939) and described by WHO (1975). The total number of fourth instar larval and pupal stages per unit area of site were related to the adult mosquitoes emerging from a unit area as estimated by using the emergence traps (WHO, 1975). In addition, the overall output of adult mosquitoes from each site in the study villages were estimated using the total area of that category of site and a

comparison made among these villages.

2.6.2 Larval survival in breeding sites and abundance of adult mosquitoes

The degree of survival of immature stages of mosquitoes is probably the most important factor in the regulation of the adult populations of *An. gambiae s.l* seasonally and between villages (Christie, 1959). Indeed, the probability of an adult female mosquito surviving through one day is assumed to remain constant with time (Macdonald, 1957), and although there are moderate changes in sporozoite rates between seasons these appear to be related more to fluctuating recruitment than to changes in survival rates of adult female mosquitoes (Christie, 1959; Davidson and Draper, 1953).

However, little attention has been paid to the factors governing the survival of immature stages of *An. gambiae s.l* (Christie, 1958, 1959; Service, 1971, 1973, 1977; Zahar, 1985) unlike other species (Mogi, *et al,* 1980; Moore *et al* 1969; Rajagopalan *et al,* 1976, 1977; Reisen and Siddiqui, 1979; Reisen *et al.* 1982, 1989; Siddiqui, 1976). Christie (1959) suggested a number of possible ways through which the survival of *An. gambiae s.l* and to a certain extent *An. funestus* could be influenced. For almost all of these there was no supporting evidence and so he suggested lines along which investigation could be carried out to demonstrate them, but they have remained virtually untouched.

In this project attention will be paid to the following factors thought to affect survival of *An. gambiae s.l* immature stages:

(a) Effect of density of larvae on the emergence of adult stages.

Breeding sites where *An. gambiae s.s.* are commonly found are shallow and relatively small temporary water bodies (Gillies and De Meillon, 1968). The density of larvae in these sites, particularly footprints and small puddles, can be very high but the influence of density on the survival of this species in nature is unknown. The question is: does increase in the density of immature stages result in increased proportion of dying immature stages, and hence reduced output of adults? i.e Is mortality in small breeding sites of *An. gambiae s.l* density dependent? If it is, what type of density dependent regulation? (Varley and Gradwell, 1970).

To test for density dependent mortality per generation the values of percent mortality or the logarithm (k) of reciprocal of percent survival are plotted against the density (e.g density of eggs, or first instar larvae) on which mortality is acting (Varley and Gradwell, 1960). If the correlation is positive and the regression coefficient is significantly greater than 0, then the population is said to be undergoing density dependent mortality (Varley and Gradwell, 1968).

Most ecological studies on this subject have involved mosquito species breeding in containers (e.g pitchers, treeholes, pods of coconuts and cocoa or water storage pots) (Service, 1993). Service (1993), has reviewed studies on density dependent mortality in container breeders. In most of these species including *Wyeomyia smithii, Aedes sierrensis, Aedes triseriatus, Trichoprosopon digitatum,* density dependent mortality has been demonstrated. While working in Thailand Southwood *et al.* (1972) concluded that *Aedes aegypti* mortality occurring between eggs and second instar larvae in water storage pots was density dependent and the most important in regulating population size, while mortality of 4th stage larvae was density independent. This conclusion was refuted by Rogers (1983) who, after re-analyzing the data of Southwood *et al.* (1972) concluded that the greatest mortality occurred between pupal stage to oviposition. However, the fact remains, and that is density dependent mortality occurred in *Aedes aegypti* in water storage containers.

A limited number of studies have been conducted to determine the type of mortality experienced by mosquito species inhabiting more permanent ground water bodies. These include the work of Chubachi, (1979) who concluded that the immature stages *Cx. tritaeniorhynchus* in rice fields were experiencing density dependent mortality and the regulatory factor was competition for food. Rajagopalan *et al.* (1976 and 1977), in India were showed that there was density dependent mortality in the immature stages of *Cx. quinquefasciatus* in wells and in drains. This was also proved to be a genuine causal relationship of survival on density as described in chapter 9. By plotting the k (log 1/survival) values against log egg density (log d), significant regression coefficients were obtained indicating the type of density dependence relationship in different seasons. In late summer, there was undercompensating density dependence relationship i.e changes made in the density of eggs or first instar larvae, led to changes in survival less than sufficient to keep adult

emergence constant. During post monsoon to early summer, density dependence relationship was fully compensating, i.e changes made in the density of eggs or first instar larvae was compensated by proportional changes in larval survival to keep numbers of emerging adult mosquitoes unchanged. In winter there was overcompensating density dependence i.e changes made in the density of first instar larvae led to changes in survival more than sufficient to keep adult density constant.

No work of this kind has been reported using *An. gambiae s.l* and therefore it was decided to carried out a field experiment on this question because strong density dependence of larval survival could prevent moderate levels of larval killing from any beneficial effect on vector populations.

(b) Changes in water level and recruitment of first instars.

The chance of an individual mosquito reaching the adult stage not only depends on the probability of survival of larvae and pupae, but also on the hatching of eggs (Christie, 1959; A. McCrae, personal comm.). In temporary breeding sites however, frequent changes in water levels on sloping margins may interfere more with egg hatching through stranding than with larval survival. Eggs of *An. gambiae s.s* are known to be more susceptible to desiccation than those of *An. melas* (Gillies and De Meillon, 1968). It therefore seems that changes in water levels in these sites could be more important than predation in controlling survival of immature stages (A. McCrae, personal comm.). This was investigated in a field experiment.

(c) Distribution of predators in breeding sites.

Absence of predators in temporary breeding sites has been proposed as the reason for the early larval population explosion of *An. gambiae s.l* in these sites, with a subsequent decline upon invasion by predators (Gillies and De Meillon, 1968). Christie (1959) found that this idea conflicted with his observations in the case of *An. funestus* which breeds in predator-infested sites. While no attempt was made to carry out experimental study of this subject, the distribution of large predators was studied longitudinally to find if there was any association between them and the breeding of *An. gambiae s.l*, *An. funestus* and Culicines.

(d) Measurement of instar durations

Instar duration is the time in days taken by each larval and pupal stage before it metamorphoses into the next instar (Service, 1976, 1993). Knowledge of instar durations and observation of existing ratios of the density of each instar have been used by Service (1971, 1973, 1977) to estimate the proportions of larvae of *An. gambiae s.l* surviving each instar over a rather short period in a limited number of breeding sites. However, this approach has never been applied to a longitudinal study of survival patterns of this species. It best suits populations with completely overlapping generations and stable age distribution, emergence being balanced by eclosion at any one moment (Service, 1976, 1993), Nevertheless, it is considered that average survivorship over a longer period can be measured in a more unstable species by combining repeated samples. According to Service (1971, 1973, 1976, 1977, 1993) estimation of instar mortalities relies on accurate estimation of instar durations and density of each instar.

However, it remains unclear how Service, (1971, 1973) determined the instar durations of *An. gambiae s.l* in the field. During November to December, 1969, the estimated instar durations in days for stages I, II, III, IV, and pupae were as follows (Service, 1971): 1.5, 3, 2, 4, and 2 giving a total duration of 12.5 days. Values estimated for different instars of *An. gambiae s.l* During November to December, 1971 were as follows (Service, 1973): 1.42 days, 2.88 days, 1.93 days, 3.75 days and 1.79 days for stage I, II, III, IV, and pupae respectively giving a total of 11.77 days. The total durations of up to 12 days appear to be longer than is expected for *An. gambiae s.l* in shallow temporary breeding sites which are considered to be preferred by this species (Gillies and De Meillon, 1968). Owing to differences in the water qualities instar durations in small water bodies are likely to differ from those in larger permanent sites. Therefore it would not be correct to use same instar durations estimated in different situations to estimate mortality of *An. gambiae s.l.*

In the present project no attempt was made to measure instar durations. Instead, the duration (in puddles - defined in chapter 3) in days from first instar to fourth stage or pupae was counted directly from data collected daily for 309 days and the details are given in chapter

10. Correct estimation of instar durations is important for serving as a guide on the interval between larvicide application.

Estimation of densities of different instars in samples appear to be not so accurate for the purpose of estimating mortality between instars although the same information may be sufficient for the evaluation of the effect of larvicides. For example, Service (1977), sampled 2nd instars in excess, while Southwood *et al.* (1972), solved that problem by pooling stage 1 and 2 of *Aedes aegypti,* Reisen, *et al.* (1982), sampled low numbers of pupae of *An. culicifacies* and Lakhani and Service (1974) sampled more pupae than fourth stage larvae. Unless equal sampling efficiency for the different instars is achieved, and instar durations estimated in natural situations estimated mortalities are bound to be inaccurate and misleading.

2.7 Antilarval measures against *An. gambiae s.l* and *An. funestus* in rural areas.

In the present project an attempt was made to control the immature stages of mosquitoes including *An. gambiae s.l, An. funestus* and culicines in one of the study villages, Mngaza. Two types of insecticides were used, temephos (Abate) (a chemical organophsphate insecticide), and *Bacillus thuringiensis israelensis* (Bti) (a microbial insecticide). In this section a review is given on the usage of these insecticides.

2.7.1. Temephos (Abate).

The generic name of this insecticide is temephos, and Abate is a trade name of American Cyanamid Company which manufacture the insecticide. Other trade names of American Cyanamid for this insecticide include: Abathion. Abat, Biothion, Swebate and Nimitex. Abate is the commonly used trade name of temephos for public health use. In this text therefore, temephos will be referred to as Abate.

Abate is an organophospate insecticide that has been used for the control of mosquito breeding for quite a long time in many parts of the world. It has also been used in the control of *Simulium* breeding in a number of African countries in place of DDT (WHO, 1984). In this

Onchocerciasis Control Programme in West Africa, Abate has been used as a 200g/l (20%) emulsion for the control of blackflies in rivers where they breed. Abate is also used for the control of body louse in a dust formulation (W.H.O, 1984).

Abate which is mainly used as a mosquito larvicide, is considered to be among the least hazardous organophosphate insecticides in current use. It is effective at low dosage, making it suitable for the treatment of potable water at a dosage rate not more than 1mg/l (1 part per million or ppm) (WHO, 1984). For example, in Latin America and the Caribbean, many of the most important habitats of *Aedes aegypti* are difficult to control with other toxicants since they consist of a variety of water storage containers including drums, tubs, buckets, cisterns as well as storage tanks (Moore *et al.*, 1978; Chadee, 1984). Therefore studies were undertaken to evaluate the safe use of Abate in domestic water containers in these countries (Gaines, 1969; Laws *et al.*, 1967, 1968). It was concluded that Abate could be used in domestic solutions for controlling *Aedes aegypti.*

Abate, has not been widely used against *An. gambiae s.l* immature stages. However, reports of organophosphate resistance have been published for adult *An. arabiensis* from Sudan (WHO, 1984). In Muheza, Tanzania, *An. gambiae s.s* is the main member of the *An. gambiae complex*. Therefore problems of organophosphate resistance in *An. gambiae s.l* larvae were not a limitation for the use of Abate as a larvicide. On the other hand organophosphate resistance in Culicines is well documented in Tanzania (Curtis and Pasteur, 1981). However, microbial insecticides and insect growth regulators may be a feasible option against organophosphate resistant culicines (Curtis and Pasteur, 1981).

2.7.2 Bacillus thuringiensis israelensis (Bti).

The need to minimize environmental impact of chemical insecticides as well as to combat insecticide resistance has increased the use of microbial insecticides in many countries (Lacey, 1984). Among the microbial insecticides is *Bacillus thuringiensis israelensis* which is Serotype H-14 of *Bacillus thuringiensis*, hence its name *Bacillus thuringiensis* H-14. Goldberg and Margalit isolated it in Israel in 1977 (Goldberg and Margalit, 1977) and H. de Barjac

(1978) designated it *Bacillus thuringiensis* var *israelensis* (Bti).

It is a spore forming bacillus with a high larvicidal activity within 12 hours following its application (Goldberg and Margalit, 1977). It has been shown to be effective as a stomach poison against a number of mosquito species in a variety of habitats (Dame *et al.*, 1981; Yu *et al.*, 1982; Mulla *et al.*, 1985). However, *Anophelines* considered to be less susceptible to Bti than culicines because of their surface feeding behaviour and the rapid settling of Bti toxin. The use of a higher dose (i.e more than 1litre/hector) resulted in increased mortality in anopheline and culicine larvae in the field (Sharma *et al.* 1983).

The efficacy of Bti is said to be affected by certain environmental factors including temperature (Mulla *et al.*, (1990a). At higher temperatures Bti was found to be less effective against *Cx. stigmatosoma* Speiser. Water quality, larval density, and their sensitivity to the insecticide may influence the efficacy of Bti (Mulla, *et al.* 1990b).

Bti was shown to be safe to a number of non-target organisms including Notonectids and *Gambusia,* and *Aplocheilus blochii* (Balaraman *et al.*, 1983). By treating the fish tanks with 100 times the dose required to kill *Cx. quinquefasciatus*, no adverse effect was observed in fish, Notonectids and other invertebrate organisms. This suggested that the insecticide could be used in integrated vector control together with fish (Balaraman *et al.*, 1983).

2.8. Mark-release recapture experiment and monitoring of adult mosquitoes

In order to find out whether immigrant mosquitoes could be influencing the adult control level achieved, a mark-release recapture experiment was conducted to estimate the degree of mosquito interchange (Service, 1976, 1993; Rawlings *et al* (1981); Rawlings and Curtis, 1980). Adult mosquito density was evaluated by the use of light traps (WHO, 1975, Lines *et al* (1991).

CHAPTER 3

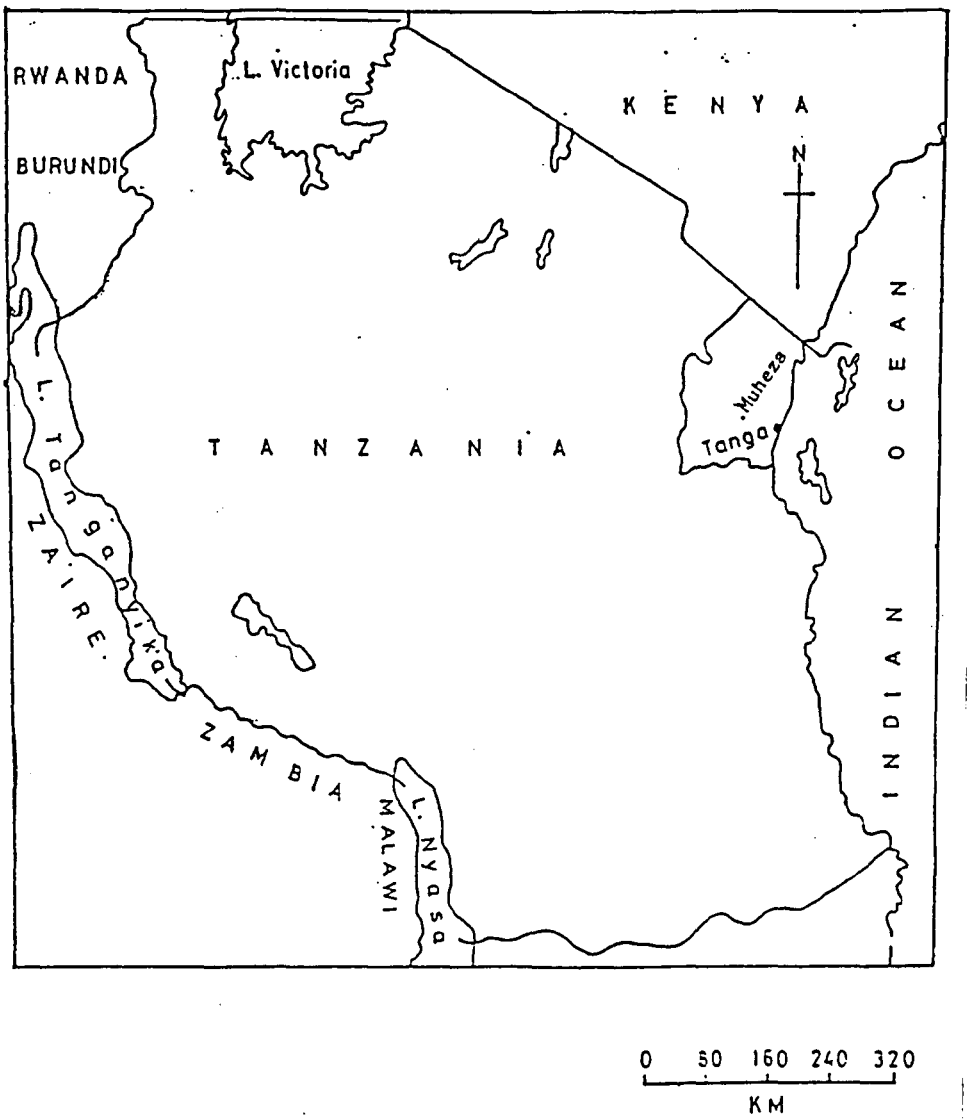
GENERAL MATERIALS AND METHODS

3.1 Study area

This project was conducted in three villages (Mngaza, Kumbamtoni and Mlingano). Owing to shortage of time work in Mlingano was dropped in the later stages. These villages are located in Muheza area, Tanga region in north-eastern Tanzania, about 40km inland from the coast of the Indian ocean (Figure 3.1a). The area has been described before by Gillies and Wilkes (1965) and Njunwa *et al.*, (1991). As mentioned in chapter 2 these villages were the site of a trial of bednets impregnated with pyrethroid insecticides conducted between 1986 and 1990. While there was success in reducing the sporozoite inoculation rate of *An. gambiae s.l* by more than 90% in the study villages the population of this species remained high enough to maintain transmission. This suggested that supplementary measures might be helpful in reducing transmission further. It was on this basis, and because of our long established contacts with the villagers, as well as the presence of a substantial amount of information on the adult mosquito population (Magesa *et al.*, 1991) that three of these villages were selected for the present study. *An. gambiae s.l* is the major vector of malaria in this part of Tanzania. The vector populations mainly consist of *An. gambiae s.s* and a small number of *An. arabiensis* (Mnzava and Kilama, 1986). *An. funestus* are present in relatively small numbers (Gillies and de Meillon, 1968; Magesa, *et al* 1991).

The area experiences two rainy seasons and two dry seasons. The main rainy season starts in late March and ends in June, while the short rains fall in November and December. The hot dry season lasts from January to late March, and the cool dry

Figure 3.1a: Map of Tanzania showing the position of Muheza, in Tanga



season runs from July to the end of October. Figure 3.1b shows the mean minimum and maximum temperatures recorded at Mlingano Agro-meteorological Station in 1990 and 1991, and Figure 3.1c shows the rainfall pattern recorded at Mlingano (1990 & 1991), Mngaza (1991) and Kumbamtoni (1991). In 1990 the lowest mean temperature was 18.6^{0}C in July, and the highest was 31.8^{0}C in December. In 1991 the situation was almost the same with a mean minimum temperature of 18.8^{0}C in September and a maximum of 33.5^{0}C in January. Generally, there was always some rain in each month which kept some of the breeding sites wet throughout the year.

3.2 Sketch mapping of the villages

Before the start of sampling for larvae the villages were mapped for their actual breeding sites and potential breeding sites in dry depressions, by the use of compass bearing and counting of paces as described by Russell, *et al.* (1963) and W.H.O (1975). The sketch maps of Mngaza and Kumbamtoni are shown in Figure 3.2a and 3.2b respectively. In all cases, the area covered was within one kilometre radius from the houses that were used for adult mosquito monitoring with CDC light traps. On the maps, important landmarks such as tall trees, and some paths, were marked to facilitate identification of specific locations. On these maps new breeding sites were added as soon as they appeared. To facilitate identification of specific locations of breeding sites, numbers were painted on the trees at intervals of 100m along the water courses. These markers constituted known reference points along each stream and swamp.

3.3 Classification of breeding sites encountered

As mentioned in chapter 2 the terminology used in many previous classification systems of mosquito breeding sites has been confusing in the sense that terms like puddles and pools have been used interchangeably without any mention of their size range and other criteria. While it was not the intention of this study to describe a new classification system for *An. gambiae s.l* breeding sites, a brief description of the breeding sites encountered in the study villages is given here. The breeding sites are

Figure 3.1b: Mean monthly temperature for 1990 and 1991 in Mlingano

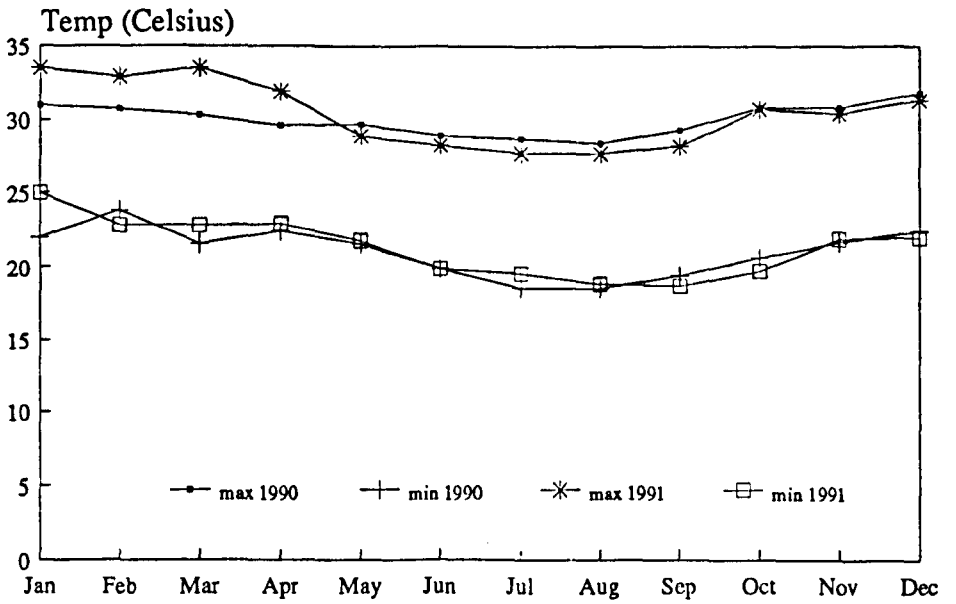


Figure 3.1c: Mean monthly rainfall for Mlingano, Mngaza, and Kumbamtoni 1990 and 1991

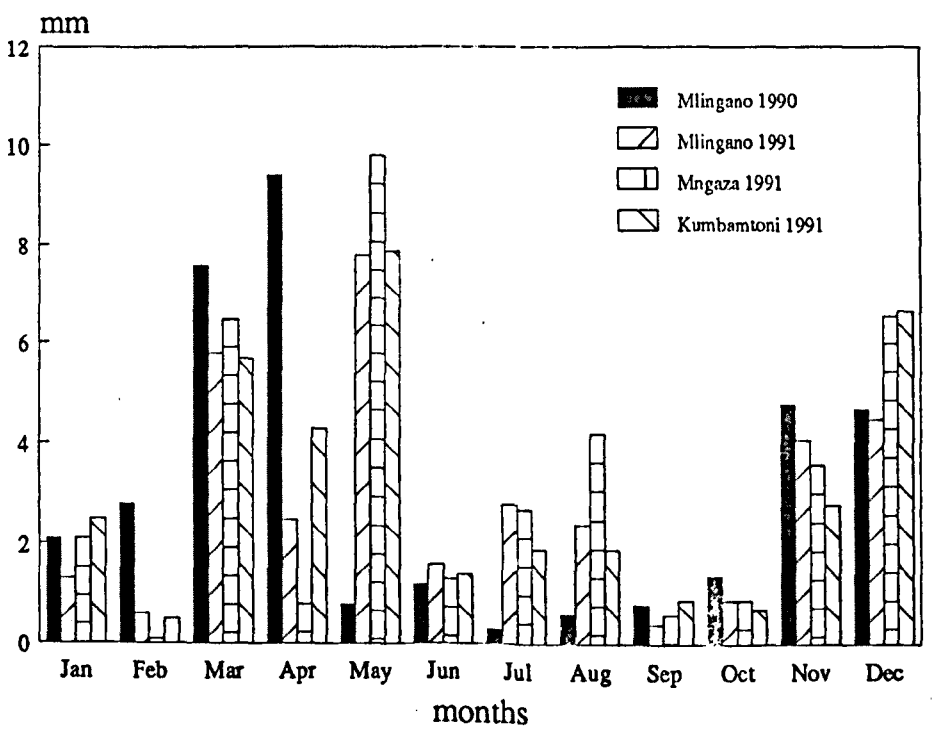


Figure 3.2a: Map of Mngaza to Show *Anopheles* and Culicine Breeding Sites

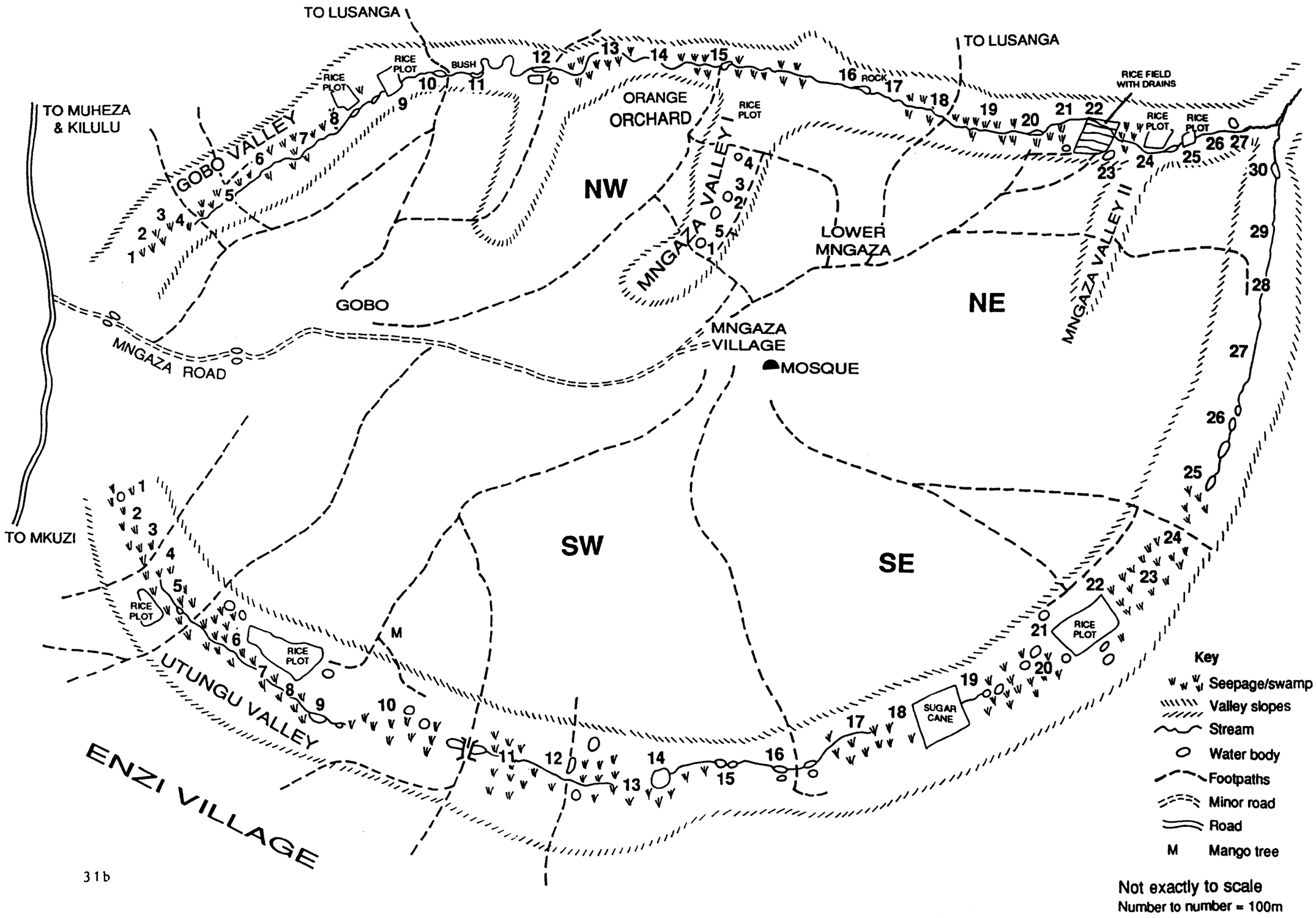
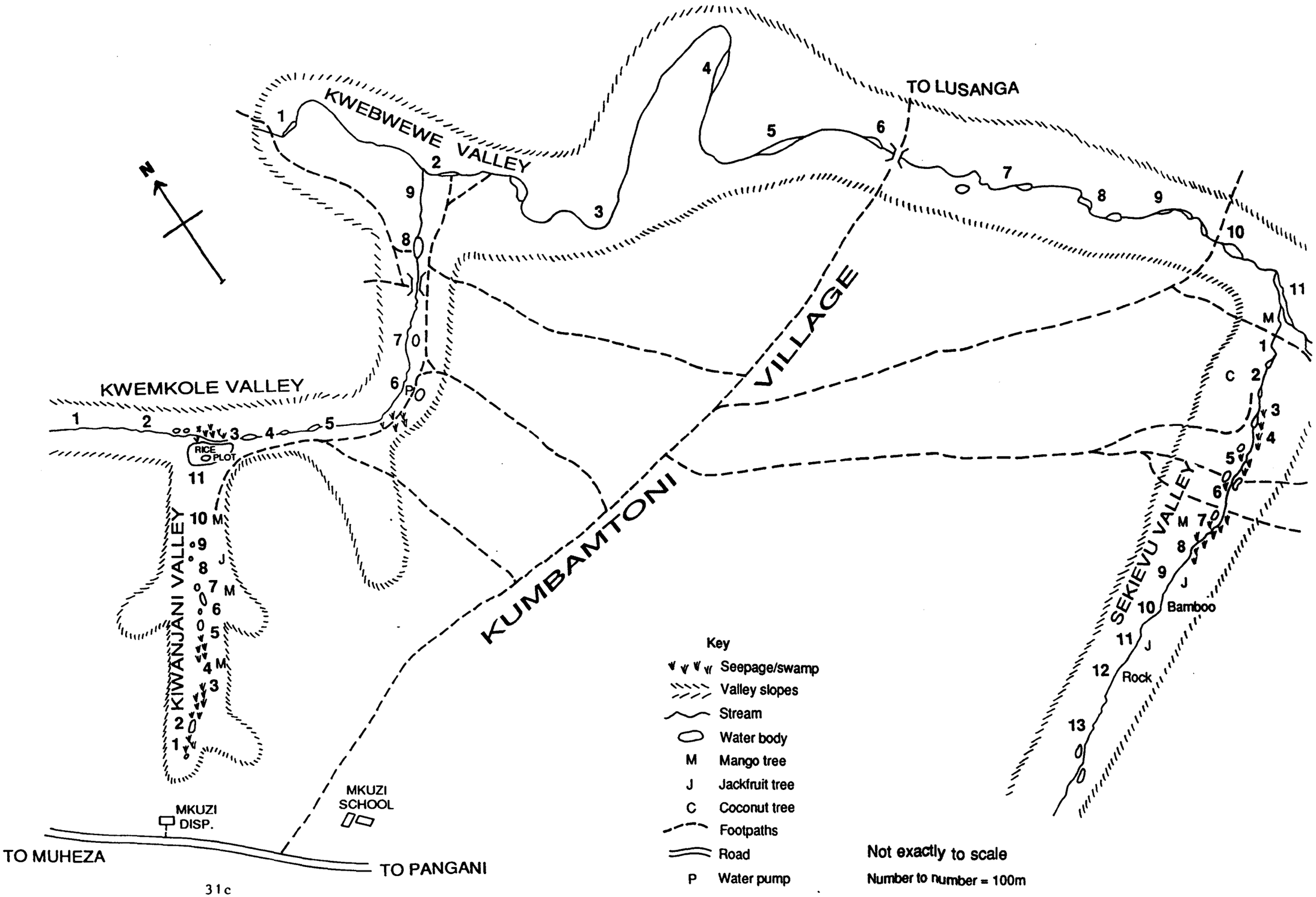


Figure 3.2b: Map of Kumbamtoni to Show *Anopheles* and Culicine Breeding Sites



defined in terms of depth and area. In the course of this study therefore the category of some breeding sites changed depending on their measurements at the time of sampling.

3.3.1 Footprints

Any body of water equal to or less than 0.25m x 0.25m was regarded as a "footprint". This category of breeding site mostly consisted of actual footprints but also hoofprints (Figure 3.3.1).

3.3.2 Puddles

These were typically turbid water collections defined as being less than 0.5m deep, and formed in surface depressions (Figure 3.3.2).

3.3.3 Pools

More or less open water surface. Usually deeper than 0.5m (Fig 3.3.3).

3.3.4 Ponds

Usually deeper than 1m. Although these tended to be more than 2m across, and more or less permanent but occasionally dry, these latter criteria were not emphasised in this classification as these features overlapped with those of pools and puddles. In that case it was the depth that distinguished the sites, with additional description of these other features (Figure 3.3.4).

3.3.5 Streams

Flowing water, usually up to 1m wide and with varying depth (Figure 3.3.5).

Figure 3.3.1: Wet footprints in a rice field

Figure 3.3.2: Puddles in a bed of dry stream

Figure 3.3.3: Pool in a pit cleared of vegetation

Figure 3.3.4: Ponds (a) with vegetation on the margin

(b) partially covered with vegetation

(c) completely covered with *Pistia*.

Figure 3.3.5: Stream

Figure 3.3.6: Swamp with *Jancus* overgrowth and pond (well) covered with *Ipomea*, on the margin.

3.3.6 Swamps

Permanently overgrown with dense vegetation with wide variation in depth and area. Since such areas were not homogeneous, a swamp was defined as a seepage ground with vegetation cover (Figure 3.3.6). For sampling purposes marginal puddles, pools, ponds, and stream channels did not fall under the category of swamp.

Commonly used terminologies such as rice fields, wells, furrows and roadside ditches were considered to be secondary criteria to those mentioned above. If prominence for these features was considered essential, then a description was given after the initial classification. For example, there were many sites classed as footprints and puddles found in rice fields. This emphasis on physical characteristics of the sites was later borne out by analysis of results.

3.4 Sampling design for immature stages of mosquitoes

3.4.1 Frequency of sampling

The study villages initially included Mngaza, Kumbamtoni and Mlingano. These were surveyed for the immature stages and for adult mosquitoes. Ideally, all the villages were intended to have been surveyed continuously to cover the period for larval development to adult emergence. However, it was soon realized that was not feasible, as the estimation of numbers of dips to be taken from each site took even more time than the actual sampling dipping. This greatly increased the time taken to cover all the sampling points in a single village. Nonetheless, it was considered preferable to sample all breeding sites in a few rounds than to sample just a few sites very frequently. The sampling visits to one village depended on how fast the sampling in the other village was done. Usually when there were more breeding sites it took longer before moving to another village. Table 3.4.1a and Table 3.4.1b shows the number of visits made to Mngaza and Kumbamtoni respectively in each quarter of 1990 and 1991. In 1990 a total of four survey rounds were made in Mngaza and Kumbamtoni.

Table 3.4.1a: Number of survey rounds made in Mngaza

Quarter and number of surveys					
Quarter	Jan-Mar 1	Apr-June 2	Jul-Sep 3	Oct-Dec 4	Total surveys
1990	1	1	1	1	4
1991	2	7	6	8	23
Total	3	8	7	9	27

Table 3.4.1b: Number of survey rounds made in Kumbamtoni

Quarter and number of surveys					
Quarter	Jan-Mar 1	Apr-Jun 2	Jul-Sept 3	Oct-Dec 4	Total surveys
1990	1	1	1	1	4
1991	-	4	3	6	13
Total	1	5	4	7	17

In order to cope with the frequency of larviciding that was done in Mngaza, starting on 1st April, 1991, 23 visits were made to this village and 13 to Kumbamtoni. This was facilitated by having two separate teams working in the two villages whereas in 1990 only two people did all the work. In July 1990, Mlingano was excluded from further larval sampling activity and only used for adult mosquito trapping. The remainder of the time was used for other experimental studies reported in this chapter, section 3.4.3 and 3.4.4, and chapters 5, 9 and 10.

3.4.2 Estimation of number samples to be taken from each site

Sampling for immature stages from the breeding sites was done by means of a white plastic ladle (WHO, 1975). However, the available methods for the determination of the number of samples that are needed to be taken from a habitat were not useful because the distribution of larvae in these habitats was not known. For example, Service (1971) was not able to obtain a generally well-defined pattern of distribution for *An. gambiae s.l* although observation suggested that the immature stages were clumped. On the other hand, studies involving most insect populations with clumped distributions (Thompson, 1983), have shown that the distribution could be adequately expressed by a negative binomial model (Anscombie, 1949; Bliss, C.I. and Fisher, R.A., 1953; Bliss and Owen, 1958; Harcourt, 1965; Ibarra, *et al.* 1965). If the clumped distribution conformed to the negative binomial model, then the required number (N) of samples to be taken would not depend on the variance (Rojas, 1964; Kuno *et al.*, 1963, both quoted in Service, 1976, 1993) but on the dispersion characteristics of the population, as shown by the negative binomial exponent k and this is represented as follows:

$$N = (1/m + 1/k) /D^2$$

where D = required level of accuracy (normally 0.1 i.e 10%)

m = arithmetic mean

Because of doubts about the applicability of this distribution to different types of breeding site of *An. gambiae s.l* in this study estimation of number of dips to be

taken was based on a non-statistical method suggested by Thompson (1983). This method depends on the principle that as more samples are taken from a habitat, the variability in the overall mean number of larvae per sample will tend to decrease and should eventually reach a stage where an arbitrarily defined degree of stability is reached. Therefore, successive sets of five dips were taken from a particular habitat and the number of larvae in every dip was recorded. Then, after every set of 5 dips, means were calculated for aggregate number of immature stages (without considering their stage) per dip from the dips so far taken i.e. the overall mean from 5, 10, 15, etc dips (see Table 3.4.2). When the percentage difference between any two successive means was equal to or less than 25% then this was taken as the appropriate number of samples for that type of site. This procedure can be represented as follows:

$$\text{If} \quad \frac{\{(S_1+S_2)/(n_1+n_2) - (S_1/n_1)\}}{S_1/n_1} \times 100 \leq 25\%$$

then the number of dips to be made = n_1+n_2

where S_1 and S_2 = numbers of immatures in successive sets of 5 dips respectively

n_1 and n_2 = successive sets of 5 dips respectively

If the difference between successive means was more than 25%, more samples (dips) were taken in sets of five units until the difference between successive was 25% or less.

In the case of footprints an estimate of the number of footprints to be sampled was based on taking a single dip per footprint. This is because a small trial showed that evacuation of a footprint and counting of all the larvae took about 10 minutes, which was excessive for a single footprint. Evacuation was reserved for situations where less than the estimated number of footprints to be sampled was found and each dip taken counted as a sample.

Table 3.4.2: Example of determination of number of samples (dips) to be taken from a breeding site

Village:
Date:
Site:
Point:
Species:

Dips	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Aggregate No. dips			5					10					15					20		
No. larvae	3	2	2	1	2	0	1	2	0	1	2	1	4	2	2	0	1	1	1	0
Aggregate No. larvae			10					14					25					28		
Aggregate no. larvae per dip			2					1.4					1.67					1.4		
% difference between means					30					19.2					16.2					

NOTE: If we choose 25% difference between successive means as an acceptable level of repeatability, it would be concluded that 15 dips were enough.

However, where many footprints were found the number of footprints to be sampled by a single dip per footprint was equal to the mean number of footprints found in a square metre. Therefore the first task was to estimate the number of footprints found in a square meter of ground. This was done by counting the number of footprints found in a 1m x 1m quadrat. A mean number of 18 ± 1 footprints was estimated per square meter.

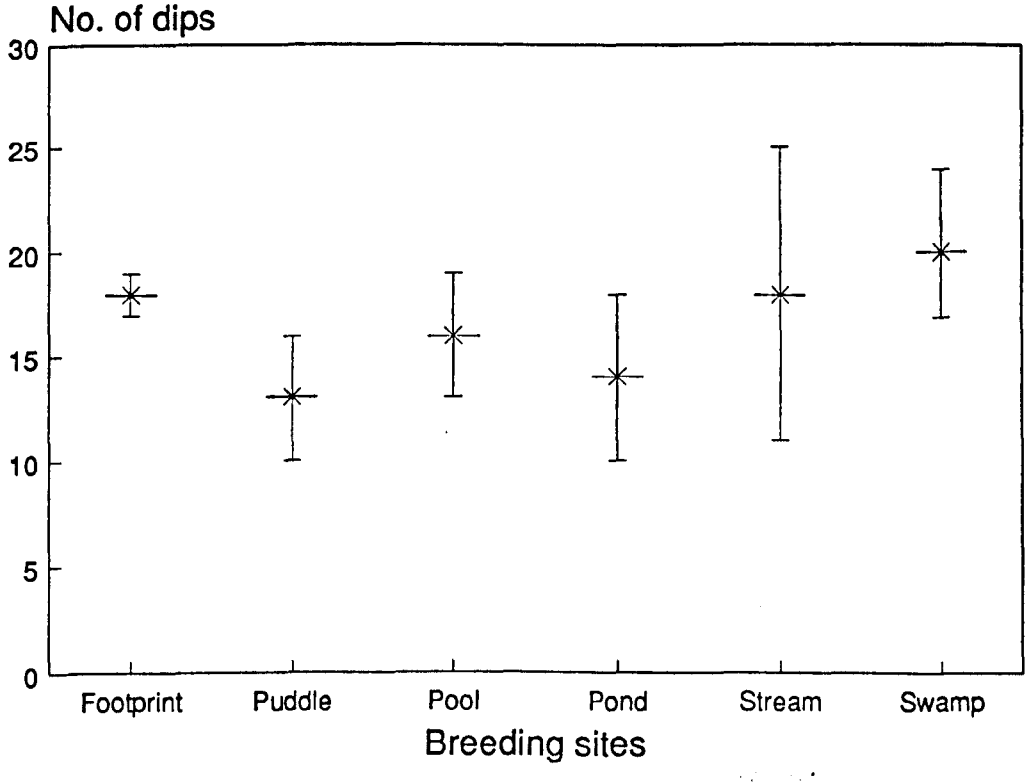
The results of estimations of number of dips and footprints are shown in Figure 3.4.2. The mean number of dips estimated ranged between 13 ± 3 for puddles to 20 for swamps.

3.4.3 Estimation of the time spent under water of different depths after initial and subsequent disturbance of *An. gambiae s.l* larvae and pupae

It is a common observation that when larvae and pupae are disturbed they dive below the water surface, staying there for some time before resurfacing. Most investigations that were performed in this project involved repetitive dipping in the same breeding site or quadrat. However, the effect of such dippings on the time spent under water by larvae and pupae was not known. Likewise it was not known if the time differed among different instars. Knowledge about the time of submersion was important for a decision to be made as to what interval between dips was necessary to allow the immature stages time to resurface. An experiment was therefore performed to determine the time of submersion, and to see if this time differed with larval instar and depth.

Three depths were arbitrarily selected. These were 5cm, 15cm and 25cm. Three pits with surface area of 50cm x 50cm were lined with plastic sheeting to prevent water from seeping through, some mud was sprinkled inside and they were filled with water to different depths as mentioned above. Then 50 larvae or pupae of *An. gambiae s.l* were introduced one stage at a time in each of the three depths.

Figure 3.4.2: Sample size estimation:- Average number of dips estimated as necessary for six types of breeding site



The larvae were then disturbed by making a single dip with the ladle. All the larvae and pupae disappeared from the surface, and the time interval between disappearance and the appearance of the first larva or pupa was noted. This was repeated ten times. The initial idea of estimating the time when 90% of the larvae or pupae had emerged was dropped because the immature stages kept diving and resurfacing. This procedure was repeated for each stage in the three depths of water.

When ten measures for one stage had been taken the pits were emptied of the water, plastic sheeting was washed to remove stranded larvae, and the sheets were replaced in the pits. The pits were then refilled with fresh water and another stage of *An. gambiae s.l* put in and sampled as described above.

Then by using One way analysis of variance, the mean times for the three kinds of depth for each stage were compared to see if there was variation among them. Where the variances were found to be not homogeneous the data was analyzed with Kruskall-Wallis analysis of variance. Then the mean emergence times of all the stages were compared using One way analysis of variance to see if emergence times varied among stages.

The results showed that there was no significant difference in the emergence times from water of the three depths for stage I ($F= 0.768$, $df= 2, 27$, $P= 0.948$), stage II ($H= 4.405$, $df=2$, $P= 0.11$), stage III ($F= 1.294$, $df= 2, 27$, $P = 0.582$, and Pupae ($F= 3.67$, $df= 2, 27$, $P= 0.687$). With stage IV there was significant difference in the emergence time from the three depths ($H= 10.25$ $df= 2$, $P< 0.01$. The Least Significant Test showed that the source of variation was the 15cm depth emergence times which had a variance of 246.8 compared with 76.4 and 47.5 for 5cm and 25cm depths respectively. Confidence limits were then attached to the means of the emergence times of stage IV as follows: 29.8 ± 6.3, 17.8 ± 10.8, 16.2 ± 4.9. The confidence limits overlapped, and therefore it was concluded that the most likely cause of variation was failure to detect the first emerging larvae which might have dived back before being noticed, and it was probably not really due to depth.

A further One way analysis of variance was done to find if there was significant difference between the means of emergence times of different stages in different depths. The results showed that there was no significant difference between the means ($F= 0.285$ $df= 2,12$ $P= 0.757$). Therefore, a common mean time of submersion for all the species was calculated and found to be equal to 30.9 ± 6.2 seconds. This was almost equal to the time spent identifying and recording the larvae collected in every dip. Therefore, it was decided not to spend more time waiting between dips. Also, it was considered that although the depth varied greatly between the breeding sites it was not necessary to change the time interval between sites. This is because the puddles and footprints which constituted a larger proportion of the sites (as found during the sketch mapping) were less than 50cm deep, and were close to 25cm deep which was used for estimation of time of submersion in this experiment.

3.4.4 Estimation of the proportion of larvae and pupae of *An. gambiae s.l* caught from quadrats driven into mud and suspended quadrats in relation to actual densities

As explained in chapter 5, estimation of the absolute number of larvae in the breeding site after estimation of larvae caught in 5 or 10 dips with replacement, involved either evacuation of all the water from a quadrat driven into the mud or continual removal of larvae from the quadrat using a pipette for at least 5 minutes until no more larvae could be seen inside the quadrat. Before doing such a calibration experiment it was considered necessary to find out if there was any trend in the number of larvae caught in successive dips, and if evacuation of larvae caught a sufficient proportion of the numbers present in both situations.

3.4.4.1 Quadrats driven into mud

Thick (3mm) aluminium quadrats with an opening measuring 25cm x 25cm were placed in a blocked drain where water had been checked and found to be devoid of any mosquito immature stages. Then 50 larvae or pupae were introduced in three

replicates (for stages I-III) and four replicates (for the 4th and pupal stages), one stage at a time, and five dips made with replacement. Then the quadrats were evacuated with addition of a little water to remove stranded larvae. This was done for all the stages of *An. gambiae s.l.*

One way analysis of variance was performed on the data collected and the results showed that there was no trend in the number of larvae collected in successive dips in the replicates of each instar (see Table 3.4.4.1a). Therefore the chance of catching the larvae did not decrease in the course of making 5 dips. A t-test was used to compare the mean number of larvae and pupae caught and those not caught (number introduced minus number caught) upon evacuation of each of the three replicates. The results are shown in Table 3.4.4.1b. With the exception of stage one, the numbers of larvae and pupae evacuated were significantly more than the numbers not caught. This suggested that there was need for paying more attention to the mud remaining at the bottom by adding more water to release stranded first instars.

3.4.4.2 Suspended quadrats

In this experiment aluminium quadrats similar to those described above but twice as tall were used. The heights used for comparison purposes were 40cm and 60cm. The quadrat was suspended from wooden rods secured onto the opposite sides of the quadrat running across the rim of a drum which had been filled with fresh water.

Twenty larvae or pupae were gently introduced, one instar at a time, into the quadrat, with change of water before introducing another instar. Five dips were then made with gentle replacement of larvae after counting and recording them. This was done in both the 40cm and 60cm deep quadrats. Then the larvae or pupae were gently removed from the quadrat with a pipette, to avoid further agitation of the water through using the dipper, for at least five minutes until no more could be seen.

Analysis by χ^2 for trend was done on the number caught in each dip and number not caught. The results are shown in Table 3.4.4.2a and b. In all the stages and in both

Table 3.4.4.1a: Mean number of larvae and pupae of *An. gambiae s.l* caught in each of the five dips taken from a quadrat driven into mud

Instar	Number of replicates	Mean number caught in each dip					F-value	df	signif.
		Dip 1	Dip 2	Dip 3	Dip 4	Dip 5			
I	3	2.3	2.0	2.3	3.0	2.7	0.1	4, 10	ns
II	3	2.3	2.0	1.7	3.7	2.7	0.89	4, 10	ns
III	3	5.7	3.0	4.0	3.7	2.3	0.82	4, 10	ns
IV	4	3.7	4.0	5.5	1.8	4.8	1.43	4, 10	ns
P	4	4.5	5.0	7.3	3.5	6.5	0.67	4, 10	ns

Table 3.4.4.1b: Mean number of larvae and pupae of *An. gambiae s.l* recovered upon evacuation of a quadrat driven into mud

Instar	Number of Replicates	No. of immature stages put in quadrat	Mean number evacuated	Mean number missed	t-value	df	Signif.
I	3	50	31.0	19.0	2.1	4	ns
II	3	50	36.3	13.7	9.1	4	***
III	3	50	42.0	8.0	5.8	4	**
IV	4	50	45.5	4.5	20.1	6	***
P	4	50	42.5	7.5	15.9	6	***

Table 3.4.4.2a: Mean number of larvae and pupae of *An. gambiae s.l* caught or not caught in each of the five dips taken from a quadrat **40cm** in depth suspended in water

Instar	No. put in quadrat		Mean number caught in each dip					χ^2-trend	df	signif.
			Dip 1	Dip 2	Dip 3	Dip 4	Dip 5			
I	20	Caught	2	1	4	0	3	0.56	1	ns
		Not caught	18	19	16	20	17			
II	20	Caught	3	3	1	0	5	0.04	1	ns
		Not caught	17	17	19	20	15			
III	20	Caught	2	3	7	0	2	0.37	1	ns
		Not caught	18	17	13	20	18			
IV	20	Caught	4	1	6	2	3	0.04	1	ns
		Not caught	16	19	14	18	17			
P	20	Caught	4	4	2	5	1	0.92	1	ns
		Not caught	16	16	18	15	19			

Table 3.4.4.2b: Mean number of larvae and pupae of *An. gambiae s.l* caught or not caught in each of the five dips taken from a quadrat **60cm** in depth suspended in water

Instar	No. put in quadrat		Mean number caught in each dip					χ^2-trend	df	signif.
			Dip 1	Dip 2	Dip 3	Dip 4	Dip 5			
I	20	Caught	4	3	0	0	2	2.96	1	ns
		Not caught	16	17	20	20	18			
II	20	Caught	1	2	2	0	3	0.27	1	ns
		Not caught	19	18	18	20	17			
III	20	Caught	6	5	6	4	6	0.03	1	ns
		Not caught	14	15	14	16	14			
IV	20	Caught	3	1	5	4	3	0.33	1	ns
		Not caught	17	19	15	16	17			
P	20	Caught	2	3	2	4	4	0.97	1	ns
		Not caught	18	17	18	16	16			

heights of quadrats there was no significant trend observed in the number of larvae caught in successive dips. Comparison of the mean numbers of different instars caught by dipping in the two quadrats showed that they were not significantly different (t= 0.505, df= 4, P= 0.640). The percentages of the larvae or pupae collected after dipping had been done in the 40cm and 60cm deep quadrats compared with the number introduced, as shown in Figure 3.4.4.2(a and b) with confidence limits attached.

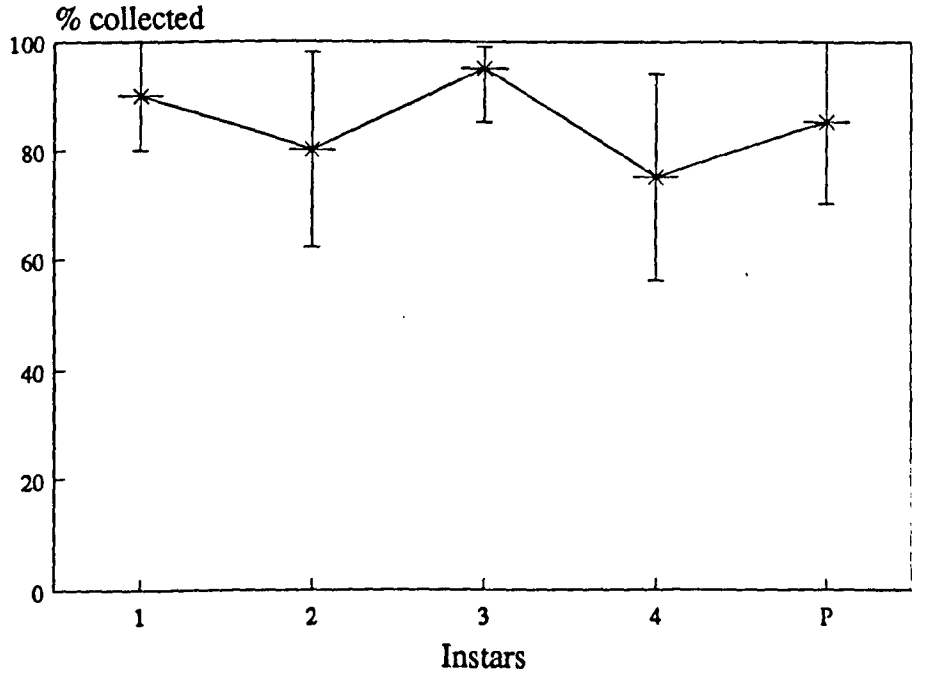
The ranges of confidence limits in both types of quadrats suggested that pipetting the larvae out caught between 50% 100% of the larvae. Pipetting was therefore used in field calibration studies with suspended quadrats.

3.4.5 Routine sampling and recording of results

Routine sampling involved making a pre-determined number of dips from each breeding site encountered during each survey. The dips were taken at intervals around a breeding site depending on its size. A single dip was taken from each footprint. Where there were fewer footprints than the estimated number of samples required to be taken, more than one dip was taken in some footprints. In the case of streams an attempt was made to take dips from equidistant points. Dips were taken from the margin as well as from the middle of all the sites.

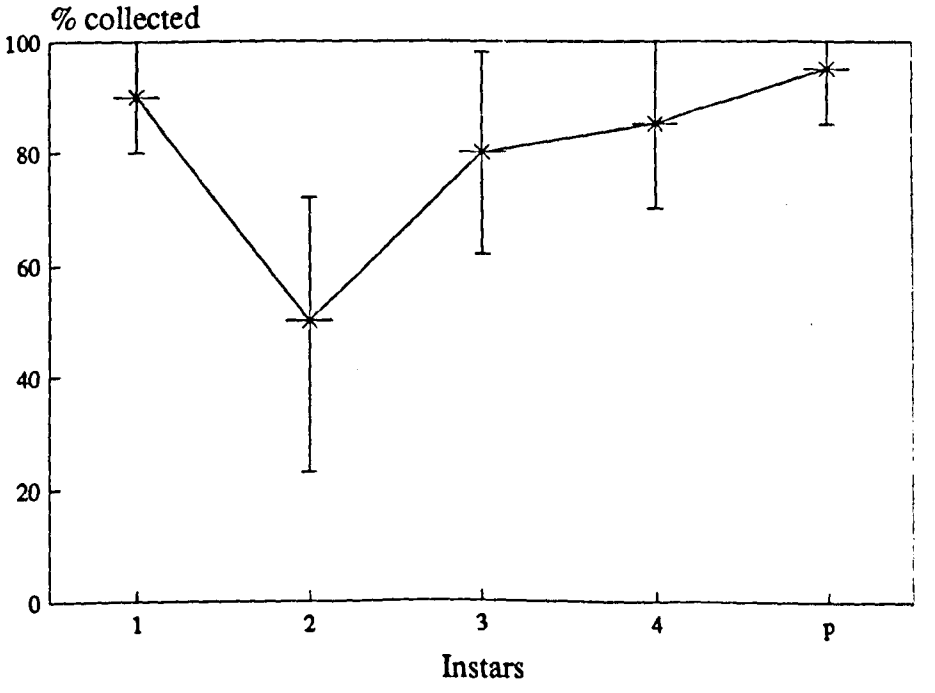
The features recorded for every point sampled are shown in the sampling form in Appendix 1. Anopheline larvae caught in every dip were identified according to Gillies and De Meillon (1968), while culicines were identified only to subfamily level, except *Culex tigripes* which is conspicuous and is important as a predator of other mosquito larvae. Identification of larvae in the field was considered necessary because it would have been very tedious to carry all the larvae sampled for laboratory identification. Initially, a small sample of each species of anophelines encountered was taken to the laboratory for confirmation under a stereo microscope, but with practice all the larval stages could be identified without a stereo microscope. Distinguishing earlier stages, however, required extra care. After identification, anopheline larval species and their number were recorded on the form. Occasionally, a distinction was

Figure 3.4.4.2a: Percentage of immatures caught in 5 minutes with pipette from quadrat 40cm in depth



An. gambiae s.l

Figure 3.4.4.2b: Percentage of immatures caught in 5 minutes with pipette from quadrat 60cm in depth



An. gambiae s.l

made between the margin and middle sections of a breeding site. Where the middle part was too far to reach with the usual length of handle of the ladle, an extension was added.

Plant species found growing in each breeding site were recorded. Initial identification of the plant species was done at the Botany department herbarium of Dar es Salaam University. Notonectid species were identified by the Natural History Museum, London.

3.5 Adult mosquito sampling

Adult mosquitoes were monitored in the three study villages using CDC light traps hung beside occupied bednets (Service, 1976; Lines *et al.*, 1991, Magesa *et al*, 1991). A sample of the form used for recording adult mosquito catches is attached (Appendix 2). Results of adult mosquito sampling are given in chapter 7.

3.6 Types of insecticide used and preparation of maize cobs and sawdust for application to the breeding sites

3.6.1 Types of insecticides used for the treatment of maize cob chips and sawdust

Maize cobs and saw dust were used as carriers of Abate and Bti for application to the breeding sites. The insecticides used were as follows:

Abate 500-E (50% w/v Emulsifiable Concentrate) from Cyanamid, Agricultural Research Division, American Cyanamid Company, Princeton, New Jersey 08540. Table 3.6.1 shows the recommended concentrations of the insecticide as given by Cyanamid. The concentrations of insecticide used range from 0.1mg/l to 1mg/l (i.e 0.1ppm to 1ppm). W.H.O (1984) also certifies the safety of up to 1ppm in drinking water.

Bti was in the form of Vectobac 12-AS (ART-NR: 4371). This was a fluid concentrate formulation from Stahler Agrochemie GMBH, Postfach 2047-2160, Stade,

Table 3.6.1: Recommended concentrations of Abate for use in various water conditions (from Cyanamid Abate manual)

Type of water	Mosquito control with Abate 500-E (ml/ha)*
Clean: standing water, shallow ponds, lakes, woodlands	100-150
Moderately polluted: tidal waters, marshes,swamps, etc	200-250
Highly polluted: drains, cesspits, and other water high in organic matter content	400-1000

* The depth of the water is assumed to be 0.1m

Germany. One millilitre of this concentrate in one litre was considered to be 1ppm. Dosages were expressed as volumes of this concentrate per litre without knowledge of the content of active toxin.

3.6.2 Maize cob chips

3.6.2.1 Chopping cobs into radial sections

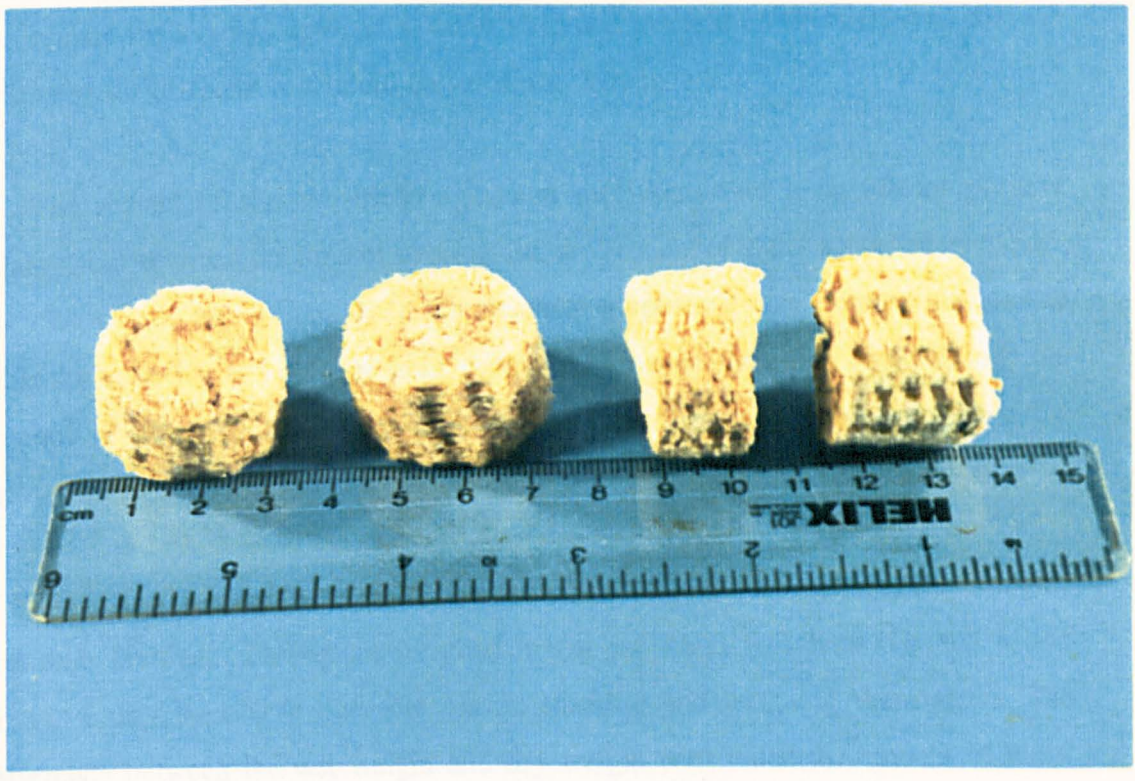
Dry maize cobs were bought from the villagers at a token price equivalent to USA $ 0.5 per 100kg sack. They were then brought to Ubwari Field station and chopped into 2cm thick chips (Figure 3.6.2.1) by placing a sharp knife on the cob and hitting the knife with a wooden block. The diameter was variable depending on the size of the cob. On average, three people chopped 3,556 per day. So the cobs chopped in 9 days were more than enough for use throughout this study.

3.6.2.2 Estimation of the amount of water absorbed by a single chip of maize cob

Before chips of maize cobs could be treated with an insecticide it was necessary to estimate the average volume of water that was needed to saturate a single chip.

A small experiment was done to assess if there was an increase in the amount of water absorbed with increasing soaking time, and if so to what extent. This was done by soaking in water pre-weighed dry chips of maize in sets of 10 for periods ranging from 10 minutes to 2 hours. Each set of chips was allowed to drip over a plastic sheeting until dripping stopped, and then weighed again. The difference between the

Figure 3.6.2.1: Pieces of maize cobs used as carriers of insecticides



drip dried wet weight and the dry weight of all the cob chips was taken as the amount of water absorbed grams (or mls).

Since the volumes of the water absorbed depended on the volume of individual sets of maize chips (which was not easy to measure for this purpose), it was decided to estimate the average amount of water absorbed per unit of dry weight so that the estimates for different sets could be justifiably compared.

The results of this experiment showed that there was a significant positive relationship between the time of soaking and the amount of water absorbed ($r= 0.815$, $F= 19.8$, $df= 1, 10$, $p= 0.00012$). For practical purposes it was therefore decided to soak the cobs for at least one hour during which time the chips could absorb the amount of water equal to their own weight. This was considered convenient for estimation of the amount of insecticide in a known weight of maize cob chip.

Therefore, further the estimation of the water or insecticide absorbed by a maize cob chip involved soaking pre-weighed maize cob chips for about one and a half hours, letting them drip over the plastic sheeting and weighing them again. The difference between the wet weight and dry weight constituted the amount of water absorbed, as described above. The mean weight of water absorbed by 100 cob chips was found to be 380.0 ± 6.6g (3.8gm/chip).

3.6.2.3 Treatment of maize cob chips with insecticide

(a)Treatment of maize cob chips with Abate:

As described in section 3.6.2.2 a single chip absorbed 3.8g of water on average. Owing to viscosity of the 50% w/v emulsifiable concentrate of Abate it was decided to dilute it by half before treating the maize cob chips. Therefore in the 3.8g of

solution of Abate absorbed by one chip of maize cob there was 3.8/4 = 0.95g (approximated as 1.0g) of active ingredient of Abate (temephos).

The mixing of insecticide and treatment of maize cob chips was done in bulk in plastic washing-up bowls. The chips were stirred thoroughly by using a stick until all the insecticide had been absorbed (Figure 3.6.2.3). Then the contents were poured onto plastic sheeting in the shade (to avoid direct sunlight that could destroy Abate) for the cobs to dry. They were continually turned over to absorb any solution from the plastic sheeting. When no more dripping could be seen they were packed in sets of 50 cobs in sealed bags.

(b) Treatment of maize cob chips with Bti

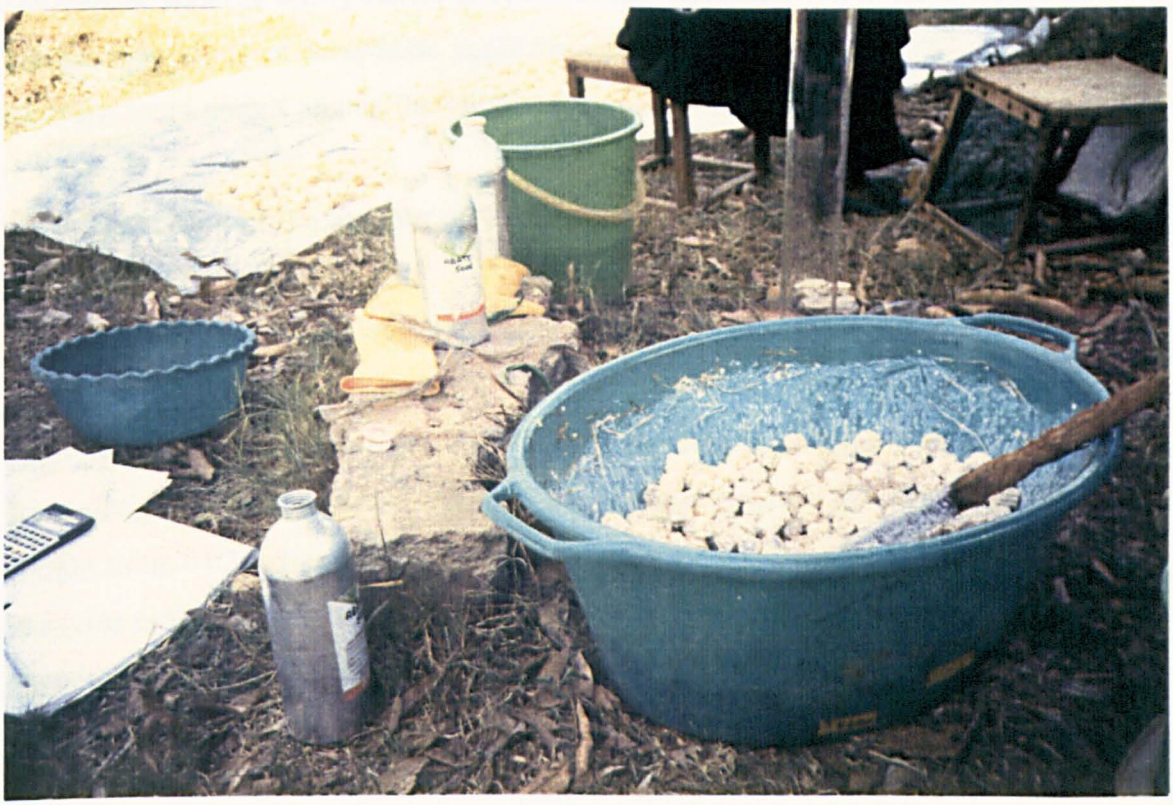
Various workers have used a wide range of concentrations of different brands of Bti (see Curtis, 1991). In this trial, however, a target concentration of 1mg/l was aimed for. Therefore, the maize cob chips described above were treated so as to absorb 1g of Bti (Vectobac).
This was done as follows:
100ml(Vectobac) + 280ml(water) + 100 (chips) produced a volume of 380ml mixture of liquid that was absorbed by the chips (each assumed to absorb 3.8g) to acquire 1g active ingredient of Bti.

(c) Estimating the number of maize cob chips required for an known volume of water in a breeding site

The aim was to apply up to 1ppm (1mg/l) and the following formula was used

$$\text{No. of chips} = \frac{\text{Water volume in a breeding site (in litres)}}{\text{Quantity of Abate in a single chip (in mg), i.e 1000mg}}$$

Figure 3.6.2.3: Mixing maize cob chips with insecticide



e.g = 3000litres

1000mg

= 3 cob chips

Thus, the estimated volume of water in cubic meters corresponded to the number of maize cob chips required. Owing to their light weight, and capacity to treat large volumes of water it was possible to conveniently carry only a few maize cob chips for a single round of insecticide application.This was a important advantage over carrying spray pumps around the village.

3.6.3 Estimation of the amount of water absorbed by saw dust

For small breeding sites saw dust was used as insecticide carrier instead of maize cobs.

A "pinch" was taken to be the smallest amount of saw dust that could be picked from a bag during insecticide application. Weighing of replicate pinches showed their weight to be 0.02g. Since the rate of absorbtion of liquid in sawdust was faster than in maize cob chips, the amount of water absorbed was estimated by pouring small quantities of water at a time onto 100g of saw dust in a washing up bowl followed by thorough mixing until it was about to start dripping. This was repeated 10 times. The average of the water absorbed by 100g of sawdust was 220 ± 7.5g.

3.6.3.1 Treatment of saw dust with insecticide

(a) Treatment of saw dust with Abate

The smallest breeding sites were footprints, and the concentration of Abate in sawdust was adjusted so that two pinches of sawdust would be required for each footprint. Therefore, for the estimation of the amount of insecticide to be used for treating a pinch, half the size of a typical human footprint (see chapter 6), was used. This volume was equal to 25cm x 5cm x 5cm(depth) = 625ml or 0.625 litre, so that

0.625mg of insecticide was required for a concentration equal to 1mg/litre (or 1ppm). 0.625mg of insecticide in 0.02g of sawdust is equivalent to 3g of active ingredient of insecticide or 6ml of 50% w/v in 100g sawdust.

Therefore, the sawdust was mixed with the insecticide and water as follows to make each pinch produce 1ppm in the above mentioned footprint size:

6ml	+	100ml	+	100g
(50% Abate concentrate)		(water)		(sawdust)

(b) Treatment of sawdust with Bti

Knowing that Bti may have shorter residual effect than Abate, the estimated weight of 3g of insecticide (mentioned above) was increased ten fold to 30g (in the case of Bti) per 100g of saw dust. It was assumed that at after flooding there would remain the equivalent of 1mg/l of insecticide concentration.

The mixing of 100g of saw dust with Bti and water was done as follows: 30ml (= 30g) Vectobac concentrate + 190ml water = 220ml of mixture which was absorbed by the 100g of sawdust.

3.7 Data handling

Most of the data was entered into Epi Info Version 5 database package where the data was "cleaned" and some analysis performed. Some other software packages that were used in the analysis of data include Epistat and SPSS/PC$^+$ Version 4.

CHAPTER 4:

EFFECTS OF ENVIRONMENTAL FEATURES ON THE BREEDING OF *AN. GAMBIAE S.L, AN. FUNESTUS, AN. COUSTANI,* AND CULICINES

4.1 INTRODUCTION

Larval sampling data collected in Mngaza, over a period of two years were analyzed to study the effects of various environmental features on the breeding of *An. gambiae* s.l, *An. funestus*, *An. coustani* and *Culex* spp in various categories of breeding site. Only the records for the sites containing water were used in this analysis. Larval stages were grouped into two categories, namely "any stage" and "final stages" (i.e fourth and pupal stages). The effect of the environmental features on the breeding of each mosquito species and culicines was assessed by testing for a significant association between these environmental features and the presence or absence of each category of larvae in the breeding sites. The environmental features noted for each site may act independently or there may be confounding effects between them. Therefore, the analysis involved testing for both overall (with confounding) and independent effects of the environmental features.

4.2 MATERIALS AND METHODS

4.2.1 Breeding sites

The features of each category of breeding site are given in chapter 3 on "general materials and methods". In summary, the following types of breeding site were distinguished in this study: footprints, puddles, pools, ponds, streams, and swamps.

4.2.2. Other environmental features

The environmental features noted in association with the larval breeding sites were as follows:

(i) Vegetation

Two dominant species were recorded if the site contained more than one plant. Samples of these plants were collected and pressed using standard botanical procedures. They were then taken to the Botany department of the University of Dar es Salaam for identification. The following plant species were found in the breeding sites: *Commelina* spp. (Commelinaceae), *Cyperus* spp. (Cyperaceae), *Cynodon* spp. (Graminae), *Ipomea aquatica* (Convolvulaceae), *Lagarosiphon* spp. (Hydrocharitaceae), *Oryza sativa* (Graminae), *Paspalum* spp. (Graminae), *Pistia* spp. (Araceae), *Jancus* spp. (Jancaceae), *Spirogyra* spp. and (Chlorophyceae), *Acrostichum aureum* (Pteridaceae). Those plants which spread over the water surface were grouped in a category called "cover", and they included *Ipomea aquatica, Lagarosiphon, Pistia,* and *Commelina.* The absence of vegetation was also noted and used in the analysis.

(ii) Suspended mud, oily surface layer, odour of water, shade, red scum surface dust, sloping muddy margins, depth, water movement, Notonectids, and dragonflies.

Suspended mud, odour of water, shade, red scum (brownish-red, flocculated, colloidal material), and surface dust, were given scores ranging between zero (for none) and four (for very much). An oily layer, sloping muddy margins (over ten centimeters in width), water movement, Notonectids and dragonflies were categorised as present or absent. Depth and water speed were left as continuous variables since they were more accurately estimated. This was done by using the graduated handle of a ladle, while the speed of the water in metres per minute was derived from the time taken for a floating object to move a distance of 0.20m along the handle of the dipper.

Analysis of data to find if there was any association between the breeding of

different mosquito species and the presence of any of the environmental features or the type of breeding site was done using "Relative Risk" statistic (Kirkwood, 1988) where the features were treated as categorical and using χ^2 for ordinal variables. Where the variances of the means of continuous variables to be compared were found not to be similar a non-parametric test (Kruskal-Wallis One Way Analyis of Variance was used, and the statistic was represented as "H" which is considered to be equivalent to χ^2. Multiple logistic regression analysis was used to assess the independent effect of individual features which together were thought to have been having a confounding effect. Multiple logistic model takes confounding effect into account as described in section 4.3 below. For example, when odour and *An. funestus* were tested for association using χ^2-test a significant negative association was found between any stage and odour but not with final stages of *An. funestus*. When multiple logistic regression was used, odour was found to have a positive association with any and final stages of this species.

4.3 RESULTS

4.3.1 *An.gambiae s.l*

4.3.1.1 Overall effect of environmental features

4.3.1.1.1 Type of breeding site

The relationships between the type of breeding site and mosquito breeding are shown in Tables 4.3.1.1.1(a-g).

An. gambiae s.l showed a significant heterogeneity in the probability of occurrence between different types of breeding site: for any larval stage and late stages (Table 4.3.1.1.1a). It was found that footprints had the highest probability of containing any larval stage and final stages (Table 4.3.1.1.1b). Also, pools showed a significantly higher than average probability of containing any stage but not with final stages (Table 4.3.1.1.1d). On the other hand, the streams showed a significantly lower than average probability of containing any stage or final stages (Table 4.3.1.1.1f).

Table 4.3.1.1.1a: Association between any stage or final stages (fourth instars and pupae) of three anopheline species and culicines with the different categories of breeding site in Mngaza

Mosquito[a] species	χ^2	df	p	Signif.
Ag-any	86.14	4	< 0.001	***
Ag-final	123.39	4	< 0.001	***
Af-any	70.33	4	< 0.001	***
Af-final	9.28	4	> 0.05	ns
Ac-any	59.74	4	< 0.001	***
Ac-final	41.47	4	< 0.001	***
Cx-any	75.04	4	< 0.001	***
Cx-final	57.51	4	< 0.001	***

[a] Any stage or final stage of *An. gambiae s.l* (Ag), *An. funestus* (Af), *An. coustani* (Ac), and Culicines (Cx).

Table 4.3.1.1.1b: Association between footprints (FP) and any stage or final stages (fourth and pupae) of three anopheline species and culicines in Mngaza

Type of breeding site	Mosquito species and stage	RR (df=1)	95% CL of RR	P	Signif.
FP	Ag-any	2.71	2.18-3.36	< 0.001	***
	Ag-final	6.13	4.33-8.69	< 0.001	***
	Af-any	0.56	0.23-1.34	> 0.05	ns
	Af-final	0.68	0.17-2.78	> 0.05	ns
	Ac-any	0.41	0.15-1.09	> 0.05	ns
	Ac-final	0.82	0.20-3.34	> 0.05	ns
	Cx-any	1.22	0.83-1.81	> 0.05	ns
	Cx-final	1.73	1.03-2.90	> 0.05	ns

Table 4.3.1.1.1c: Association between puddles (PD) and any stage or final (fourth and pupal) stages in Mngaza

Type of breeding site	Mosquito species and stage	RR (df=1)	95% CL of RR	P	Signif.
PD	Ag-any	0.93	0.78-1.12	> 0.05	ns
	Ag-final	0.86	0.61-1.21	< 0.05	ns
	Af-any	0.55	0.41-0.75	< 0.001	***
	Af-final	0.75	0.44-1.30	> 0.05	ns
	Ac-any	0.86	0.64-1.15	> 0.05	ns
	Ac-final	0.92	0.50-1.66	> 0.05	ns
	Cx-any	1.23	1.0-1.52	> 0.05	ns
	Cx-final	0.95	0.69-1.29	> 0.05	ns

Table 4.3.1.1.1d: Association between pools (Pl) and any stage or final (fourth and pupal) stages in Mngaza

Type of breeding site	Mosquito species and stage	RR (df=1)	95% CL of RR	P	Signif.
PL	Ag-any	1.84	1.32-2.57	< 0.001	***
	Ag-final	1.38	0.63-3.03	> 0.05	ns
	Af-any	3.42	2.18-5.36	< 0.001	***
	Af-final	3.02	1.23-7.38	< 0.05	*
	Ac-any	3.13	2.0-4.89	< 0.001	***
	Ac-final	3.65	1.48-9.02	< 0.05	*
	Cx-any	2.32	1.66-3.26	< 0.001	***
	Cx-final	2.86	1.73-4.72	< 0.001	***

Table 4.3.1.1.1e: Association between ponds (PN) and any stage or final (fourth and pupal) stages in Mngaza

Type of breeding site	Mosquito species and stage	RR (df=1)	95% CL of RR	P	Signif.
PN	Ag-any	1.15	0.59-2.24	> 0.05	ns
	Ag-final	1.04	0.27-4.04	> 0.05	ns
	Af-any	4.99	3.0-8.28	< 0.001	***
	Af-final	2.66	0.68-10.5	> 0.05	ns
	Ac-any	4.57	2.76-7.58	< 0.001	***
	Ac-final	8.59	3.61-20.4	< 0.001	***
	Cx-any	3.39	2.35-4.89	< 0.001	***
	Cx-final	4.66	2.71-8.01	< 0.001	***

Table 4.3.1.1.1f: Association between streams and any stage or final (fourth and pupal) stages in Mngaza

Type of breeding site	Mosquito species and stage	RR (df=1)	95% CL of RR	P	Signif.
ST	Ag-any	0.68	0.53-0.86	< 0.01	**
	Ag-final	0.32	0.71-0.58	< 0.001	***
	Af-any	1.16	0.82-1.65	> 0.05	ns
	Af-final	1.15	0.61-2.13	> 0.05	ns
	Ac-any	0.73	0.5-1.07	> 0.05	ns
	Ac-final	0.44	0.17-1.11	> 0.05	ns
	Cx-any	0.49	0.36-0.66	< 0.001	***
	Cx-final	0.52	0.33-0.82	< 0.01	**

Table 4.3.1.1.1g: Association between swamps (SW) and any stage or final (fourth and pupal) stages in Mngaza

Type of breeding site	Mosquito[a] species and stage	RR (df=1)	95% CL of RR	P	Signif.
SW	Ag-any	0.57	0.37-0.87	< 0.01	**
	Ag-final	0.45	0.18-1.08	> 0.05	ns
	Af-any	1.17	0.7-1.95	> 0.05	ns
	Af-final	0.68	0.21-2.16	> 0.05	ns
	Ac-any	1.07	0.64-1.77	> 0.05	ns
	Ac-final	0.53	0.13-2.18	> 0.05	ns
	Cx-any	0.57	0.35-0.91	< 0.05	*
	Cx-final	0.53	0.25-1.12	> 0.05	ns

4.3.1.1.2 Vegetation

Tables 4.3.1.1.2(a-o) show the relationship between mosquito breeding and different types of vegetation found in the breeding site. The degree of association of *An. gambiae s.l* with various plant species varied between plants and between early and late stages (4th and pupae). There was a significant positive association between all the stages of *An. gambiae s.l* only with rice *(Oryza sativa)* (Table 4.3.1.1.2a). A significant negative association of all stages was found with *Ipomea aquatica* (Table 4.3.1.1.2c), *Pistia* (Table 4.3.1.1.2d), and overall cover of the water surface (which includes *Ipomea aquatica, Lagarosiphon, Pistia,* and *Commelina* (Table, 4.3.1.1.2n). The absence of vegetation did not seem to have much influence on the presence or absence of all stages of *An. gambiae s.l* (Table 4.3.1.1.2(o)). However, the mere presence of vegetation on the margin (alone) (Table 4.3.1.1.2(l)), or on the margin and in the middle of the water body (Table, 4.3.1.1.2m), was inhibitory.

The presence of the final stages of this mosquito species was positively associated only with rice (Table 4.3.1.1.2a). A significant negative association was found with *Ipomea aquatica* (Table 4.3.1.1.2c), *Cyperus* (Table 4.3.1.1.2e), *Jancus* (Rushes) (Table 4.3.1.1.2b), and overall cover of the water surface (Table, 4.3.1.1.2n). The absence of vegetation was significantly positively associated with the late stages (Table 4.3.1.1.2 (o)) while the presence of vegetation on the water margin had a negative effect (Table 4.3.1.1.2(o)). The effect of the presence of vegetation in the middle of the water body was not significant (Table 4.3.1.1.2m).

4.3.1.1.3 Suspended mud, oily surface layer odour of water, shade, site, red scum, surface dust, slope, depth, water movement including speed, notonectids, and dragon flies.

The types of association between these features and the occurrence of different mosquito species are shown in Tables 4.3.1.1.3(a-l). The influence of suspended mud on *An. gambiae s.l* was more pronounced in the late stages than the early stages

Table 4.3.1.1.2a: Association between any stage or final stages (fourth instars and pupae) and *Oryza* spp (Rice).

Plant species	Mosquiito species	RR (df=1)	95% CL of RR	P	Signif.
Oryza	Ag-any	2.59	2.06-3.24	< 0.001	***
	Ag-final	5.05	3.47-7.35	< 0.001	***
	Af-any	0.72	0.32-1.59	> 0.05	ns
	Af-final	0.36	0.05-2.56	> 0.05	ns
	Ac-any	0.32	0.11-1.0	> 0.05	ns
	Ac-final	0.41	0.06-2.96	> 0.05	ns
	Cx-any	1.69	1.2-2.37	< 0.01	**
	Cx-final	1.84	1.1-3.08	< 0.05	*

Table 4.3.1.1.2b: Association between any stage or final stages (fourth instars and pupae) and *Jancus* spp (Rushes).

Plant species	Mosquito species	RR (df=1)	95% CL of RR	P	Signif.
Jancus	Ag-any	0.83	0.67-1.01	> 0.05	ns
	Ag-final	5.50	0.32-0.78	< 0.01	**
	Af-any	1.20	0.87-1.65	> 0.05	ns
	Af-final	0.99	0.55-1.79	> 0.05	ns
	Ac-any	2.01	1.50-2.01	< 0.001	***
	Ac-final	1.41	0.76-2.59	> 0.05	ns
	Cx-any	0.82	0.65-1.03	> 0.05	ns
	Cx-final	0.99	0.20-1.38	> 0.05	ns

Table 4.3.1.1.2c: Association between any stage or final stages (fourth instars and pupae) and *Ipomea* spp.

Plant species	Mosquito species	RR (df=1)	95% CL of RR	P	Signif.
Ipomea	Ag-any	0.76	0.62-0.92	< 0.01	**
	Ag-final	0.53	0.35-0.81	< 0.01	**
	Af-any	1.20	0.88-1.64	> 0.05	ns
	Af-final	1.13	0.64-1.98	> 0.05	ns
	Ac-any	1.55	1.16-2.07	< 0.01	**
	Ac-final	1.03	0.90-2.96	> 0.05	ns
	Cx-any	1.40	1.14-1.72	< 0.01	**
	Cx-final	1.54	1.12-2.10	< 0.01	**

Table 4.3.1.1.2d: Association between any stage or final stages (fourth instars and pupae) and *Pistia* spp.

Plant species	Mosquito species	RR (df=1)	95% CL of RR	P	Signif.
Pistia	Ag-any	0.12	0.02-0.86	< 0.05	*
	Ag-final	0.40	0.06-2.77	> 0.05	ns
	Af-any	0.32	0.05-2.26	> 0.05	ns
	Af-final	0.96	0.14-6.76	> 0.05	ns
	Ac-any	0.29	0.04-2.02	> 0.05	ns
	Ac-final	1.14	0.16-8.08	> 0.05	ns
	Cx-any	0.94	0.44-1.99	> 0.05	ns
	Cx-final	1.02	0.34-3.06	> 0.05	ns

Table 4.3.1.1.2e: Association between any stage or final stages (fourth instars and pupae) and *Cyperus* spp.

Plant species	Mosquito species	RR (df=1)	95% CL of RR	P	Signif.
Cyperus	Ag-any	0.87	0.72-1.05	> 0.05	ns
	Ag-final	0.51	0.35-0.76	< 0.001	***
	Af-any	0.93	0.68-1.27	> 0.05	ns
	Af-final	0.98	0.56-1.71	> 0.05	ns
	Ac-any	1.31	0.98-1.76	> 0.05	ns
	Ac-final	0.99	0.54-1.82	> 0.05	ns
	Cx-any	0.66	0.53-0.82	< 0.001	***
	Cx-final	0.51	0.36-0.74	< 0.001	***

Table 4.3.1.1.2f: Association between any stage or final stages (fourth instars and pupae) and *Spirogyra* spp.

Plant species	Mosquito species	RR (df=1)	95% CL of RR	P	Signif.
Spiro-gyra	Ag-any	1.42	0.91-2.21	> 0.05	ns
	Ag-final	2.14	1.05-4.38	> 0.05	ns
	Af-any	0.72	0.24-2.2	> 0.05	ns
	Af-final	0.71	0.1-5.03	> 0.05	ns
	Ac-any	1.13	0.48-2.64	> 0.05	ns
	Ac-final	0.89	0.12-6.24	> 0.05	n
	Cx-any	2.29	1.56-3.35	< 0.001	***
	Cx-final	3.19	1.88-5.42	> 0.001	***

Table 4.3.1.1.2g: Association between any stage or final stages (fourth instars and pupae) and *Acrostichum aureum* (Fern).

Plant species	Mosquito species	RR (df=1)	95% CL of RR	P	Signif.
Acros-tichum.	Ag-any	0.83	0.23-2.99	> 0.05	ns
	Ag-final	1.33	0.2-8.79	> 0.05	ns
	Af-any	1.09	0.16-7.18	> 0.05	ns
	Af-final	3.04	0.45-20.5	> 0.05	ns
	Ac-any	1.0	0.15-6.62	> 0.05	ns
	Ac-final	3.98	0.59-26.8	> 0.05	ns
	Cx-any	0.48	0.07-3.18	> 0.05	ns
	Cx-final	0.76	0.11-5.17	> 0.05	ns

Table 4.3.1.1.2h: Association between any stage or final stages
(fourth instars and pupae) and *Lagarosiphon* spp.

Plant species	Mosquito species	RR (df=1)	95% CL of RR	P	Signif.
Lagaro-siphon	Ag-any	1.81	0.58-0.92	> 0.05	ns
	Ag-final	2.88	0.48-17.4	> 0.05	ns
	Af-any	2.01	0.33-12.4	> 0.05	ns
	Af-final	6.11	0.98-38.3	> 0.05	ns
	Ac-any	1.85	0.3-11.52	> 0.05	ns
	Ac-final	7.25	1.15-45.6	> 0.05	ns
	Cx-any	2.26	0.72-7.03	> 0.05	ns
	Cx-final	4.91	1.57 15.4	< 0.05	*

Table 4.3.1.1.2i: Association between any stage or final stages (fourth instars and pupae) and *Commelina* spp.

Plant species	Mosquito species	RR (df=1)	95% CL of RR	P	Signif.
Commelina	Ag-any	1.36	0.41-4.53	> 0.05	ns
	Ag-final	1.91	0.3-12.2	> 0.05	ns
	Af-any	1.77	0.28-11.1	> 0.05	ns
	Af-final	4.75	0.73-30.7	> 0.05	ns
	Ac-any	1.44	0.23-9.2	> 0.05	ns
	Ac-final	5.63	0.87-36.6	> 0.05	ns
	Cx-any	0.84	0.13-5.29	> 0.05	ns
	Cx-final	1.83	0.29-11.5	> 0.05	ns

Table 4.3.1.1.2j: Association between any stage or final stages (fourth instars and pupae) and *Cynodon* spp.

Plant species	Mosquito species	RR (df=1)	95% CL of RR	P	Signif.
Cynodon	Ag-any	2.71	0.68-10.9	> 0.05	ns
	Ag-final	8.61	2.13-34.8	> 0.05	ns
	Af-any	7.05	1.75-28.4	> 0.05	ns
	Af-final	21.4	5.22-88.0	< 0.05	*
	Ac-any	6.51	1.61-26.2	> 0.05	ns
	Ac-final	25.42	6.2-104	< 0.05	*
	Cx-any	3.38	0.84-15.8	> 0.05	ns
	Cx-final	7.34	1.82-29.6	> 0.05	ns

Table 4.3.1.1.2k Association between any stage or final stages (fourth instars and pupae) and *Palsparum* spp.

Plant species	Mosquito species	RR (df=1)	95% CL of RR	P	Signif.
Palspa-rum	Ag-any	0.98	0.79-1.22	> 0.05	ns
	Ag-final	0.92	0.60-1.42	> 0.05	ns
	Af-any	0.91	0.62-1.35	> 0.05	ns
	Af-final	0.85	0.42-1.73	> 0.05	ns
	Ac-any	0.50	0.32-0.80	< 0.01	**
	Ac-final	0.41	0.15-1.13	> 0.05	ns
	Cx-any	0.96	0.78-1.16	> 0.05	ns
	Cx-final	0.64	0.41-1.01	> 0.05	

Table 4.3.1.1.21: Association between any stage or final stages (fourth instars and pupae) and vegetation along the margin.

Plant species	Mosquito species	RR (df=1)	95% CL of RR	P	Signif.
On the margin	Ag-any	0.54	0.42-0.69	< 0.001	***
	Ag-final	0.47	0.3-0.76	< 0.01	**
	Af-any	2.96	1.11-7.88	< 0.05	*
	Af-final	1.92	0.47-7.83	> 0.05	ns
	Ac-any	1.8	0.86-3.78	> 0.05	ns
	Ac-final	1.61	0.39-8.58	> 0.05	ns
	Cx-any	0.86	0.6-1.24	> 0.05	ns
	Cx-final	0.9	0.51-1.59	> 0.05	ns

Table 4.3.1.1.2m: Association between any stage or final stages (fourth instars and pupae) and vegetation in the middle

Plant species	Mosquito species	RR (df=1)	95% CL of RR	P	Signif.
In the middle	Ag-any	0.74	0.62-0.89	< 0.01	**
	Ag-final	0.8	0.56-1.13	> 0.05	ns
	Af-any	1.26	0.9-1.77	> 0.05	ns
	Af-final	0.82	0.47-1.43	> 0.05	ns
	Ac-any	1.05	0.77-1.44	> 0.05	ns
	Ac-final	1.0	0.53-1.89	> 0.05	ns
	Cx-any	1.05	0.85-1.31	> 0.05	ns
	Cx-final	1.36	0.97-1.97	> 0.05	ns

Table 4.3.1.1.2n: Association between any stage or final stages (fourth instars and pupae) and plant "cover" including *Spirogyra, Ipomea aquatica, Lagarosiphon, Pistia* and *Commelina* spp.

Plant species	Mosquito species	RR (df=1)	95% CL of RR	P	Signif.
Cover	Ag-any	0.78	0.84-0.95	< 0.05	*
	Ag-final	0.61	0.41-0.9	< 0.05	*
	Af-any	1.16	0.85-1.58	> 0.05	ns
	Af-final	0.95	0.54-1.67	> 0.05	ns
	Ac-any	1.37	1.02-1.83	< 0.05	*
	Ac-final	1.51	0.83-2.73	> 0.05	ns
	Cx-any	1.55	1.27-1.90	< 0.001	***
	Cx-final	1.99	1.46-2.76	< 0.001	***

Table 4.3.1.1.2o: Association between any stage or final stages (fourth instars and pupae) and the absence (none) of vegetation.

Plant species	Mosquito[a] species	RR (df=1)	95% CL of RR	P	Signif.
None	Ag-any	1.04	0.87-1.25	> 0.05	ns
	Ag-final	1.93	1.33-2.88	< 0.001	***
	Af-any	1.15	0.84-1.57	> 0.05	ns
	Af-final	1.81	0.99-3.33	> 0.05	ns
	Ac-any	0.55	0.41-0.74	< 0.001	***
	Ac-final	1.05	0.57-1.92	> 0.05	ns
	Cx-any	0.96	0.78-1.18	> 0.05	ns
	Cx-final	0.97	0.71-1.33	> 0.05	ns

Table 4.3.1.1.3a : Association between any stage or final stages (fourth instars and pupae) of different mosquito species and mud suspended in water

Feature	Mosquito species and stage	χ^2 (χ^2 trend)	df	P	Sign.	+ve/ -ve
Mud	Ag-any	26.0 (20.6)	4 1	< 0.001 < 0.001	*** ***	+
	Ag-final	47.7 (31.0)	4 1	< 0.001	*** ***	+
	Af-any	39.2 (16.2)	4 1	< 0.001 < 0.001	*** ***	-
	Af-final	12.0 (3.6)	4 1	< 0.05	* ns	
	Ac-any	27.0 (8.6)	4 1	< 0.001 < 0.01	*** **	-
	Ac-final	7.8	1	> 0.05	ns	
	Cx-any	22.0 (14.6)	4 1	< 0.001 < 0.001	*** ***	+
	Cx-final	23.1 (10.7)	4 1	< 0.001 < 0.01	*** **	-

Table 4.3.1.1.3b: Association between any stage or final stages (fourth and pupae) of different mosquito species and odour of water

Feature	Mosquito species and stage	χ^2 (χ^2 trend)	df	P	Sign.	+ve/ -ve
Odour	Ag-any	2.0	4	> 0.05	ns	
	Ag-final	1.3	4	> 0.05	ns	
	Af-any	29.8 (10.0)	4 1	< 0.001 < 0.01	*** **	-
	Af-final	11.0 (4.6)	4 1	< 0.05 < 0.01	* **	-
	Ac-any	14.3 (10.5)	4 1	< 0.01 < 0.01	** **	-
	Ac-final	5.5	1	> 0.05	ns	
	Cx-any	20.2 (16.8)	4 1	< 0.001 < 0.001	*** ***	-
	Cx-final	36.8 (27.5)	4 1	< 0.001 < 0.001	*** ***	-

Table 4.3.1.1.3c: Association between any stage or final stages (fourth instars and pupae) of different mosquito species and shade over the water surface

Feature	Mosquito species and stage	χ^2 (χ^2 trend)	df	P	Sign.	+ve/ -ve
Shade	Ag-any	52.2 (42.3)	4 1	< 0.001 < 0.001	*** ***	-
	Ag-final	46.9 (31.6)	4 1	< 0.001 < 0.001	*** ***	-
	Af-any	9.3	4	> 0.05	ns	
	Af-final	7.6	4	> 0.05	ns	
	Ac-any	4.2	4	> 0.05	ns	
	Ac-final	4.3	4	> 0.05	ns	
	Cx-any	14.4 (13.1)	4 1	< 0.01 < 0.001	** ***	-
	Cx-final	11.6 (7.7)	4 1	< 0.001 < 0.01	*** **	-

Table 4.3.1.1.3d: Association between any stage or final stages (fourth instars and pupae) of different mosquito species and redscum

Feature	Mosquito species and stage	χ^2 (χ^2 trend)	df	P	Sign.	+ve/ -ve
Redscum	Ag-any	12.0 (4.0)	4 1	< 0.05 < 0.05	* *	-
	Ag-final	4.9	4	> 0.05	ns	
	Af-any	3.2		> 0.05	ns	
	Af-final	2.6	4	> 0.05	ns	
	Ac-any	5.7	4	> 0.05	ns	
	Ac-final	7.8	1	> 0.05	ns	
	Cx-any	1.6	4	> 0.05	ns	
	Cx-final	2.7	4	> 0.05	ns	

Table 4.3.1.1.3e: Association between any stage or final stages (fourth instars and pupae) of different mosquito species and dust floating on the water surface

Feature	Mosquito species and stage	χ^2 (χ^2 trend)	df	P	Sign.	+ve/ -ve
Surface-dust	Ag-any	9.3	4	> 0.05	ns	
	Ag-final	12.7 (0.87)	4 1	< 0.05 > 0.05	* ns	
	Af-any	20.9 (10.5)	4 1	< 0.001 < 0.01	*** **	-
	Af-final	10.6 (5.8)	4 1	< 0.05 < 0.05	* *	-
	Ac-any	34.6 (23.7)	4 1	< 0.001 < 0.001	*** ***	-
	Ac-final	15.3 (6.3)	4 1	< 0.01 < 0.05	** *	-
	Cx-any	18.4 (8.7)	4 1	< 0.01 < 0.05	** *	-
	Cx-final	8.9	4	> 0.05	ns	-

Table 4.3.1.1.3f: Association between any stage or final stages (fourth instars and pupae) of different mosquito species and stillness of water

Feature	Mosquito species	RR (df=1)	95% CL of RR	P	Signif.
Stillness of water	Ag-any	1.12	0.89-1.41	> 0.05	ns
	Ag-final	1.88	1.11-3.18	< 0.05	*
	Af-any	0.54	0.39-0.75	< 0.001	***
	Af-final	0.50	0.28-0.81	< 0.05	*
	Ac-any	0.86	0.61-1.22	> 0.05	ns
	Ac-final	1.55	0.66-3.66	> 0.05	ns
	Cx-any	1.74	1.27-2.37	< 0.001	***
	Cx-final	1.64	1.03-2.59	< 0.05	*

Table 4.3.1.1.3g: Association between any stage or final stage (fourth instars and pupa) of different mosquito species and speed of water flow (excluding still water).

Feature	Mosquito species and stage	Kruskal-Wallis Test H	df	P	Sign.	+ve/-ve
Speed of water flow	Ag-any	11.8	1	< 0.001	***	-
	Ag-final	10.4	1	< 0.01	**	-
	Af-any	11.1	1	< 0.001	***	-
	Af-final	3.5	1	> 0.05	ns	
	Ac-any	8.0	1	< 0.01	**	-
	Ac-final	1.8	1	> 0.05	ns	
	Cx-any	9.1	1	< 0.01	**	-
	Cx-final	2.8	1	> 0.05	ns	

Table 4.3.1.1.3h: Association between any stage or final stages (fourth instars and pupae) of different mosquito species and depth of the water

Feature	Mosquito species and stage	Kruskal-Wallis Test H	df	P	Sign.	+ve/ -ve
Depth	Ag-any	0.02	1	> 0.05	ns	
	Ag-final	2.95	1	> 0.05	ns	
	Af-any	22.3	1	< 0.001	***	+
	Af-final	9.2	1	< 0.01	**	+
	Ac-any	27.8	1	< 0.001	***	+
	Ac-final	12.8	1	< 0.001	***	+
	Cx-any	16.8	1	< 0.001	***	+
	Cx-final	4.0	1	< 0.05	*	+

Table 4.3.1.1.3i: Association between any stage or final stages (fourth instars and pupae) of different mosquito species and oily water surface layer

Feature	Mosquito species	RR (df=1)	95% CL of RR	P	Signif.
Oily layer	Ag-any	0.97	0.71-1.31	> 0.05	ns
	Ag-final	1.0	0.56-1.78	> 0.05	ns
	Af-any	0.85	0.52-1.38	> 0.05	ns
	Af-final	0.81	0.3-2.24	> 0.05	ns
	Ac-any	1.22	0.77-1.92	> 0.05	ns
	Ac-final	0.98	0.35-2.72	> 0.05	ns
	Cx-any	1.27	0.94-1.73	> 0.05	ns
	Cx-final	1.39	0.88-2.2	> 0.05	ns

Table 4.3.1.1.3j: Association between any stage or final stages (fourth instars and pupae) of different mosquito species and sites with sloping muddy margins

Feature	Mosquito species	RR (df=1)	95% CL of RR	P	Signif.
Slope	Ag-any	2.28	1.92-2.70	< 0.001	***
	Ag-final	2.44	1.74-3.42	< 0.001	***
	Af-any	1.79	1.31-2.54	< 0.001	***
	Af-final	1.59	0.84-2.66	> 0.05	ns
	Ac-any	1.38	1.01-1.89	< 0.05	*
	Ac-final	1.96	1.07-3.58	< 0.05	*
	Cx-any	1.24	1.0-1.55	> 0.05	ns
	Cx-final	1.45	1.05-2.02	< 0.05	*

Table 4.3.1.1.3k: Association between any stage or final stages (fourth instars and pupae) of different mosquito species and Notonectids

Feature	Mosquito species	RR (df=1)	95% CL of RR	P	Signif.
Notone-ctids	Ag-any	2.2	1.82-2.67	< 0.001	***
	Ag-final	1.14	0.69-1.86	> 0.05	ns
	Af-any	3.10	2.25-4.27	< 0.001	***
	Af-final	3.67	2.08-6.48	< 0.001	***
	Ac-any	4.64	3.5-6.15	< 0.001	***
	Ac-final	2.84	1.48-5.47	< 0.01	**
	Cx-any	2.65	2.15-3.28	< 0.001	***
	Cx-final	2.67	1.91-3.75	< 0.001	***

Table 4.3.1.1.3l: Association between any stage or final stages (fourth instars and pupae) of different mosquito species and presence of dragon fly larvae

Feature	Mosquito[a] species	RR (df=1)	95% CL of RR	P	Signif.
Dragon fly larvae	Ag-any	1.77	1.4-2.25	< 0.001	***
	Ag-final	1.55	0.94-2.56	> 0.05	ns
	Af-any	5.12	3.79-6.92	< 0.001	***
	Af-final	4.48	2.5-8.03	< 0.001	***
	Ac-any	3.47	2.53-4.78	< 0.001	***
	Ac-final	1.74	0.75-4.08	> 0.05	ns
	Cx-any	2.3	1.8-2.93	< 0.001	***
	Cx-final	3.52	2.51-4.95	< 0.001	***

(Table 4.3.1.1.3a). The odour of the water did not significantly influence the distribution of either early or late stages (4.3.1.1.3b). On the other hand shade had a strong influence on both early and late stages (Table 4.3.1.1.3c).

In order to determine if there was a tendency of increased breeding with increase in intensity of each of these features a test of chi-square for trend was carried out. The results of these tests showed that there was an increase in the probability of occurrence of final stages with increase in the amount of mud (Table 4.3.1.1.3a). An increase in the amount of shade was associated with a reduced probability of occurrence of any stages and of final stages (Table 4.3.1.1.3c).

Taking into consideration only the final stages, significant association was also found with the following features:
Surface dust (Table 3.1.1.3e), sloping muddy margin (4.3.1.1.3j) and stagnant-water (Table 4.3.1.1.3f). Water-speed had a negative effect (Table 4.3.1.1.3g).

The presence of predators, that is Notonectids and dragon fly larvae, did not show significant association with the probability of occurrence of the final stages of *An. gambiae s.l* although there was a positive association with the probability of occurrence of any stage (Tables 4.3.1.1.2k & l).

4.3.1.1.4 Independent effect of environmental features

As mentioned in section 4.2.2 above, the independent effect of environmental features can best be assessed by means of a logistic regression model since most of the dependent variables are binary (i.e. presence or absence of larvae) (Kirkwood, 1988). A multiple logistic regression model directly estimates the probability of an event occurring and interactively selects coefficients of the variables with maximum likelihood of bringing about the event (Kirkwood, 1988). By use of such a model those factors (confounding factors) without a direct effect on the event are systematically eliminated.

In this study the analysis was done by single entry of all the factors into the logistic model. The "Odds Ratio" (OR), i.e the ratio of the probability that the event will occur to the probability that it will not occur, was used for describing the relative importance of features found to have a direct influence on breeding. The significance of departure of odds ratio from unity was tested by χ^2 (Wald statistic) (Kirkwood, 1988). The following features were found to exert independent influence on the breeding of this mosquito species (see Tables 4.3.1.1.4a and b)

(i) Footprints had a positive association, streams had a negative influence

(ii) *Pistia:* this was the only plant found to have a direct negative influence (iii) Mud: had positive association (iv) shade: negative (v) red scum: negative (vi) sloping muddy margin: positive.

4.3.2 *An. funestus.*

Late stages of this species were caught in rather small numbers. This may have been due to their actual rarity and/or evasive behaviour. For the purpose of this presentation emphasis is mainly on presence or absence of any stage of this species.

4.3.2.1 Overall effect of environmental features

4.3.2.1.1 Breeding sites

An. funestus showed a significant heterogeneity in probability of occurrence in the different types of site for any stage but not for the final stages (Table 4.3.1.1.1a). Among individual categories of breeding site, only pools had a significant association with the final stages and this was a positive one (Table 4.3.1.1.1d). The following categories of breeding sites had significant association with any stage of *An. funestus*:

(i) Puddles: Negative association (Table 4.3.1.1.1c).

(ii) Pools: Positive association (4.3.1.1.1d)

(iii) Ponds: Positive association (4.3.1.1.1e)

4.3.2.1.2. Vegetation

Table 4.3.1.1.4a: Features having independent influence on any stage of *An. gambiae sl.*

Feature	Odds (Exponent B) of features	Log_e B	S.E of B	Wald χ^2	df	Signif.	Regression coefficient R
Pistia	0.1373	-1.9857	1.0022	3.93	1	< 0.05 *	-0.0304
Suspended mud	1.0883	0.0846	0.0423	4.01	1	< 0.05 *	0.0310
Shade	0.8314	-0.1847	0.0527	12.3	1	< 0.001 ***	-0.0701
Redscum	0.7355	-0.3072	-0.1080	8.09	1	< 0.01 **	-0.0540
Slope	2.5307	0.9285	0.1226	57.39	1	< 0.001 ***	0.1628
Water speed	0.9213	-0.0819	0.0370	4.91	1	< 0.05 *	-0.0373
Footprint	3.2644	1.1831	0.2107	31.5	1	< 0.001 ***	0.1189
Pool	1.7082	0.5354	0.2627	4.2	1	< 0.05 *	0.0321
Constant		-1.6444	0.1229	178.9	1	< 0.001 ***	

Table 4.3.1.1.4b: Features having independent influence on the final stages(fourth instars and pupae) of *An. gambiae s.l.*

Feature	Odds (Exponent B) of features	Log_e B	S.E of B	Wald χ^2	df	Signif.	Regression coefficient R
Suspended mud	1.2226	0.2010	0.0680	8.75	1	< 0.01 **	0.0431
Shade	0.7398	-0.3013	0.0933	12.43	1	< 0.01 **	-0.0933
Redscum	0.6740	-0.3945	0.2010	3.85	1	< 0.05 *	-0.0437
Slope	2.0509	0.7183	0.1993	12.98	1	< 0.001 ***	0.1605
Foot print	4.5710	1.5197	0.2589	34.45	1	< 0.001 ***	0.1830
Stream	0.4098	-0.8920	0.3274	7.42	1	< 0.01 *	-0.0748
Constant		-2.9828	0.2144	193.47	1	< 0.001 ***	

No individual plant species was significantly associated with *An. funestus* (Tables 4.3.1.1.2a-o) although the presence of vegetation along the margin of breeding sites had a positive effect to all stages (see Tables 4.3.1.1.2n).

4.3.2.1.3 Other environmental features

As shown in Tables 4.3.1.1.3(a-l) most of the environmental features recorded showed association with presence /absence of this species. Among the most striking associations were presence/absence with water movement (Table 4.3.1.1.3f), predators (4.3.1.1.3.k & l), shade (Table 4.3.1.1.3c), depth (Table 4.3.1.1.3h) and mud. *An.funestus* showed a negative association with stillness of water, but no association with speed of water flow. On the other hand, it showed a positive of association with presence of predators (4.3.1.1.3g). Shade did not seem to have any significant effect on the distribution of this mosquito (Table 4.3.1.1.3c). Depth had a strong positive association with probability of occurrence of any stage (Table 4.1.1.3a)as well as final stages.

4.3.2.1.4 Independent effect of environmental features

The results of a multiple logistic regression model (as described above for *An. gambiae s.l*) showed that only two features were independently associated with the final stages of *An. funestus* (see Tables 4.3.2.1.4a and b). These were surface-dust on water and odour .

4.3.3 Effect of overall and independent effect of environmental features on *An. coustani* and culicines

4.3.3.1 *An. coustani*

This mosquito species, which is generally considered only a secondary vector of malaria, was caught in much smaller numbers than *An. gambiae s.l* or *An. funestus*.

Table 4.3.2.1.4a: Features having independent influence on any stage of *An. funestus*.

Feature	Odds (Exponent B) of features	Log_e B	S.E of B	Wald χ^2	df	Signif.	Regression coefficient R
Suspended mud	1.1607	0.1490	0.06588	5.1301	1	< 0.05 *	0.0529
Redscum	0.6980	0.3595	0.1781	4.07	1	< 0.05 *	-0.043
Slope	1.8733	0.6277	0.1833	11.1	1	< 0.001 ***	0.0902
Depth	2.2299	0.8020	0.3585	5.0	1	< 0.05 *	0.0518
Odour	1.2026	0.1845	0.0743	6.16	1	< 0.05 *	0.0610
Pool	3.3350	1.2045	0.3370	12.8	1	< 0.001 ***	0.0981
Pond	4.176	1.4294	0.4601	9.7	1	< 0.01 **	0.0827
Stream	2.081	0.7330	0.2183	11.3	1	< 0.001 ***	0.0910
Swamp	2.1003	0.7421	0.3212	5.34	1	< 0.05 *	0.0548
Constant		-3.9361	0.2247	306.78	1	< 0.001 ***	

Table 4.3.2.1.4b: Features having independent influence on the final stages (fourth instar and pupae) of *An. funestus*.

Feature	Odds (Exponent B) of features	Log_e B	S.E of B	Wald ^{2}	df	Signif.	Regression coefficient R
Surface dust	1.2832	0.2493	0.1176	4.49	1	< 0.05 *	0.0718
Depth	1.9291	0.6571	0.3441	3.65	1	> 0.05 ns	0.0583
Odour	1.2310	0.2078	0.1045	3.95	1	< 0.05 *	0.0635
Constant		-4.3624	0.2456	315.6	1	< 0.001 ***	

Nevertheless, the results (see Tables 4.3.1.1.1a-h; 4.3.1.1.2a-o and 4.3.1.1.3a-l) show that final stages are more likely to be found in pools (Table 4.3.1.1.1d) which have sloping muddy margins (Table 4.3.1.1.3j). *Juncus* (rushes) (Table 4.3.1.1.2a) and "cover"(Table 4.3.1.1.2n) were positively associated with any stage and final stage. The final stages were found to have negative association with dust on the water surface (Table 4.3.1.1.3e) but a positive association with the depth of the water (Table 4.3.1.1.3h)). Stillness of the water does not have any apparent influence on the distribution of *An. coustani.* However, its probability of occurrence appears be associated with the presence of predators (Table 4.3.1.1.3f and Table 4.3.1.1.3l). This species was not significantly associated with mud, odour or shade (Tables: 4.3.1.1.3a, 4.3.1.1.3b and 4.3.1.1.3c).

The test for independent action of the environmental features (see Table 4.3.3.1a and b) showed that the presence of dust on the water surface in pools and pond as compared with other types of site were positively associated with this species.

4.3.3.2 Culicines

The final stages of culicines (see Tables 4.3.1.1.1a-h) showed a positive association with pools (Table 4.3.1.1.1d) and ponds (Table 4.3.1.1.1e). On the other hand there was a negative association of culicines with streams (Table 4.3.1.1.1f). The following vegetation types (see Tables 4.3.1.1.2a-m) showed a positive association with the final stages of culicines: *Spirogyra* (Table 4.3.1.1.2f), *Ipomea* (Table 4.3.1.1.2c), *Lagarosiphon* (Table 4.3.1.1.2h) *Oryza sativa* (Table 4.3.1.1.2a). *Cyperus* had a negative association with culicines (Table 4.3.1.1.2e). Probability of occurrence of Culicines had a significantly negative association with strong odour(Table 4.3.1.1.3b), much suspended mud (Table 4.3.1.1.3a), and heavy shade (Tables 4.3.1.1.3c)

Estimation of independent action of these features on culicines (see Tables 4.3.3.2a and b) indicated that the following features had a positive association with the probability of occurrence of the final stages of culicines: *Oryza sativa* , *Spirogyra*, *Lagarosiphon*, odour. On the other hand, shade showed a negative association with the

Table 4.3.3.1a: Features having independent influence on any stage of *An. coustani*.

Features	Odds (Exponent B) of features	Log_e B	S.E of B	Wald χ^2	df	Signif.	Regression coefficient R
Ipomea	1.8918	0.6375	0.1898	12.16	1	< 0.001 ***	0.0926
Cyperus	1.8709	0.6264	0.1794	12.19	1	< 0.001 ***	0.927
Jancus	2.4217	0.8845	0.1699	27.09	1	< 0.001 ***	0.1455
Supended mud	1.1477	0.1377	0.0594	5.37	1	< 0.05 *	0.0533
Surface - dust	1.3457	0.2969	0.07012	17.92	1	< 0.001 ***	0.1159
Pool	3.9505	1.3739	0.3085	19.83	1	< 0.001 **	0.1227
Pond	5.0636	1.6221	0.4103	15.63	1	< 0.001 ***	0.1072
Constant		-3.9361	0.2247	306.78	1	< 0.001 ***	

Table 4.3.3.1b: Features having independent influence on the final stages (fourth instars and pupae) of *An. coustani*.

Feature	Odds (Exponent B) of features	Log_e B	S.E of B	Wald χ^2	df	Signif.	Regression coefficient R
Surface dust	1.2792	0.2463	0.1249	3.89	1	< 0.05 *	0.668
Pool	3.8107	1.3376	0.5029	7.08	1	< 0.01 **	0.1095
Pond	10.2570	2.3280	0.5216	19.92	1	< 0.001 ***	0.2058
Constant		-4.3498	0.2196	392.25	1	< 0.001 ***	

Table 4.3.3.2a: Features having independent influence on any stage of Culicines.

Feature	Odds (Exponent B) of features	Log_e B	S.E of B	Wald χ^2	df	Signif.		Regression coefficient R
Ipomea	1.3113	0.2710	0.1330	4.15	1	< 0.05	*	0.0343
Spirogyra	2.4513	0.8966	0.2981	9.05	1	< 0.01	**	0.0620
Suspended mud	1.1428	0.1335	0.0459	8.45	1	< 0.01	**	0.0593
Shade	0.8313	-0.1847	0.0562	10.81	1	< 0.01	**	-0.0693
Odour	1.0993	0.0946	0.0537	3.10	1	> 0.05	ns	0.0245
Pool	2.2704	0.8200	0.2611	9.87	1	< 0.01	**	0.0655
Pond	3.7666	1.3262	0.3713	12.76	1	< 0.001	***	0.0766
Stream	0.4896	-0.7142	0.1818	15.44	1	< 0.001	***	-0.0856
Swamp	0.4947	-0.7037	0.2784	6.39	1	< 0.05	*	-0.0489
Constant		-1.7968	0.1359	174.9	1	< 0.001	***	

Table 4.3.3.2b: Features having independent influence on the final stages (fourth instars and pupae) of Culicines.

Feature	Odds (Exponent B) of features	Log_e B	S.E of B	Wald χ^2	df	Signif.	Regression coefficient R
Oryza	1.9147	0.6496	0.3162	4.22	1	< 0.05 *	0.0400
Spirogyra	3.2373	1.1747	0.3522	11.12	1	< 0.001 ***	0.0914
Lagarosi-siphon	6.8390	1.9226	0.9001	4.56	1	< 0.05 *	0.0484
Shade	0.8260	-0.1911	0.0789	5.86	1	< 0.05 *	-0.0594
Odour	1.3570	0.3053	0.0649	22.09	1	< 0.001 ***	0.1356
Pool	3.3704	1.2150	0.3214	14.29	1	< 0.001 ***	0.1060
Pond	5.5844	1.7200	0.4087	17.71	1	< 0.001 ***	0.1199
Constant		-2.9082	0.1620	322.3	1	< 0.001 ***	

probability of occurrence of the final stages of culicines. Pools and ponds showed a strong independent positive association with the probability of occurrence of culicines.

4.4 DISCUSSION

The results show that *An. gambiae s.l* prefers to breed in water without vegetation or shade, and which is muddy and not moving. This is in agreement with the description of Gillies and de Meillon (1968). The red scum which is usually found in seepage water that has persisted for some time seems to be inimical to *An. gambiae s.l* breeding. This suggests that this species is more likely to be found in relatively freshly collected water than water which has been lying for some time.

A possible explanation of why there was much breeding of *An. gambiae s.l* in rice fields is that there were many footprints left by the cultivators in these fields. Further evidence of the importance of footprints is shown below. Odour did not have a significant association with *An. gambiae s.l.*, most probably because it is only in the breeding sites near human habitations that vegetable wastes and human excreta pollute the water.

An. funestus final stages were mostly found in the pools in the study area since these were the most common permanent breeding sites. The final stages of this mosquito species were not associated with mud, probably because the pools in which they are commonly found tend to have little suspended mud due to their permanent nature. *An. funestus* was found not to be associated with shade. However, shade is a difficult parameter to define, since even in the absence of overhead shade a few small plants in the water may create enough shade for the mosquito larvae. In that case presence of vegetation appears to be more decisive than overhead shade alone.

An. coustani tends to be found breeding in the same sites as *An. funestus*. These

sites are pools and ponds in this study area, since these are the deeper water bodies. Such sites usually contain predators. Although it is considered that Culicines are usually associated with odour of water and suspended mud in the water, it was not so in the present study with the final stages of these mosquitoes. The probable explanation is that, in breeding sites away from human dwellings the most common cause of organic pollution was rotting vegetation. The odour of water with that type of pollution was not as strong as that of pit latrines or cesspits with human excreta. Also, the colour of such water was not always so dark, although the organic content of water may have been high enough for the culicines to breed in large numbers. In that case categorizing the water as being less muddy and the odour as being not unpleasant, was likely to lead to this type of results. Therefore, the presence of dead plants in the water should be scored highly in terms of mud even if the water appears less turbid.

CHAPTER 5:

ESTIMATION OF PRODUCTIVITY OF *AN. GAMBIAE S.L* PER UNIT AREA OF WATER.

5.1 INTRODUCTION

It is common practice to determine the mean number of immature mosquitoes per dip in breeding sites of very different types and sizes (Zahar, 1975). However, it is not possible to relate this to productivity of immature and adult stages from each type of site unless these relative estimates are converted to absolute estimates per unit area of water.

Estimations of absolute numbers of larvae and pupae of *An. gambiae s.l* have rarely been attempted by field workers in routine larval surveys. This can be attributed to the large amount of work involved where the breeding site may have to be completely or partially evacuated, or larvae marked,released and recaptured (Service, 1976). Nevertheless, as mentioned in chapter 2, absolute estimations of larval and pupal populations do not have to be made routinely. Relatively few estimations should be sufficient for providing a calibration factor with which to convert relative densities into absolute densities of larvae and pupae as proposed by Service (1971) and Zahar (1985).

This study, therefore, aimed at relating the average number per dip for a given site to the absolute density in such a site, i.e. at estimating a calibration factor that could be multiplied by the number of larvae per dip from normal surveys of sites with known area to estimate the absolute number of fourth stage larvae and pupae present in each type of site. It was hoped that the number of these final stages could then be related to the number of adult mosquitoes caught emerging from a unit area of the

site on a given night to estimate the number emerging from a known number of final stages and hence the relative productivity of adult mosquitoes of each type of site could be established. Thus only fourth instar larvae, pupae, and adult *An. gambiae s.l* will be discussed in this chapter.

5.1.1 Static quadrats

Various workers have described methods for the estimation of absolute numbers of larvae and pupae in breeding sites (Service, 1976). Cambournac (1939) in Portugal and Bates (1941) in Albania used static quadrats in the estimation of mosquito larval populations in rice fields or other breeding sites. In these studies, Cambournac (1939) used square quadrats consisting of rectangular metal frames enclosing $0.1m^2$. When secured in the mud, larvae and pupae were confined inside and could be exhaustively dipped out and counted. From this study, he was able to conclude that when all the water has been removed from the quadrat, and the bottom mud washed with small amounts of water to remove any stranded larvae, the total count of larvae should be very close to the absolute number that were present in the area of the quadrat.

5.1.1.1 Height of quadrats and depth of breeding sites

Cambournac (1939) used a set of quadrats, all of the same height because there was not much variation in depth in the rice fields where he worked. However, a different situation arises when working in deeper breeding sites. Bates (1941) attempted to solve that problem by using various different heights of quadrats for different depths. This method is, however, subject to two main limitations: (a) the depths of breeding sites vary considerably and it would be inconvenient to carry numerous sizes of quadrat around for calibration purposes, (b) in deeper breeding sites evacuation of a quadrat inserted in the mud under one meter of water would take a long time. Such a situation was overcome by Goma (1958) while quantifying *Anopheles* larvae and pupae in the swamps of Uganda by attaching prongs at the corners of square quadrats of a single height measuring 0.4m x 0.4m x 0.18m. It was found that after bailing out

a relatively small proportion of the water, no more larvae could be recovered by further bailing. This agreed with the findings reported above in chapter 3, that the proportion of larvae and pupae that were picked by pipette for a minimum of five minutes from suspended quadrats was almost equal to the known numbers of larvae that had been introduced. This suggested that even if water was not completely evacuated from suspended quadrats, the numbers of larvae collected, as described above, was a good estimate of the absolute population present.

5.1.1.2 Effect of repeated dipping

Estimation of the relative density in terms of number of larvae and pupae caught per dip entails repeated dipping from a breeding site or a quadrat. It is expected that if the larvae are not returned to the quadrat after each dip there would tend to be a decline in the number of larvae and pupae caught in subsequent samples since the number caught in each dip is related to the size of the population present (Service,1976). This decrease in the number of individuals in successive samples is theoretically expected to be in geometric progression (Le Pelley, 1935). In order to avoid this situation, larvae from each dip were returned to the quadrat after being counted, in the estimate of average number per dip.

In carrying out repeated dipping from a quadrat it is necessary that the chances of larvae being caught do not change with time of dipping (Service,1976). However, habituation and/or exhaustion of the larvae might occur. Therefore, Service (1976) proposed the use of a series of quadrats so that consecutive dips are made from different quadrats, to give time for larvae to surface. However, as reported in chapter 3, the time spent under water by each larval and pupal stage after being disturbed did not differ significantly $F = 0.285$, $p_{(\text{two tailed})} > 0.05$, $df = 2, 12$. The mean time before surfacing of each stage was found to be 30.95 ± 6.2 seconds. Since the time spent identifying and recording larvae from every dip was greater than this estimate, it was concluded that the time wasted by waiting for larvae to surface would not be a very important factor if small numbers of dips were all that was required. The results of a small experiment to see if there was any trend in the numbers caught during

repeated dipping in a quadrat were reported in chapter 3, and it was shown in this experiment that there was no such significant trend. In view of these findings it was decided to use single height quadrats for each linked estimate of larvae per dip and of absolute number of larvae in a quadrat placed in a given breeding site.

5.2 MATERIALS AND METHODS

5.2.1 Calibration dipping and evacuation

Estimation of the absolute number of larvae and pupae in the breeding site involved two main activities (a) dipping, counting of the numbers of each stage and replacement of larvae (calibration dipping), and (b) complete removal of larvae and pupae (evacuation). In sites where water was completely evacuated, the bottom mud was washed with about one litre (three ladle-fulls) of water to detach any stranded larvae or pupae. Sites for calibration work were selected in which there were considerable numbers of larvae.

5.2.1.1 Footprints

Calibration dipping in footprints was done by making five dips in a given footprint, making a record of the number of larvae of each stage caught in each dip and returning them. Then the whole footprint was evacuated and the number of larvae recorded.

5.2.1.2 Other sites

In all other sites calibration dipping was done inside a quadrat that had been pressed into the mud. The quadrats used in the calibration dipping and evacuation were made from thick (3mm) aluminium sheeting and they measured 0.25m x 0.25m x 0.4m (height) (see Figure 5.1B). Individual estimation of the required number of dips to obtain a reliable mean was not attempted as this would have damaged some of the

Figure 5.1: Quadrats for trapping emerging adult mosquitoes (A) and for evacuation studies (B).

larvae even before calibration work started. Therefore, it was decided to make five dips where larval density was high and ten dips where the density was low to estimate the relative density. Then the water in the quadrat was evacuated. If the site was too deep for the quadrat to reach the bottom without being submerged, the quadrat was suspended from poles by means of rubber bands. In this case five or ten dips were gently made, and the water was returned into the quadrat so slowly as not to cause the larvae to dive or be driven below the bottom of the quadrat. Then the larvae were continually removed from the quadrat using a pipette for at least five minutes until no more could be seen. The larvae so collected were taken as the total number in the column of water defined by the quadrat.

5.2.2 Estimation of adult emergence per unit area of water.

Estimation of adult mosquitoes emerging from a breeding site was attempted by covering an area of water with a mosquito net measuring 1m x 1m x 1m with cloth sleeves sewn on the sides through which mosquitoes could be collected (WHO, 1975). Unfortunately, application of this method had a number of setbacks particularly theft of the nets and infestation with ants. The ants found on the nets were suspected of predating the emerging mosquitoes and leading to very small numbers, or complete absence, of adult mosquitoes being found in the net on the following morning, even in the presence of large numbers of pupae in the breeding site. Therefore, this method was changed and a square quadrat measuring 0.5m x 0.5m x 0.5m substituted (Figure 5.1A) . The quadrat had a ring of grease applied inside and outside near the top edge to act as ant trap. It also had a netting top. These quadrats were placed over the sites which had previously been evacuated and which had then been allowed to refill with water. In deeper breeding sites the quadrat was supported on poles with rubber bands as already described. Wet cotton wool soaked in glucose was placed on the netting in the evening for emerging mosquitoes to feed on. The emerged mosquitoes were collected the following morning.

5.3. RESULTS

5.3.1 Estimation of calibration factor

Meaningful calibration factors could only be calculated if, for each type of breeding site, a significant positive regression could be demonstrated of the number of larvae and pupae per dip against the corresponding total number of larvae and pupae from evacuation. In such cases the regression coefficients provided the calibration factors. For practical purposes, however, a common calibration factor for all types of sites would be better than different calibration factors for different types of breeding site. Such a common calibration factor could justifiably be estimated if the confidence limits of all of these regression coefficients for different types of site overlapped. Figure 5.2a-d shows the data of the number of larvae and pupae per dip (on the y axis) against larvae and pupae evacuated (on the x axis) for different breeding sites. The calculated regression lines are shown below with variance ratios for the proportions of the total variance explained by regressions. The regression lines were forced through the origin to take into account the obvious fact that when larvae and pupae were not found in the breeding sites upon evacuation of a quadrat none would have been obtained by dipping.

Footprint $Y = 0.0 + 0.072x$

$F_{(1,9)} = 37.3 \quad p < 0.01$

95% confidence limits of regression coefficient

$= 0.045$ and 0.099

Puddle $Y = 0.0 + 0.139x$

$F_{(1,40)} = 42.9 \quad P < 0.01$

95% confidence limits of regression coefficient

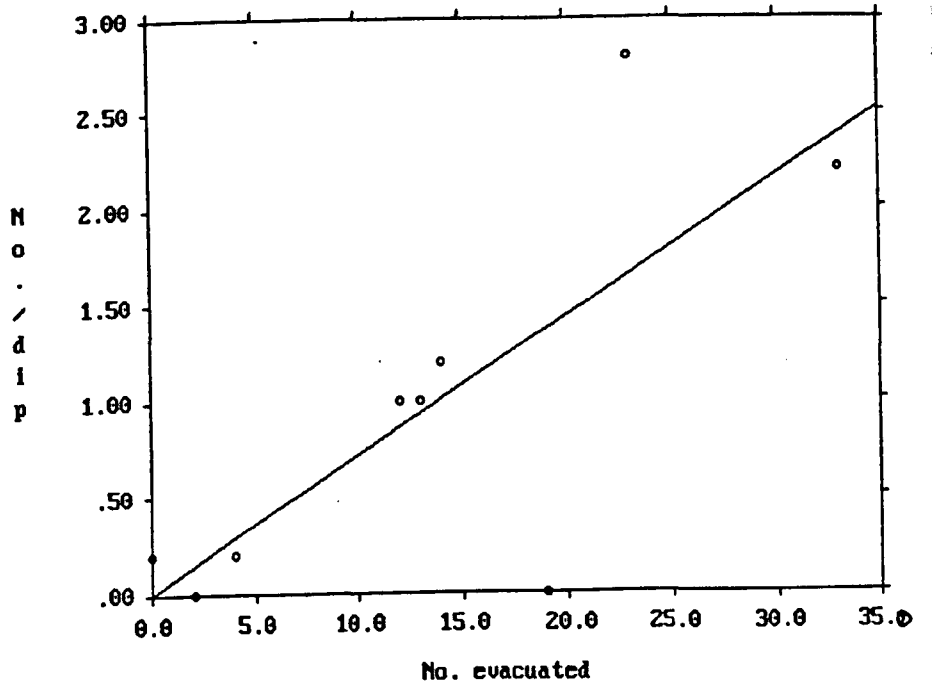
$= 0.096$ and 0.182

Pool $Y = 0.0 + 0.178x$

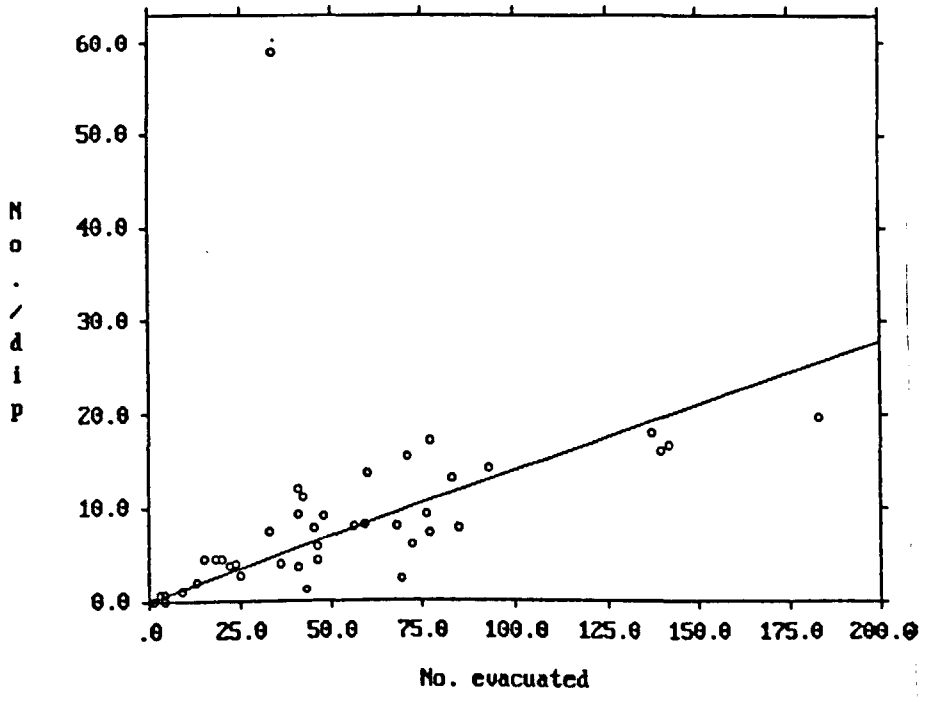
$F_{(1,7)} = 20.0 \quad p < 0.01$

Figure 5.2: Regression lines of number of larvae and pupae of *An. gambiae s.l* per dip against total number of 4th stage larvae and pupae evacuated from quadrat:-

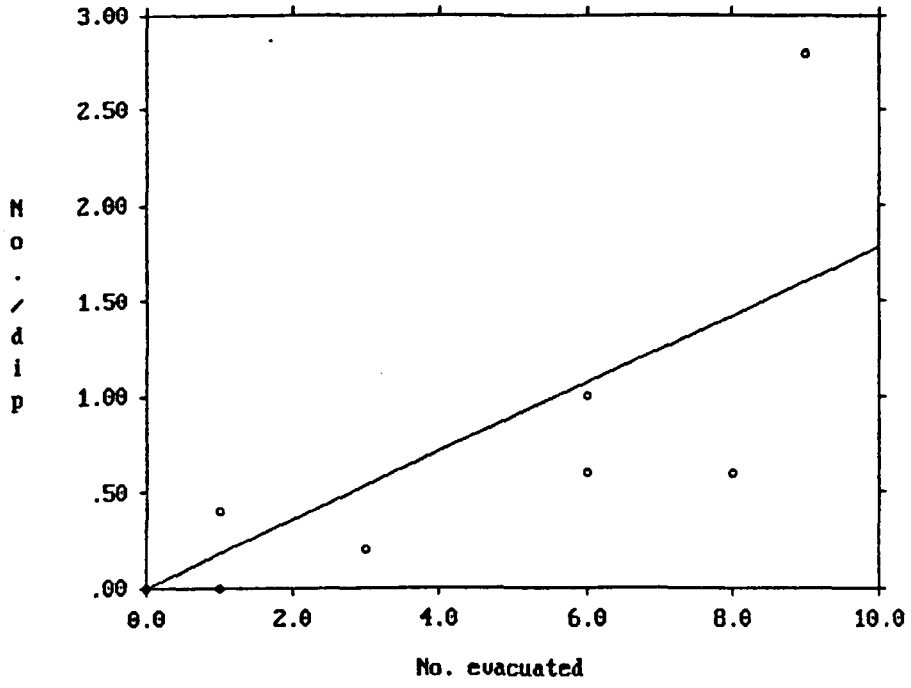
(a) 1.Footprint



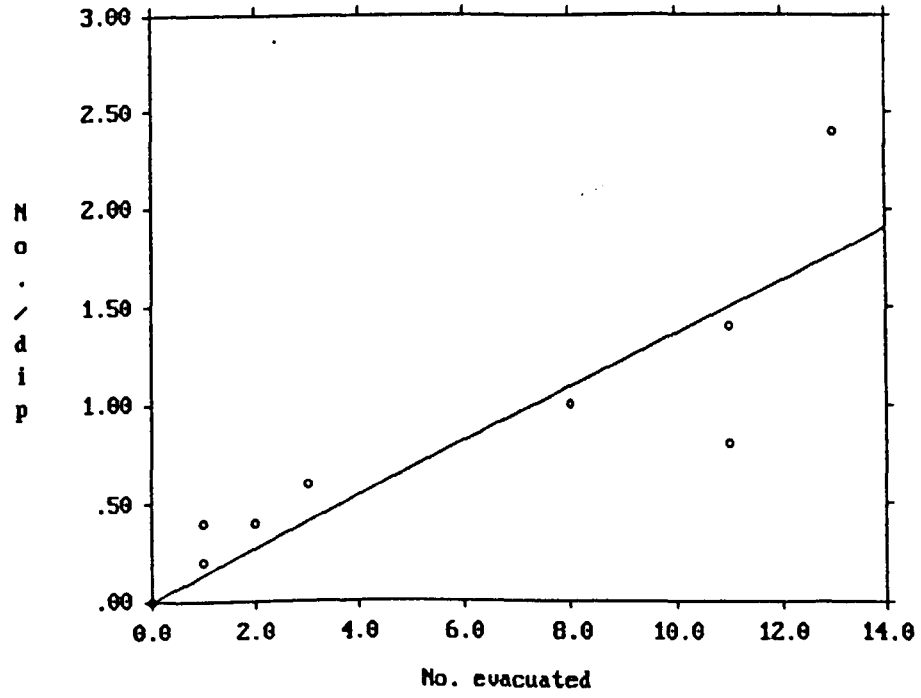
2. Puddle



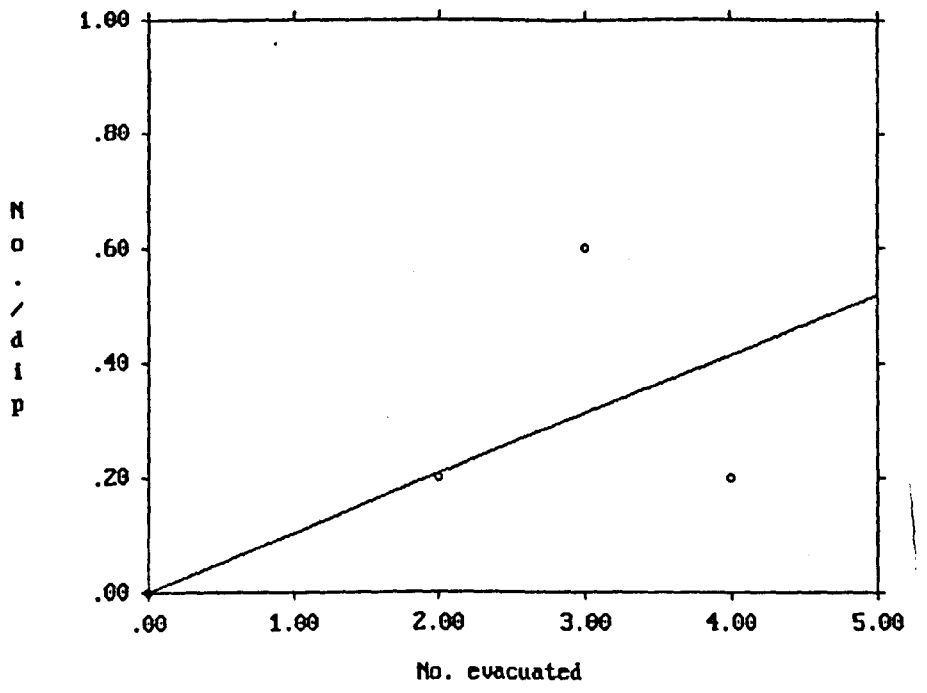
(b) 1. Pool



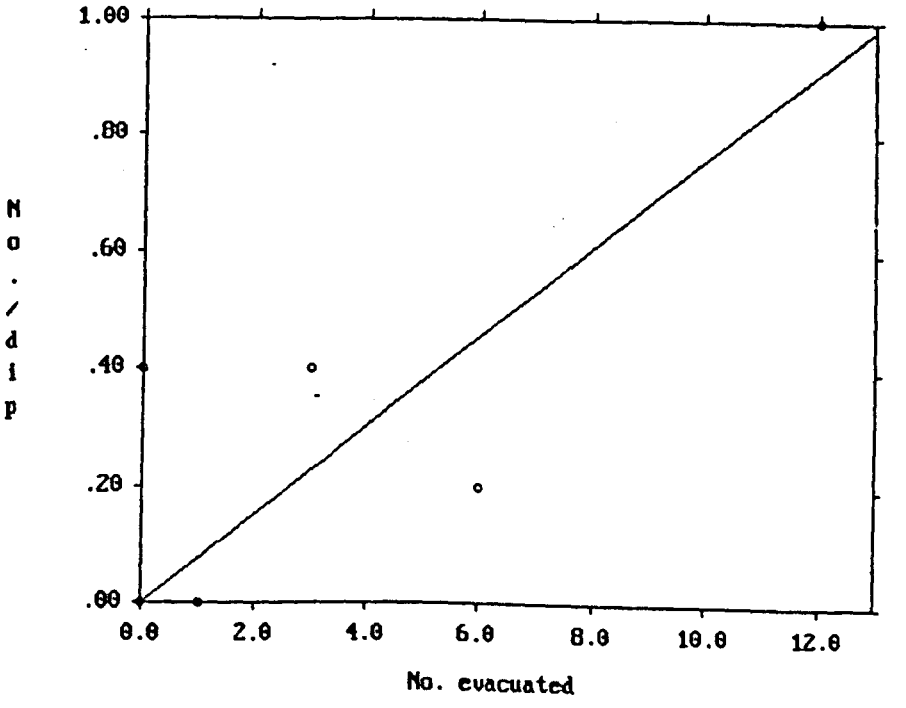
2. Pond



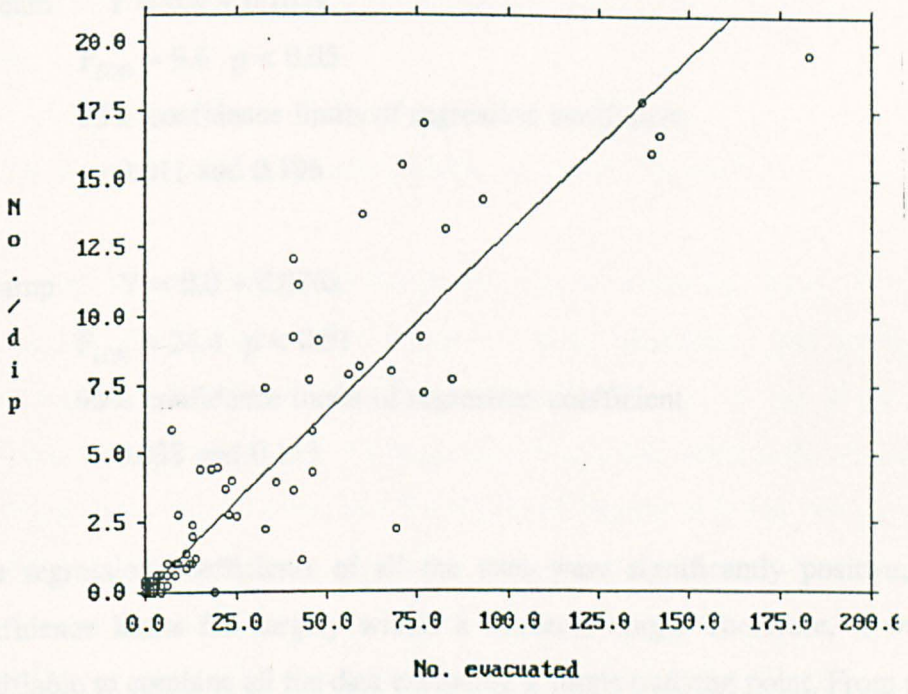
(c) 1. Stream



2. Swamp



(d) Combined data



95% confidence limits of regression coefficient

= 0.084 and 0.272

Pond $Y = 0.0 + 0.136x$

$F_{(1,8)} = 70.5$

95% confidence limits of regression coefficient

= 0.099 and 0.173

Stream $Y = 0.0 + 0.103x$

$F_{(1,4)} = 9.6 \quad p < 0.05$

95% confidence limits of regression coefficient

= 0.011 and 0.196

Swamp $Y = 0.0 + 0.076x$

$F_{(1,6)} = 24.4 \quad p < 0.01$

95% confidence limits of regression coefficient

= 0.038 and 0.113

The regression coefficients of all the sites were significantly positive, and their confidence limits fell largely within a common range. Therefore, it was thought justifiable to combine all the data excluding a single outlying point. From this pooled data a single regression coefficient was obtained as follows:

Combined sites: $Y = 0.0 + 0.130x$

$F_{(1,78)} = 570.6 \quad P < 0.001$

95% confidence limits of regression coefficient

= 0.119 and 0.141

The reciprocal of the regression coefficient, that is 7.69 was equivalent to the calibration factor with which to multiply larvae per dip to estimate larvae per unit area. Since the calibration used quadrats with a surface area of $0.0625m^2$ the calibration factor for estimating the number of larvae per square meter was obtained

by multiplying 7.69 with the reciprocal of 0.0625 (i.e. 16) which gives 123.0.

It was of interest to compare this calibration factor with what would be obtained by simply dividing the average number of fourth instar and pupae evacuated (30.58) by the average number of larvae and pupae per dip (4.15). The calibration factor from such direct division was 7.39 which when multiplied by 16 gives 118.2 which was surprisingly close to the value of 123.0 calculated above from the regression method. This may have been fortuitous and more comparisons would be needed before direct division could confidently be used for the estimation of calibration factors in routine work.

5.3.2 Estimation of a calibration factor for adult mosquitoes

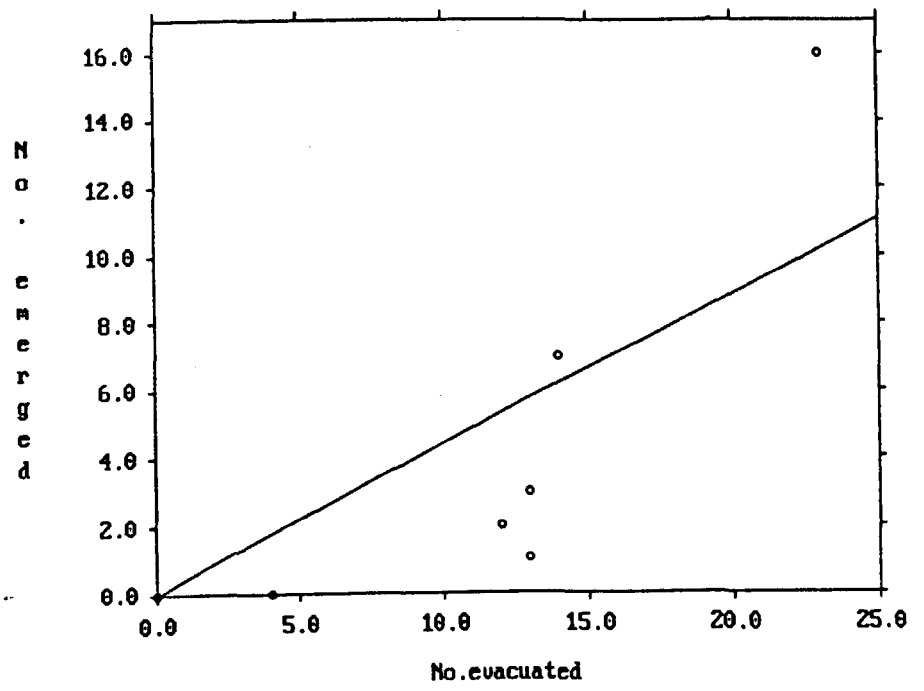
An attempt was made to relate the number of larvae and pupae recovered upon evacuation of a quadrat to the number of adult mosquitoes that emerged on the following day. Regression analysis was then performed to see if the regression coefficient was significantly different from zero and thus whether the number of adults emerging could be predicted from the estimated number of larvae and pupae present. As shown in Figure 5.3a-c a significant relationship was obtained in all sites except in puddles suggesting that the density of fourth instar larvae and pupae was positively related to the output of the adult mosquitoes. However, since the estimation of adult emergence was considered to be less reliable than that of absolute numbers of larvae and pupae owing to desiccation sinking of dead adult mosquitoes, it was decided to base estimation of prevalence of *An. gambiae s.l* for different sites on the densities of the fourth and pupal stages only.

5.4 DISCUSSION

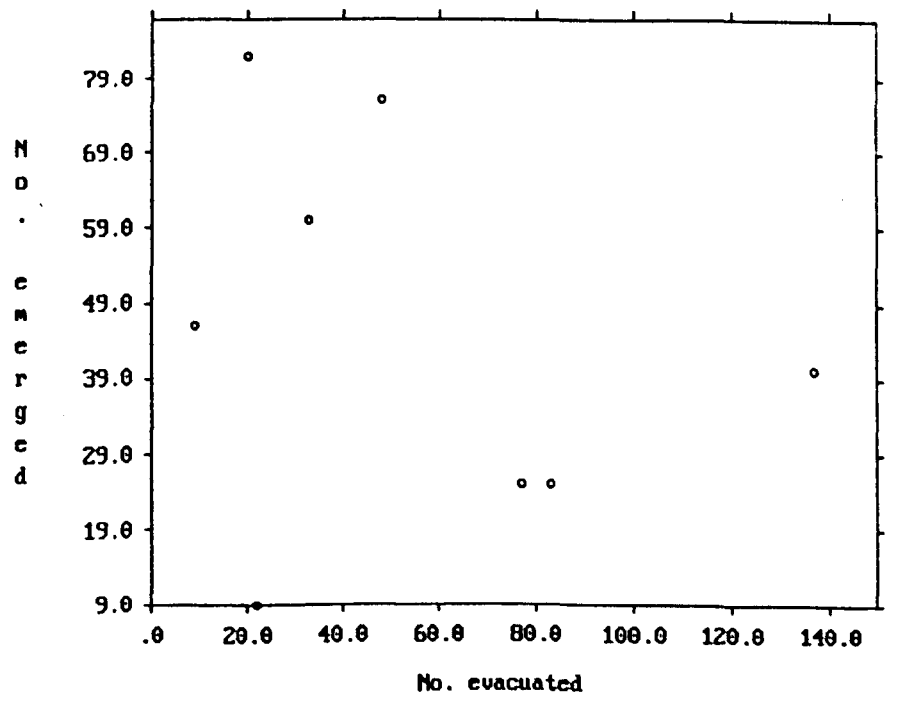
These results show that it is quite possible to estimate the 4th instar and pupal productivity of different sites for *An. gambiae s.l.* The use of numbers per dip as a measure of density seems justified, and can be used to estimate the absolute numbers per unit area in the different types of site studied. Horsfall (1946) found that quadrats

Figure 5.3: Regression lines of number of adult *An. gambiae s.l* that emerged against total number 4th stage larvae and pupae evacuated from quadrats

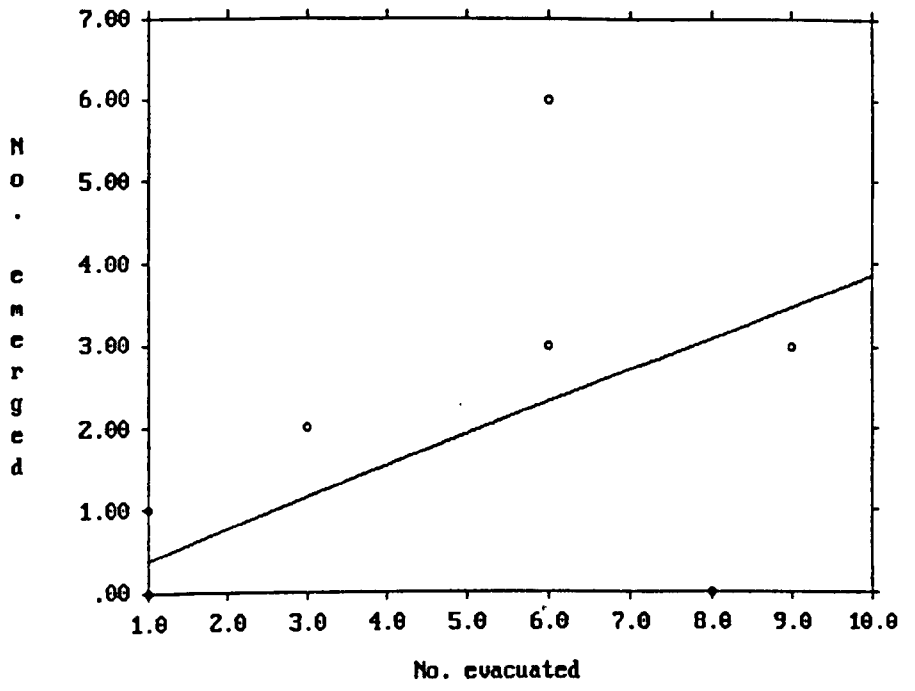
(a) 1.Footprint



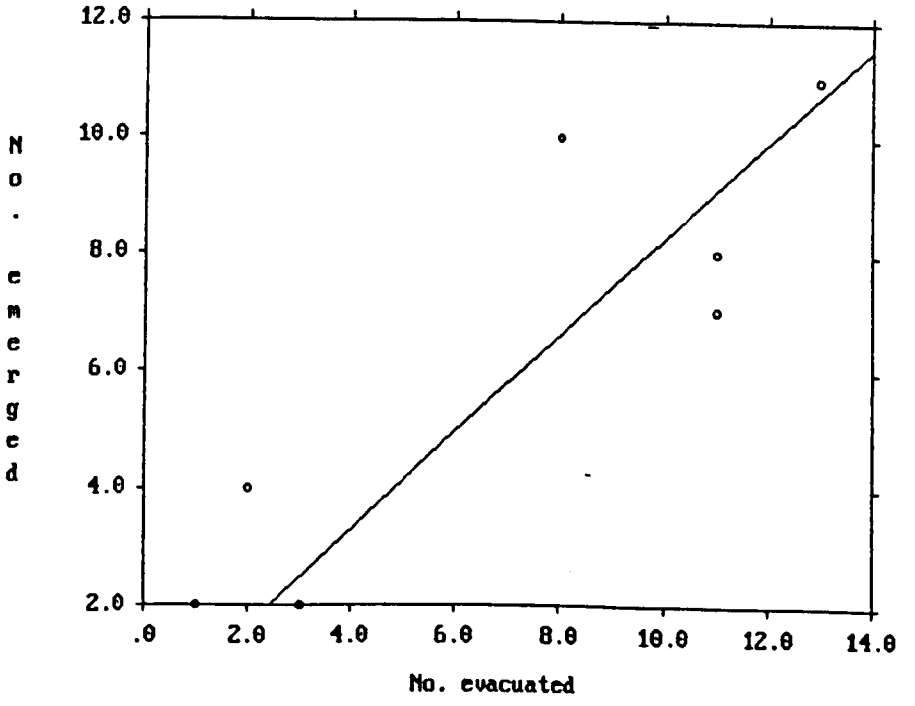
2. Puddle



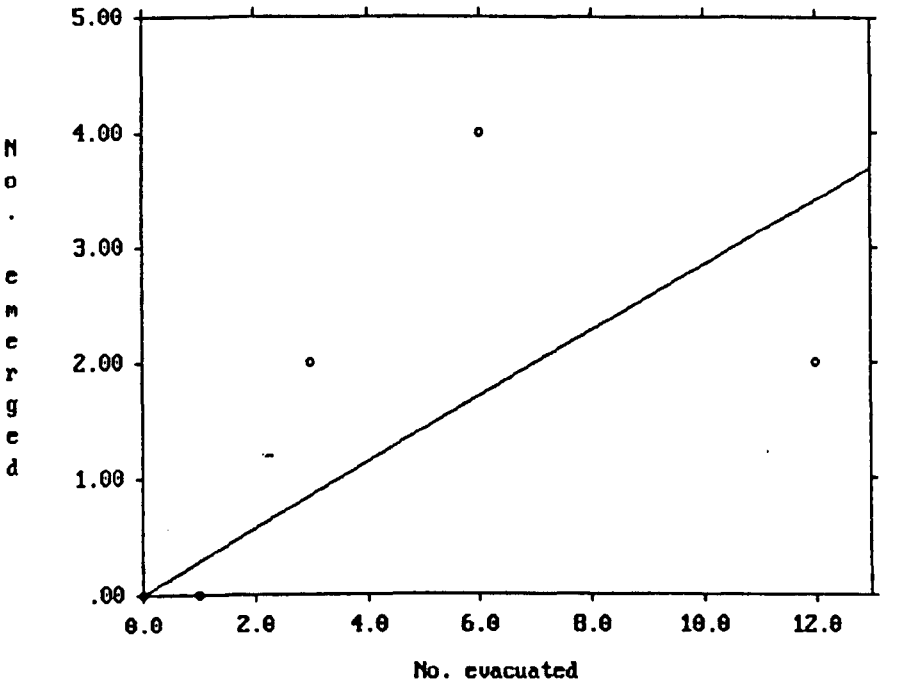
(b) 1. Pool



2. Pond



(c) Swamp.


5.00
4.00
3.00
2.00
1.00
.00
No. emerged
0.0
2.0
4.0
6.0
8.0
10.0
12.0
No. evacuated

gave a more accurate estimate of larvae than dipping, but for routine work it would not be feasible for quadrats to replace dipping. By estimation of a calibration factor for converting numbers per dip to numbers per unit area, absolute densities can nonetheless be estimated in routine surveys.

The similarity observed in the regression coefficients and hence calibration factors, for various breeding sites, suggested that the numbers of larva and pupae per dip for different types of breeding site were an adequate estimate of larvae in the various breeding sites. The estimation of a common calibration factor that could be used for all the sites had the advantage of basing it on a large sample size so the confidence limits were fairly narrow. Nevertheless, it would be desirable to check occasionally if there is important variation in this factor over time and space and to indicate if there is a need for changing it from time to time during routine work.

Although unit estimates of the number of adult mosquitoes emerging per area per night would have been a more desirable measure of relative productivity for different sites, it was considered a less reliable option than the use of final stages of the mosquito species. This is because estimates of emerged mosquitoes seemed to be less than the actual number of emerged mosquitoes owing to desiccation and sinking. For that reason, it is considered that estimation of the relative prevalence of mosquito species in different breeding sites based on late larval and pupal stages would be the best functional measure for planning and assessing larvicidal operations.

CHAPTER 6

PREVALENCE OF FOURTH INSTAR LARVAE AND PUPAE OF MOSQUITOES IN DIFFERENT TYPES OF BREEDING SITE AND THE EFFECT OF LARVICIDING USING ABATE AND *BACILLUS THURINGIENSIS ISRAELENSIS*

6.1 Prevalence of fourth instar larvae and pupae of *An. gambiae s.l*

6.1.1.Introduction

Following the calculation of a common calibration factor of 123 (see chapter 5) to relate numbers per dip to numbers per unit area, an attempt was made to estimate the prevalence of fourth instar larvae and pupae (i.e final aquatic stages) in the breeding sites around Mngaza and Kumbamtoni in order to assess the relative importance of these sites for the breeding of *An. gambiae s.l.* It was also intended to observe the variation in the prevalence of larvae between the sites and over time, and whether these variations were most influenced by changes in larval density per square meter or by the increase or decrease in the total area of the breeding sites.

6.1.2 Materials and methods

The total area of a breeding site was estimated during sampling by counting paces or by eye. In the case of footprints an estimate was made of the number of footprints in a square meter and the actual area containing water in the footprint. A typical human footprint measured about 0.1m x 0.25m = 0.025m^2. As described in chapter 3, one square meter of rice field was found to contain about 18 ± 1 footprints. This was equivalent to an area of 18 x 0.025m^2 = 0.45m^2 of wet footprints in a square meter of rice field. Therefore, the total area of rice field recorded as containing footprints was multiplied by 0.45 to estimate the actual area with water. In the case of other types of site the total area of each type of breeding site, was then used to estimate the

prevalence of the final aquatic stages of *An. gambiae s.l* in these sites in each quarter of the year as follows:

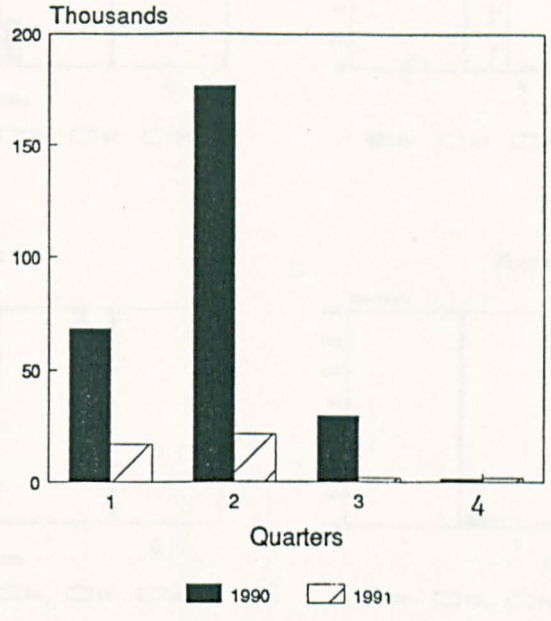
Average area of water surveyed	x	mean no. of final stages per dip	x	calibration factor, i.e. 123

Unfortunately in 1990 only one visit could be made per quarter. Also, in Kumbamtoni no visit was made during the first quarter of 1991 before insecticiding started in Mngaza. Nevertheless, since the average of the total area of water monitored, and the mean number of larvae per dip during each quarter are used in the calculation of prevalence of the final stages it is considered justifiable to make comparisons between corresponding quarters of 1990 and 1991. For this purpose, the absolute and percentage prevalence of each site for each quarter were calculated.

6.1.3 Results

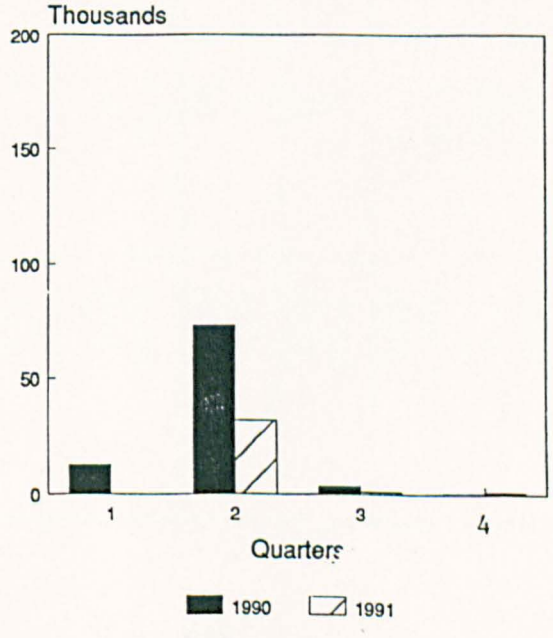
The estimated overall absolute numbers of the final aquatic stages of *An. gambiae s.l* for Mngaza and Kumbamtoni are shown in Figures 6.1.3a and b respectively. There was seasonal variation in the numbers of the final stages both in 1990 and 1991. In Mngaza in 1990 there was more output of the final stages during the first and second quarters than in 1991. In the third and fourth quarters of the two years very small numbers of the final stages were produced. Figures 6.1.3c(1 and 2) show the estimated numbers of the final aquatic stages of *An. gambiae s.l* for each type of site on a quarterly basis in Mngaza. Table 6.1.3a shows the same data in terms of percent contribution of each type of breeding site for *An. gambiae s.l.* In the first, second and third quarters of 1990 the footprints had the highest output of the final stages. The other sites contributed well below 20% of the final stages during the same period. However, in the fourth quarter of the same year, pools and ponds contributed 61.8% and 38.2% of the final aquatic stages respectively but the total produced in that quarter was very small. In 1991, in the first quarter, i.e. before larviciding was started, the puddles and swamps were the most important type of breeding site. No water-filled footprints were found during this time due to drought. However, in the second quarter,

Figure 6.1.3a: Estimated numbers of final stages of An. gambiae s.l



All sites: Mngaza 1990 and 1991

Figure 6.1.3b: Estimated numbers of final stages of An. gambiae s.l



All sites: Kumbamtoni 1990 and 1991

Figure 6.1.3c: Estimated mean number of *An. gambiae s.l* in each type of site in each quarter of 1990 and 1991 in Mngaza & Kumbamtoni

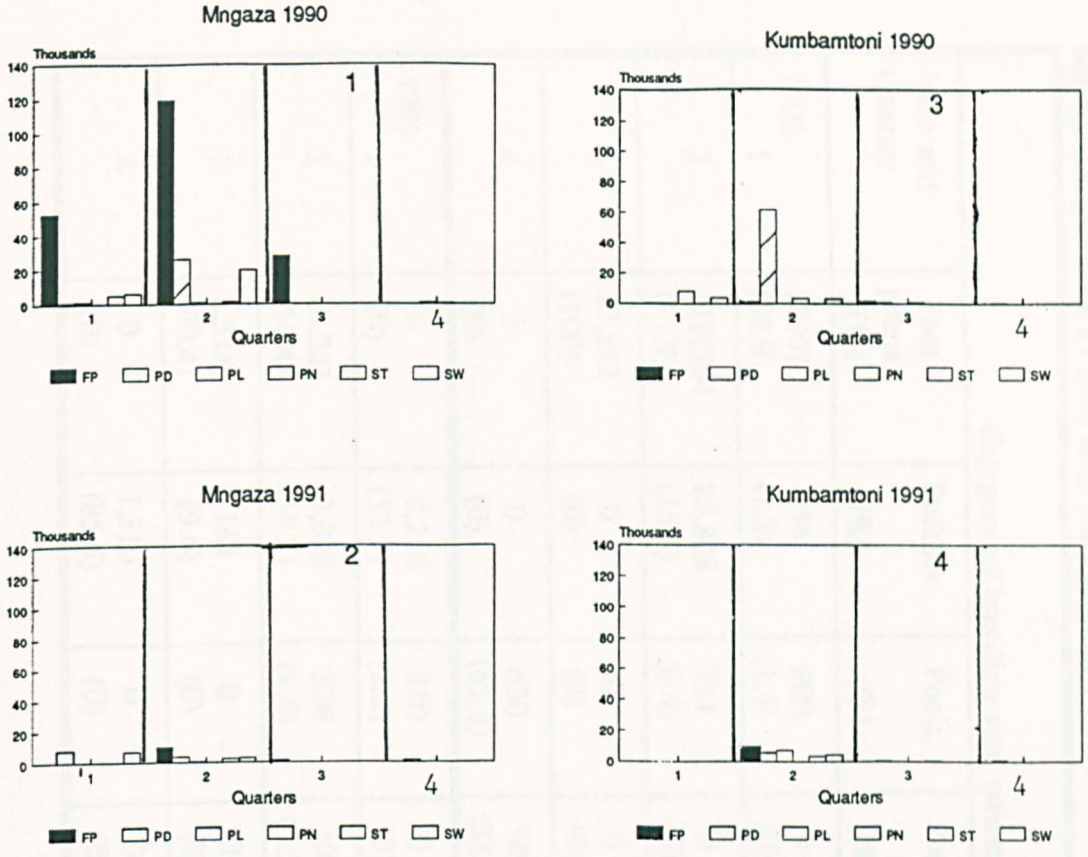


Table 6.1.3a: Quarterly estimates of total and percent contribution of each category of breeding site for *An. gambiae s.l* final stages in **Mngaza**

Category of breeding site, quarterly totals and (percentages)							
Year and Quarter	Foot-prints (%)	Puddles (%)	Pools (%)	Ponds (%)	Streams (%)	Swamps (%)	Quarterly subtotals (%)
1990 1	52970 (80.9)	754 (1.2)	900 (1.4)	0 (0)	4,800 (7.3)	6,039 (9.2)	65,463 (25)
2	119,584 (71.7)	25,808 (15.5)	711 (0.4)	0 (0)	1,171 (0.7)	19,526 (11.7)	166,800 (63.9)
3	27,812 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	27,812 (10.7)
4	0 (0)	0 (0)	630 (61.8)	389 (38.2)	0 (0)	0 (0)	1,019 (0.4)
1991 1	0 (0)	8,216 (52.7)	310 (2.0)	0 (0)	0 (0)	7,073 (45.3)	15,599 (39.9)
2	10,225 (50.4)	3,998 (19.7)	326 (1.6)	103 (0.5)	2,585 (12.5)	3,066 (15.1)	20,303 (51.9)
3	1,518 (90.4)	162 (9.6)	0 (0)	0 (0)	0 (0)	0 (0)	1,680 (4.3)
4	0 (0)	1,310 (85.5)	0 (0)	0 (0)	222 (14.5)	0 (0)	1,532 (3.9)

Table 6.1.3b: Quarterly estimates of total and percent contribution of each category of breeding site for *An. gambiae s.l* final stages in **Kumbamtoni**

Category of breeding site, quarterly totals and (percentages)							
Year and Quarter	Foot-prints (%)	Puddles (%)	Pools (%)	Ponds (%)	Streams (%)	Swamps (%)	Quarterly subtotals (%)
1990 1	77 (0.6)	374 (3.1)	282 (2.3)	7,824 (63.7)	0 (0)	3,718 (30.3)	12,275 (14.4)
2	1,107 (1.6)	61,928 (89.5)	0 (0)	3108 (4.5)	0 (0)	3,030 (4.4)	69,173 (81.3)
3	1,660 (49.9)	792 (23.8)	0 (0)	875 (26.3)	0 (0)	0 (0)	3,327 (4.0)
4	0 (0)	246 (100)	0 (0)	0 (0)	0 (0)	0 (0)	246 (0.3)
1991 1	-	-	-	-	-	-	-
2	9,620 (31.8)	5,760 (19.1)	7,163 (23.7)	0 (0)	3,334 (11.0)	4,356 (14.4)	30,233 (93.8)
3	0 (0)	439 3(9.6)	131 (11.7)	0 (0)	554 (49.3)	0 (0)	1,124 (3.5)
4	0 (0)	862 (100)	0 (0)	0 (0)	0 (0)	0 (0)	862 (2.7)

during larviciding, the footprints alone yielded about 50% (10,225/20303) of the final stages, and they yielded over 90% (1518/1680) of them in the third quarter. In the fourth quarter puddles, and to a small extent streams, were the most important type of breeding site.

In Kumbamtoni, the pattern of variation of prevalence of the final stages was similar to that of Mngaza (Figure 6.1.3c1 and 4). In 1990 there were more final stages produced than in 1991. The first and the second quarters had the highest output of the final stages in 1990. In 1991, the second quarter showed by far the highest output of the year. The overall output of final stages in Kumbamtoni was much lower than that of Mngaza. The productivity and percent contribution of the different categories of breeding sites for *An. gambiae s.l* in Kumbamtoni varied over the year as shown in Figures 6.1.3c(3 and 4) and Table 6.1.3b respectively. In the first quarter of 1990 the more permanent breeding sites namely ponds and swamps were the most productive, accounting for 63.7% (7,824/12,275) and 30.3% (3,718/12,275) of the total output during that quarter. During the second quarter of the same year, puddles accounted for 89.5% (61,928/69173). The puddles included flooded rice fields and accounted for 72.7% (8/11) of all the breeding sites which had rice growing. In the third quarter, wet footprints contained more of the final stages than the rest of the sites, but even there the numbers were very small. In the second quarter of 1991 the footprints were the most important site with 31.8% (9,620/30,233) of the estimated number of final stages during that quarter.

6.1.4 Discussion

The prevalence of fourth instar larvae and pupae showed marked variation during the year with a highest peak during the second quarter which coincided with the long rainy seasons in both Kumbamtoni and Mngaza. The increase in the prevalence of these final stages during this time was not only influenced by the increase in the total area of the breeding site (see Tables 6.1.4a1-6 and Tables 6.1.4b1-6) but also in the density of larvae. For example, Table 6.1.4a1 shows that although the footprints had only about half as much area in the second quarter of 1990 as 1991 the estimated

Table 6.1.4a1: Relationship between mean area of footprints, *An. gambiae s.l* final aquatic stages per dip (Agf/dip) and prevalence of final aquatic stages in **Mngaza**

Site (a)	Year and quarter (b)	No. of surveys (c)	Total area of water m^2 (d)	Mean area of water m^2 d/c (e)[a]	Mean Agf/dip (f)	Mean Agf/m^2. f x 123[k] (g)	Prevalence of Agf (g x e)
Footprints	1990 1	1	1995	897.8	0.48	59.0	52,970
	2	1	8305	3,737	0.26	32.0	119,584
	3	1	910	409	0.55	68.0	27,812
	4	1	0	0	0	0	0
	1991 1	2	0	0	0	0	0
	2	7	18,495	1,189	0.07	8.6	10,225
	3	6	3,376	253	0.05	6.0	1,518
	4	8	115	6	0.0	0	0

[a] For footprints mean area with water = d/c x 0.45

[k] Calibration factor

Table 6.1.4a2: Relationship between mean area of puddles, *An. gambiae s.l* final aquatic stages per dip (Agf/dip) and prevalence of final aquatic stages in **Mngaza**

Site (a)	Year and quarter (b)	No. of surveys (c)	Total area of water m^2 (d)	Mean area of water m^2 d/c (e)[a]	Mean Agf/dip (f)	Mean Agf/m^2. f x 123[k] (g)	Prevalence of Agf (g x e)
Puddles	1990 1	1	613	613	0.01	1.23	754
	2	1	5,267	5,267	0.04	4.9	25,808
	3	1	721	721	0.0	0.0	0
	4	1	283	283	0.0	0.0	0
	1991 1	2	669	334	0.2	24.6	8,216
	2	7	5,711	816	0.04	4.9	3,998
	3	6	3,889	648	0.002	0.25	162
	4	8	8,517	1,065	0.01	1.23	1,310

[a] For footprints mean area with water = d/c x 0.45

[k] Calibration factor

Table 6.1.4a3: Relationship between mean area of pools, *An. gambiae s.l* final aquatic stages per dip (Agf/dip) and prevalence of final aquatic stages in **Mngaza**

Site (a)	Year and quarter (b)	No. of surveys (c)	Total area of water m^2 (d)	Mean area of water m^2 d/c (e)[a]	Mean Agf/dip (f)	Mean Agf/m^2. f x 123[k] (g)	Prevalence of Agf (g x e)
Pools	1990 1	1	366	366	0.02	2.46	900
	2	1	578	578	0.01	1.23	711
	3	1	630	630	0.0	0.0	0
	4	1	105	105	0.05	6.0	630
	1991 1	2	504	252	0.01	1.23	310
	2	7	618	88	0.03	3.7	326
	3	6	0	0	0.0	0.0	0
	4	8	0	0	0	0.0	0

[a] For footprints mean area with water = d/c x 0.45

[k] Calibration factor

Table 6.1.4a4: Relationship between mean area of ponds, *An. gambiae s.l* final aquatic stages per dip (Agf/dip) and prevalence of final aquatic stages in **Mngaza**

Site (a)	Year and quarter (b)	No. of surveys (c)	Total area of water m^2 (d)	Mean area of water m^2 d/c (e)[a]	Mean Agf/dip (f)	Mean Agf/m^2. f x 123[k] (g)	Prevalence of Agf (g x e)
Ponds	1990 1	1	57	57	0	0	0
	2	1	313	313	0	0	0
	3	1	361	361	0	0	0
	4	1	105	105	0.03	3.7	389
	1991 1	2	148	74	0	0	0
	2	7	284	41	0.02	2.5	103
	3	6	0	0	0	0.0	0
	4	8	0	0	0	0.0	0

[a] For footprints mean area with water = d/c x 0.45

[k] Calibration factor

Table 6.1.4a5: Relationship between mean area of streams, *An. gambiae s.l* final aquatic stages per dip (Agf/dip) and prevalence of final aquatic stages in **Mngaza**

Site (a)	Year and quarter (b)	No. of surveys (c)	Total area of water m^2 (d)	Mean area of water m^2 d/c (e)[a]	Mean Agf/dip (f)	Mean Agf/m^2. f x 123[k] (g)	Prevalence of Agf (g x e)
Streams	1990 1	1	1,920	1,920	0.02	2.5	4,800
	2	1	3,165	3,165	0.003	0.37	1,171
	3	1	3,096	3,096	0	0	0
	4	1	343	343	0	0	0
	1991 1	2	2033	1,016	0	0	0
	2	7	14,717	2,102	0.01	1.23	2,585
	3	6	9,200	1,533	0	0	0
	4	8	7,101	888	0.002	0.25	222

[a] For footprints mean area with water = d/c x 0.45

[k] Calibration factor

Table 6.1.4a6: Relationship between mean area of swamps, *An. gambiae s.l* final aquatic stages per dip (Agf/dip) and prevalence of final aquatic stages in **Mngaza**

Site (a)	Year and quarter (b)	No. of surveys (c)	Total area of water m^2 (d)	Mean area of water m^2 d/c (e)[a]	Mean Agf/dip (f)	Mean Agf/m^2. f x 123[k] (g)	Prevalence of Agf (g x e)
Swamps	1990 1	1	4,910	4,910	0.01	1.23	6,039
	2	1	15,875	15,875	0.01	1.23	19,526
	3	1	10,006	10,006	0	0	0
	4	1	11,063	11,063	0	0	0
	1991 1	2	11,500	5,750	0.01	1.23	7,073
	2	7	17,452	2,493	0.01	1.23	3,066
	3	6	9,125	1,521	0	0	0
	4	8	1,060	132	0	0	0

[a] For footprints mean area with water = d/c x 0.45

[k] Calibration factor

Table 6.1.4b1: Relationship between mean area of footprints, *An. gambiae s.l* final aquatic stages per dip (Agf/dip) and prevalence of final aquatic stages in **Kumbamtoni**

Site (a)	Year and quarter (b)	No. of surveys (c)	Total area of water m^2 (d)	Mean area of water m^2 d/c (e)[a]	Mean Agf/dip (f)	Mean Agf/m^2. f x 123[k] (g)	Prevalence of Agf (g x e)
Footprints	1990 1	1	14	6.3	0.1	12.3	77
	2	1	100	45	0.2	24.6	1,107
	3	1	300	135	0.1	12.3	1,660
	4	1	0	0	0	0	0
	1991 1	-	-	-	-	-	-
	2	4	2,600	650	0.12	14.8	9,620
	3	3	25	8.3	0	0	0
	4	6	0	0	0	0	0

[a] For footprints mean area with water = d/c x 0.45

[k] Calibration factor

Table 6.1.4b2: Relationship between mean area of puddles, *An. gambiae s.l* final aquatic stages per (Agf/dip) and prevalence of final aquatic stages in **Kumbamtoni**

Site (a)	Year and quarter (b)	No. of surveys (c)	Total area of water m^2 (d)	Mean area of water m^2 d/c (e)[a]	Mean Agf/dip (f)	Mean Agf/m^2. f x 123[k] (g)	Prevalence of Agf (g x e)
Puddles	1990 1	1	304	304	0.01	1.23	374
	2	1	7,201	7,201	0.07	8.6	61,928
	3	1	317	317	0.02	2.5	792
	4	1	200	200	0.01	1.23	246
	1991 1	-	-	-	-	-	-
	2	4	1,441	360	0.13	16	5,760
	3	3	1,073	357	0.01	1.23	439
	4	6	527	88	0.08	9.8	862

[a] For footprints mean area with water = d/c x 0.45

[k] Calibration factor

Table 6.1.4b3: Relationship between mean area of pools, *An. gambiae s.l* final aquatic stages per dip (Agf/dip) and prevalence of final aquatic stages in **Kumbamtoni**

Site (a)	Year and quarter (b)	No. of surveys (c)	Total area of water m^2 (d)	Mean area of water m^2 d/c (e)[a]	Mean Agf/dip (f)	Mean Agf/m^2. f x 123[k] (g)	Prevalence of Agf (g x e)
Pools	1990 1	1	229	229	0.01	1.23	282
	2	1	156	156	0	0	0
	3	1	264	264	0	0	0
	4	1	122	122	0	0	0
	1991 1	-	-	-	-	-	-
	2	4	423	106	0.55	67	7,163
	3	3	106	35.3	0.03	3.7	131
	4	6	0	0	0	0	0

[a] For footprints mean area with water = d/c x 0.45

[k] Calibration factor

Table 6.1.4b4: Relationship between mean area of ponds, *An. gambiae s.l* final aquatic stages per dip (Agf/dip) and prevalence of final aquatic stages in **Kumbamtoni**

Site (a)	Year and quarter (b)	No. of surveys (c)	Total area of water m^2 (d)	Mean area of water m^2 d/c (e)[a]	Mean Agf/dip (f)	Mean Agf/m^2. f x 123[k] (g)	Prevalence of Agf (g x e)
Ponds	1990 1	1	489	489	0.13	16	7,824
	2	1	420	420	0.06	7.4	3,108
	3	1	350	350	0.02	2.5	875
	4	1	306	306	0	0	0
	1991 1	-	-	-	-	-	-
	2	4	330	82.5	0	0	0
	3	3	0	0	0	0	0
	4	6	0	0	0	0	0

[a] For footprints mean area with water = d/c x 0.45

[k] Calibration factor

Table 6.1.4b5: Relationship between mean area of streams, *An. gambiae s.l* final aquatic stages per dip (Agf/dip) and prevalence of final aquatic stages in **Kumbamtoni**

Site (a)	Year and quarter (b)	No. of surveys (c)	Total area of water m^2 (d)	Mean area of water m^2 d/c (e)[a]	Mean Agf/dip (f)	Mean Agf/m^2. f x 123[k] (g)	Prevalence of Agf (g x e)
Streams	1990 1	1	753	753	0	0	0
	2	1	2,300	2300	0	0	0
	3	1	395	395	0	0	0
	4	1	126	126	0	0	0
	1991 1	-	-	-	-	-	-
	2	4	3,606	901	0.03	3.7	3,334
	3	3	1,350	450	0.01	1.23	554
	4	6	0	0	0	0	0

[a] For footprints mean area with water = d/c x 0.45

[k] Calibration factor

Table 6.1.4b6: Relationship between mean area of swamps, *An. gambiae s.l* final aquatic stages per dip (Agf/dip) and prevalence of final aquatic stages in **Kumbamtoni**

Site (a)	Year and quarter (b)	No. of surveys (c)	Total area of water m^2 (d)	Mean area of water m^2 d/c (e)[a]	Mean Agf/dip (f)	Mean Agf/m^2. f x 123[k] (g)	Prevalence of Agf (g x e)
Swamps	1990 1	1	3,023	3,023	0.01	1.23	3,718
	2	1	1,212	1,212	0.02	2.5	3,030
	3	1	1,850	1,850	0	0	0
	4	1	0	0	0	0	0
	1991 1	-	-	-	-	-	-
	2	4	3,559	889	0.04	4.9	4,356
	3	3	0	0	0	0	0
	4	6	0	0	0	0	0

[a] For footprints mean area with water = d/c x 0.45

[k] Calibration factor

number of the final stages in the same quarter of 1990 was more than ten times that in 1991. This difference was due to the much lower larval density recorded in the second quarter of 1991 as will be shown in section 6.2. The same argument applied to almost all types of breeding site.

Although there was a variable and unpredictable relationship between the density of larvae and the total area of each type of breeding site as shown in Tables 6.1.4a and b, the variations in the areas of breeding sites tended to contribute significantly to variation in the total output of the final stages. It is therefore reasonable to consider the possibility of reducing the area of the most productive types of breeding sites during a larviciding programme. It might be feasible to drain swamps and fill in some of the puddles, pools and ponds. However, so long as irrigated rice is cultivated by hand labour without mechanisation there will inevitably be very large numbers of water filled footprints, which are ideal breeding sites for *An. gambiae s.l.*

The observed variation in the relative importance of different types of breeding site during the year implies that it may be possible to concentrate on particular sites during different times of the year for larval control purposes. Nonetheless, wet footprints which are usually highly productive of the final stages would need constant monitoring.

6.2. Effect of larviciding with Abate and Bti in Mngaza

6.2.1 Introduction

It was mentioned in chapter 2 that there seems to be no very strong evidence for the exclusion of larviciding from malaria vector control and, on the contrary, integration of control methods is now strongly emphasised. However, larviciding has never been reported from any rural area in Tanzania. In this chapter a description is given of larviciding work that was conducted in Mngaza village using Abate and Bti in the last three quarters of 1991 to assess its impact on larval and pupal stages of *An. gambiae s.l* and other mosquito species as well as Notonectids including *Enthares*

sobrina and *Nychia limpida, Culex tigripes and* dragon fly larvae.

One of the aspects that needed to be considered before carrying out larviciding in the villages, where the terrain was not easy to cover comprehensively, was the method of application of the insecticides. As described in chapter 3, it was considered that slow-release solid formulations were more convenient to use than liquids which would have required the carrying of spray pumps as well as cans of insecticide with which to refill them.

6.2.1.1 Effect of Abate on the taste of water

Some of the pools around Mngaza are used for drinking water in the dry season when the piped water supply fails. These pools sometimes contain larvae and were therefore included in the sites for Abate treatment. A trial was therefore carried out of the often stated belief that some people object to the taste of Abate in the water. Each of a series of adult subjects was presented with one normal water sample and one treated with 1 mg/l Abate (the WHO recommended treatment dose which is certified non-toxic to humans) (WHO, 1984). Each subject was told that one of the samples had been treated.

Each subject was asked to say "yes" if she/he detected any change in the taste of water, and "no" if he/she considered the taste was normal. In the first test which involved seven people, 6 said "no" for untreated water and only one said "yes". On the other hand 4 said "no" for treated water while 3 said "yes" it was treated. Although in this pilot test there was not a significant association of the response "yes" to the treated sample ($X^2 = 0.35$, df = 1, $P > 0.05$) it was felt necessary to repeat the experiment on a larger scale was felt. The test was therefore repeated seven months later and involved twenty people. This time all said "no" for untreated water and 12 (60%) said "yes" for treated water. The difference between these responses was highly significant ($X^2 = 14$, df = 1, $P < 0.001$) and it is concluded that many people can genuinely taste Abate. Fortunately, in the village trial no one complained about its presence.

6.2.2 Materials and methods

The types of Abate and Bti that were used have been described in chapter 3. Also described in chapter 3 is the method by which the maize cobs and sawdust were impregnated with the insecticides. These insecticides were used to impregnate the maize cobs for use in larger sites and saw dust for use in very small water collections to produce a target concentration of 1mg/l (1ppm). The maize cobs and sawdust were usually treated a day before they were needed and kept in labelled plastic bags to catch the dripping insecticide. The maize cobs and sawdust were carried to the field in the same bags for application to the breeding sites.

For the purpose of application of insecticide, Mngaza village was divided into two sections, one treated with Abate and another with Bti. The two sections of the village consisted of valleys separated from each other by a ridge on which Mngaza village is located (see Figure 3.2a). The presence of the ridge made it unlikely for the water in the two valleys to intermix. The breeding sites were visited every week during the time of larviciding. Both saw-dust and maize cobs treated with insecticide were applied by hand to the appropriate breeding sites after estimating the volume of water. On some occasions some breeding sites treated with Bti and Abate were followed up to determine the time taken before the first instars appeared and whether these developed to the final stages. However, counting the number of larvae per dip was too time consuming for the team who had to carry out larviciding.Therefore rounds in which dips were counted were alternated with rounds in which only positivity or negativity of sites for larvae was recorded.

An attempt was made to carry out some bioassay tests (W.H.O, 1975) using water from previously treated breeding sites and *Cx. quinquefasciatus* collected from soakage pits. Distilled water was used in the controls.

6.2.3 Results

6.2.3.1 Effect of Abate and Bti on larvae and pupae

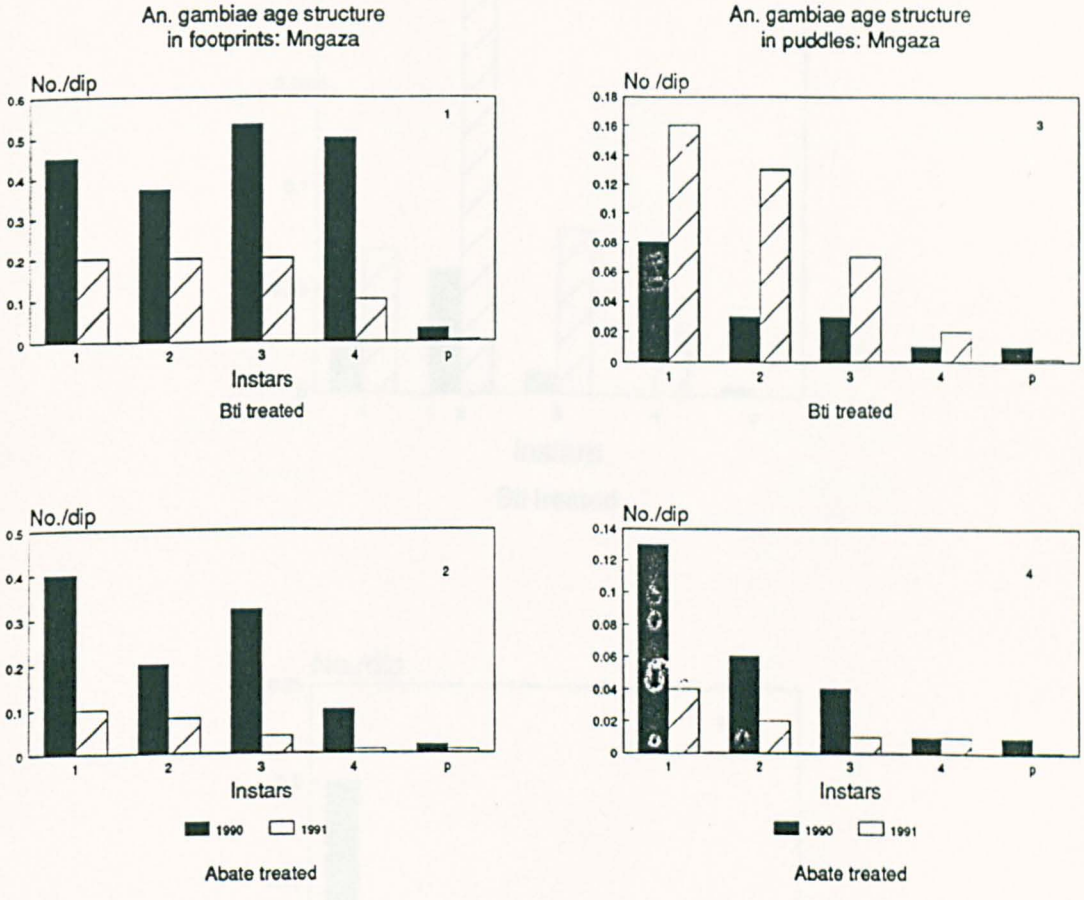
The mean number of larvae of different stages per dip before (April-December, 1990) and after (April-December, 1991) larviciding with Abate and Bti around Mngaza are shown in Figures 6.2.3.1(a and b) for *An. gambiae s.l* and culicines respectively. Corresponding data for the same two years in the control village Kumbamtoni, are shown in Figures 6.2.3.1 c and d. *An. funestus* larval numbers were rather small and after insecticide application they virtually disappeared and could not be meaningfully presented graphically. Instead they are shown in Tables 6.2.3.1a-c and Tables 6.2.3.1.d-f for Mngaza (treated) and Kumbamtoni (untreated) villages respectively.

(a) Effect on *An. gambiae s.l*

The data on *An. gambiae s.l* in Kumbamtoni, where there was no larviciding, show that there were more larvae per dip in footprints in 1990 than 1991. However, there was marked reduction in the number of larvae per dip in footprints after treatment with Abate and Bti in 1991 in Mngaza compared with the numbers in the previous year. Abate treated puddles had generally lower densites of larvae than before they were treated. In Bti treated puddles however, such a reduction was not observed in larvae, but no pupae were seen after treatment. On the other hand, in Kumbamtoni, there was an increase in the number of larvae per dip in puddles during the period of treatment in Mngaza. In pools, Bti did not seem to cause any impact against the immature stages except that no pupae were seen. In contrast, Abate had a marked effect against the immature stages in pools reducing the numbers per dip much lower than in Bti treated pools or those in Kumbamtoni. The numbers of *An. gambiae s.l* caught in ponds in Mngaza were too small for meaningful comparison. In Kumbamtoni, however, the numbers caught in ponds during the treatment year in Mngaza were larger than the ones in the pre-treatment period. In both Mngaza and Kumbamtoni, the numbers caught in streams and swamps were too small for detailed comparison, but generally in Kumbamtoni there was a higher yield of immature stages in the treatment period than in the pre-treatment period and Mngaza.

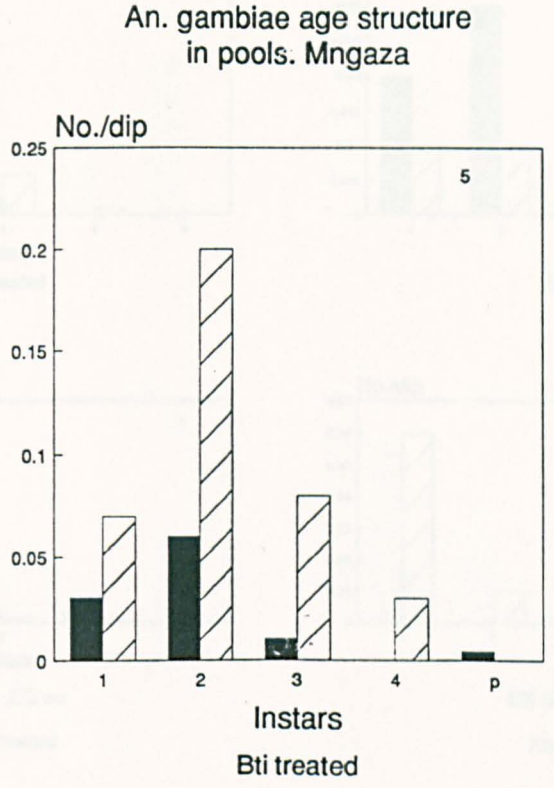
(b) Effect on culicines

Figure 6.2.3.1 a: *An. gambiae s.l.* age structure in different categories of breeding site in Mngaza before (1990) and after (1991) application of Abate and Bti



continued

Figure 6.2.3.1a. continued



continued

Figure 6.2.3.1a. continued

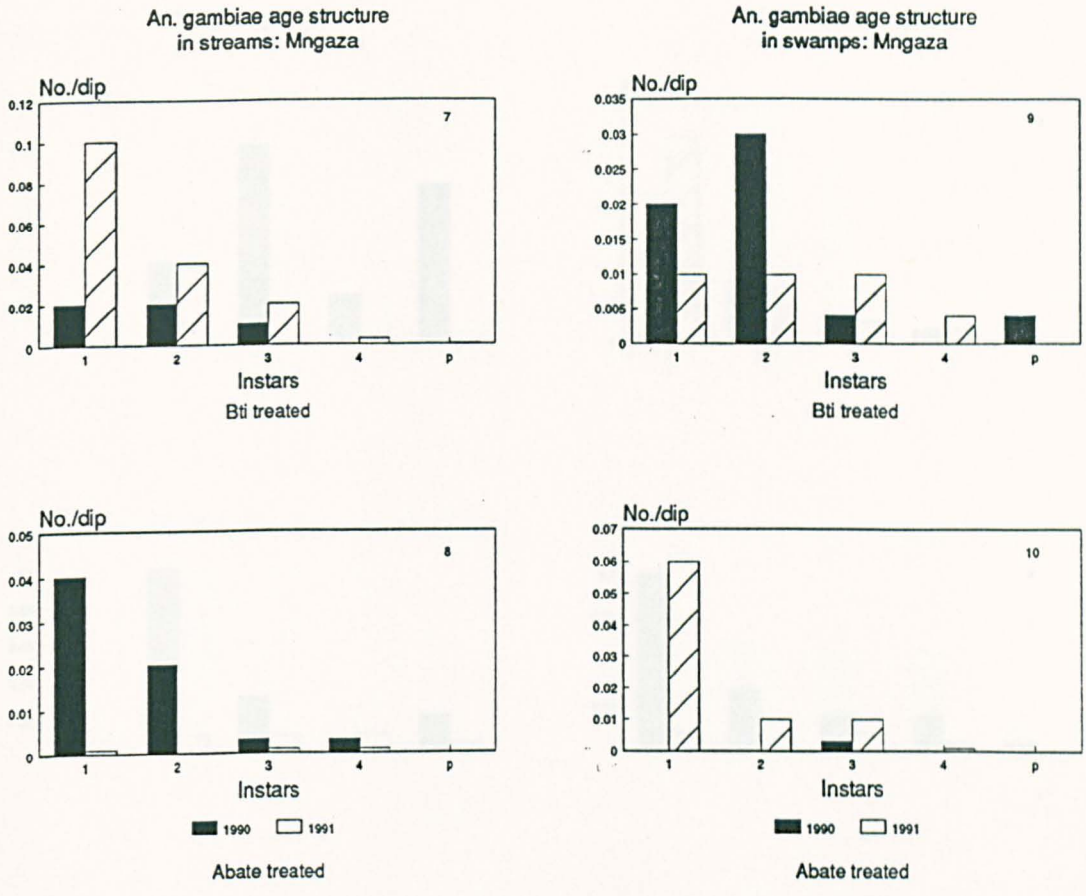
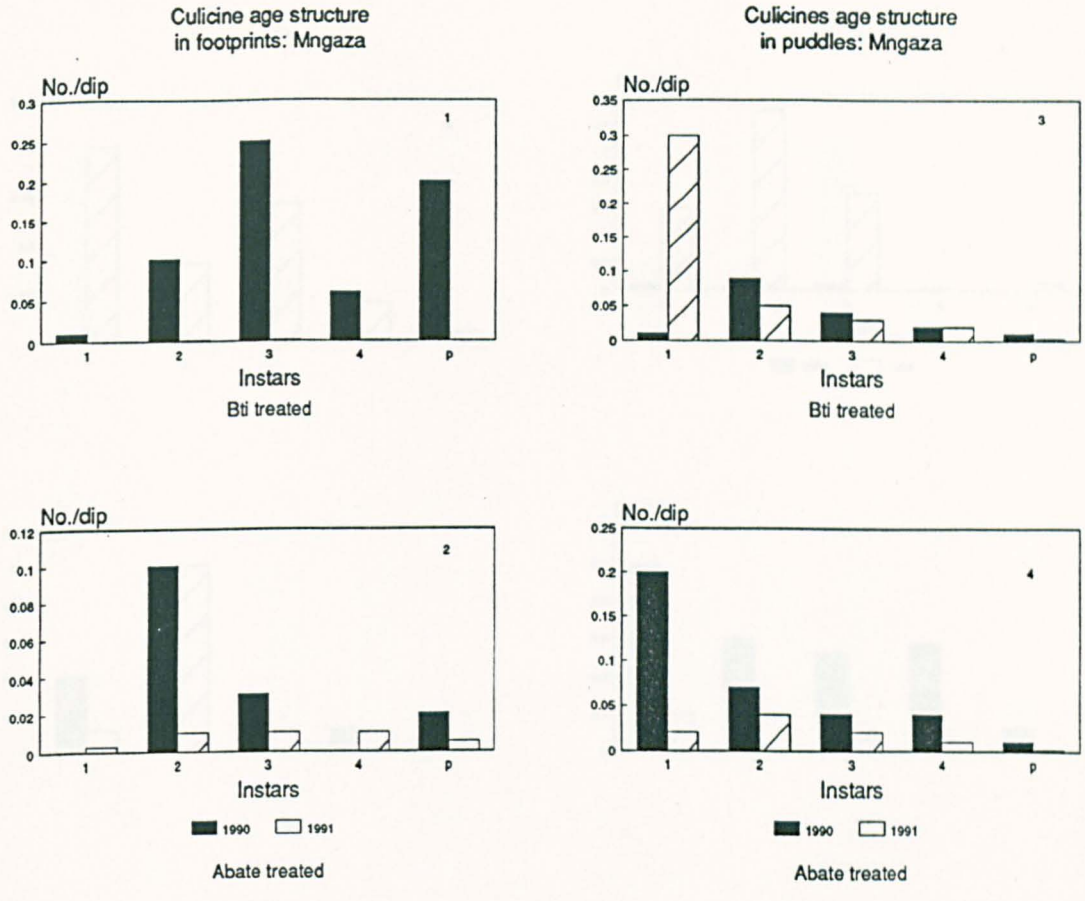
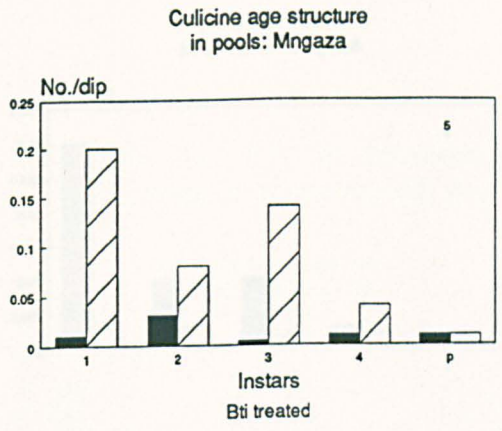


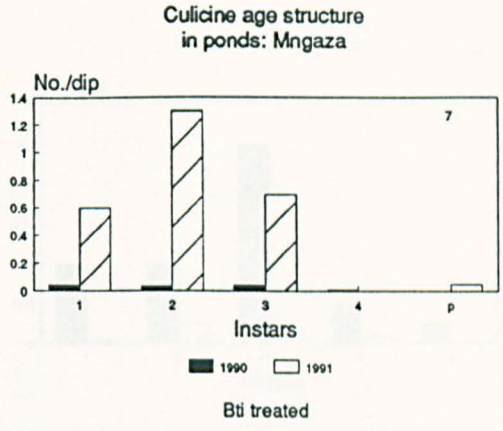
Figure 6.2.3.1b: *Culicine* age structure in different categories of breeding site in Mngaza before (1990) and after (1991) application of Abate and Bti

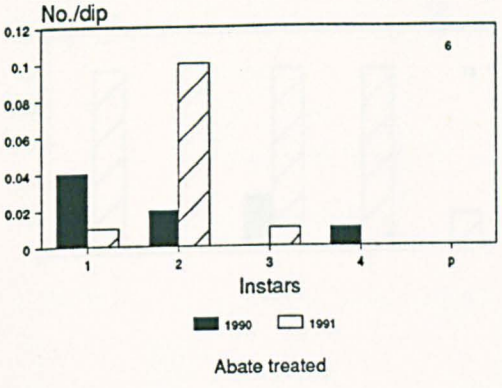


continued

Figure 6.2.3.1b. continued







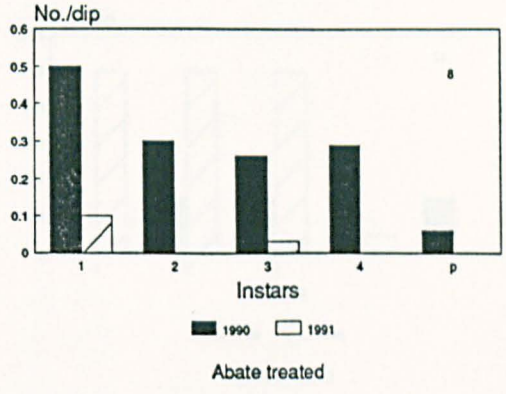


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Figure 6.2.3.1b. continued

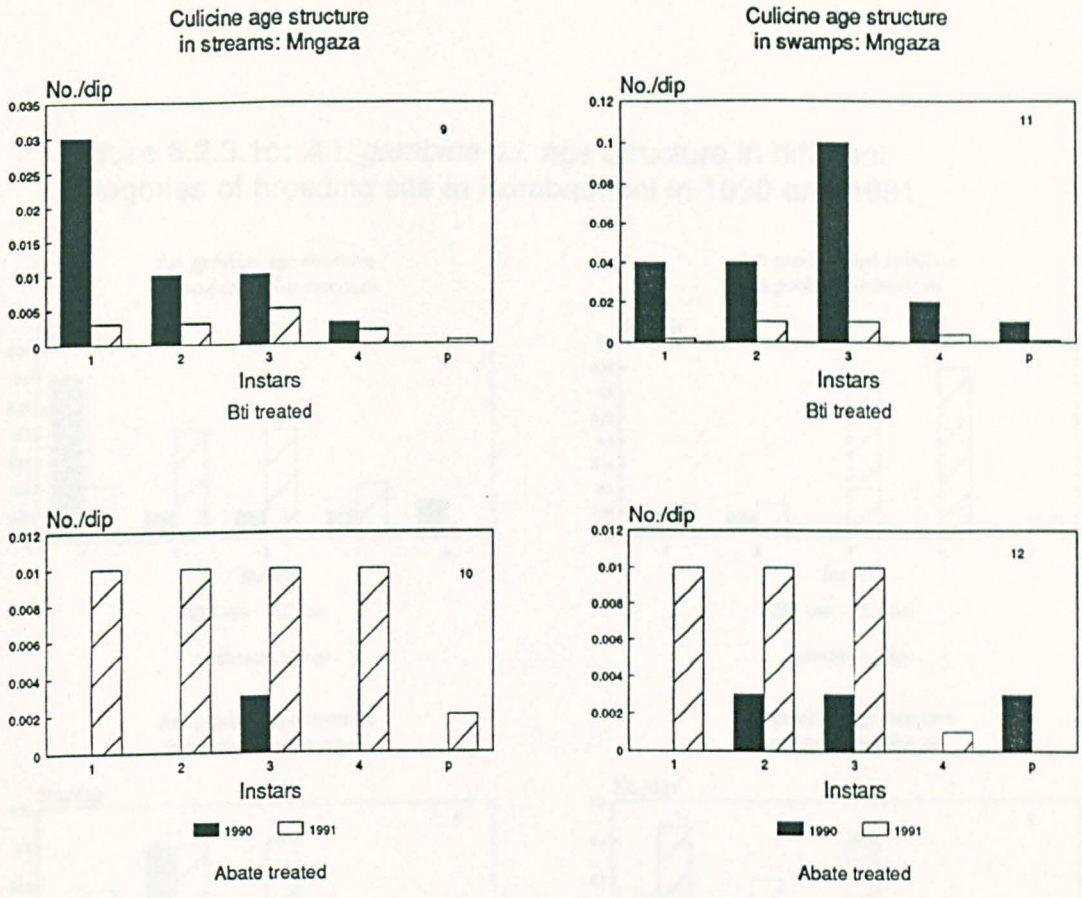
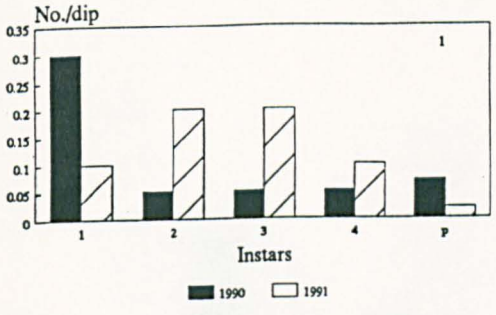


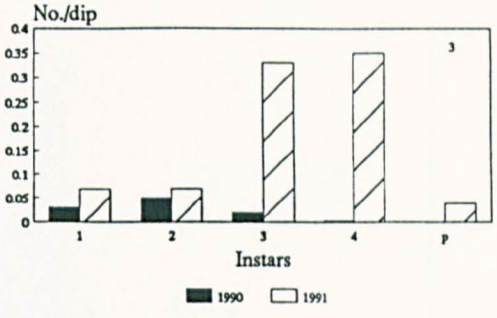
Figure 6.2.3.1c: *An. gambiae s.l.* age structure in different categories of breeding site in Kumbamtoni in 1990 and 1991

An. gambiae age structure
in footprints: Kumbamtoni



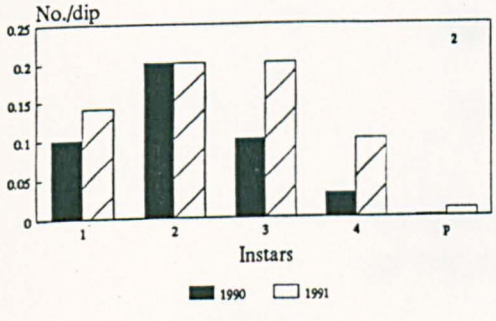
Untreated village

An. gambiae age structure
in pools: Kumbamtoni



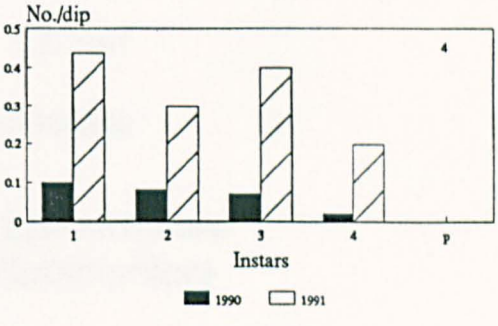
Untreated village

An. gambiae age structure
in puddles: Kumbamtoni



Untreated village

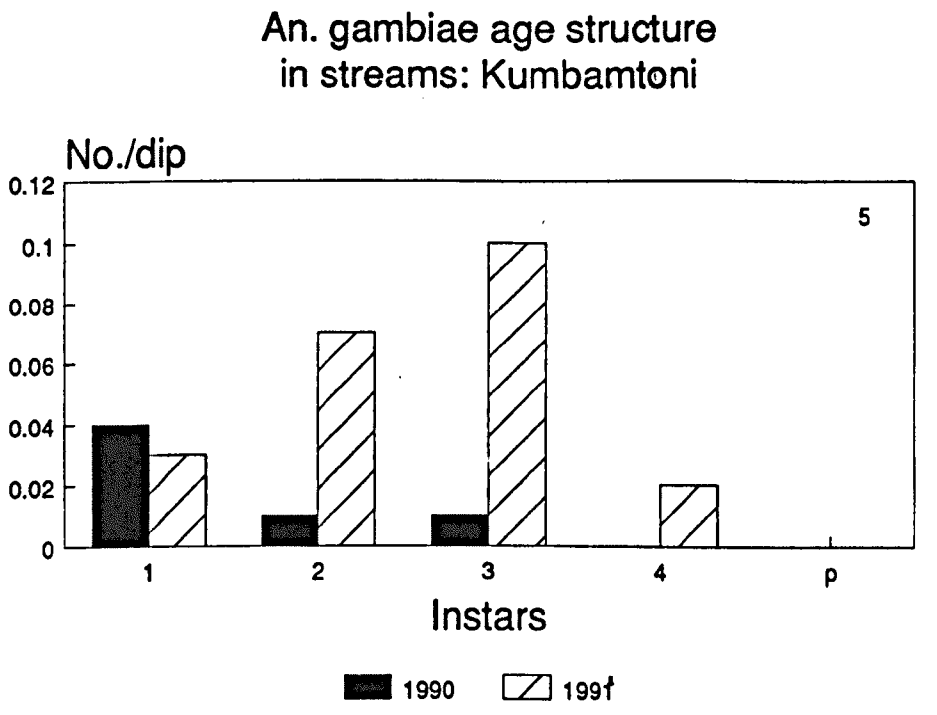
An. gambiae age structure
in ponds: Kumbamtoni



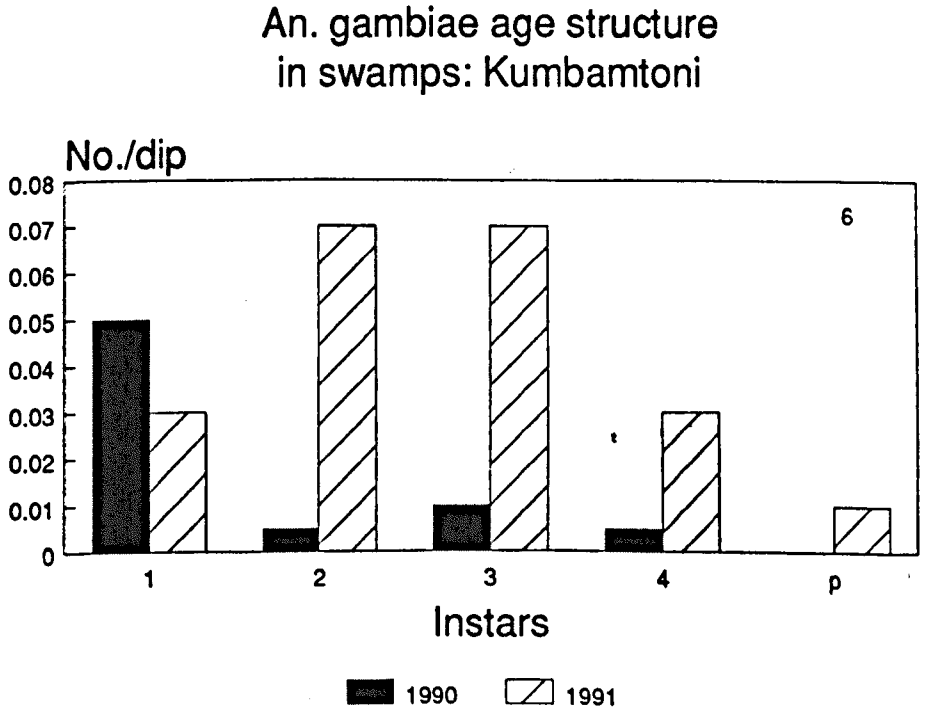
Untreated village

continued

Figure 6.2.3.1c. continued

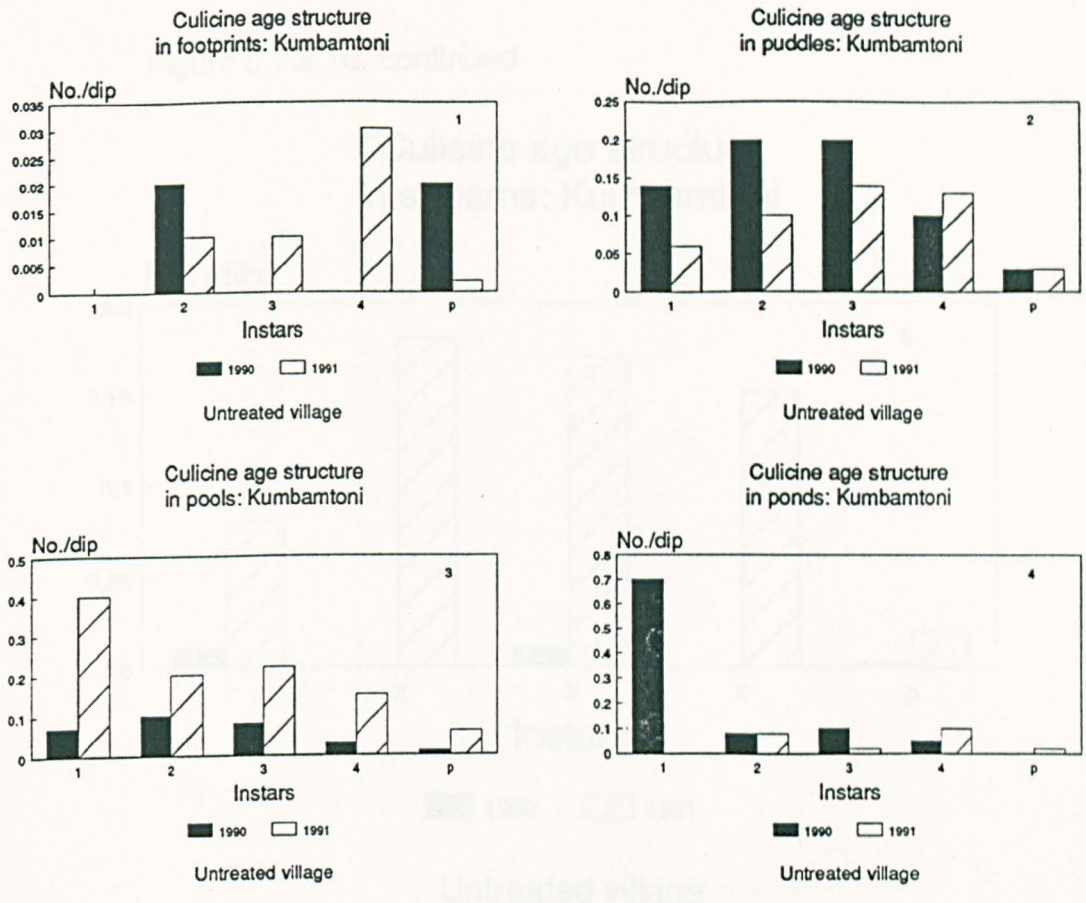


Untreated village



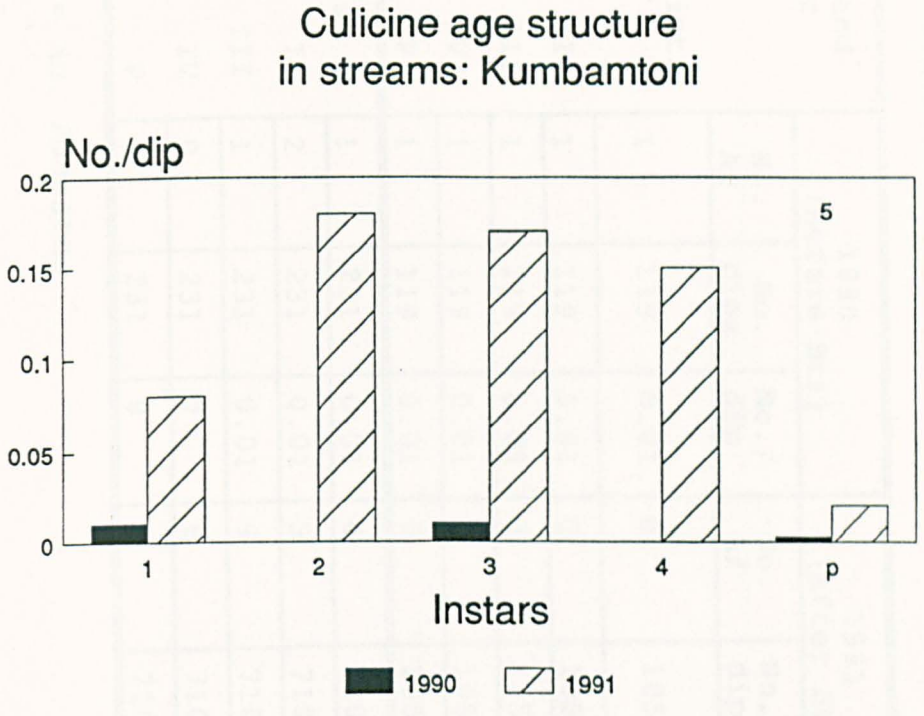
Untreated village

Figure 6.2.3.1d: *Culicine* age structure in different categories of breeding site in Kumbamtoni in 1990 and 1991

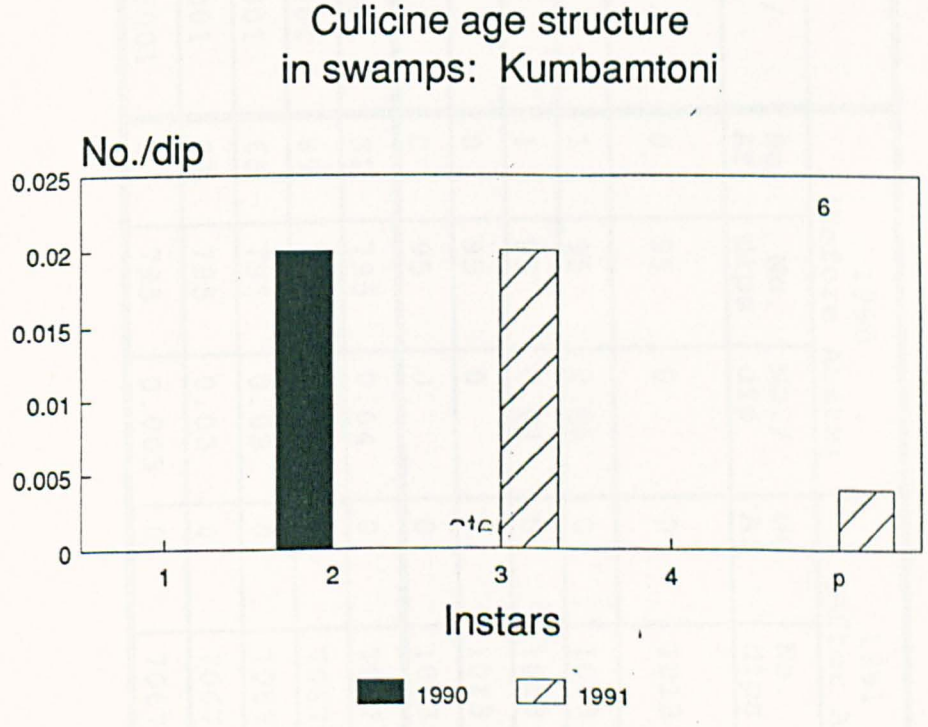


continued

Figure 6.2.3.1d. continued



Untreated village



Untreated village

Table 6.2.3.1a: Density of *An. funestus* in footprints and puddles in 1990 and 1991 in Mngaza

Site and Instar	1990 (Before Bti)			1991 (After Bti)			1990 (Before Abate)			1991 (After Abate)		
	No. Af	No. dips	No./ dip	No. Af	No. dips	No./ dip	No. Af	No. dips	No./ dip	No. Af	No. dips	No./ dip
Footprints I	1	119	0.01	0	1056	0	0	95	0	0	1013	0
II	1	119	0.01	0	1056	0	1	95	0.01	0	1013	0
AF III	1	119	0.01	0	1056	0	1	95	0.01	0	1013	0
IV	1	119	0.01	0	1056	0	0	95	0	0	1013	0
P	1	119	0.01	0	1056	0	0	95	0	0	1013	0
Puddles I	1	231	0.01	4	7107	0.001	31	795	0.04	0	7067	0
II	2	231	0.01	5	7107	0.001	89	795	0.11	0	7067	0
AF III	1	231	0.01	9	7107	0.001	65	795	0.08	0	7067	0
IV	0	231	0	6	7107	0.001	22	795	0.03	0	7067	0
P	0	231	0	1	7107	0.0001	2	795	0.003	0	7067	0

AF = *An. funestus*

Table 6.2.3.1b: Density of *An. funestus* in pools and ponds in 1990 and 1991 in Mngaza

Site and Instar		1990 (Before Bti)			1991 (After Bti)			1990 (Before Abate)			1991 (After Abate)		
		No. Af	No. dips	No./ dip	No. Af	No. dips	No./ dip	No. Af	No. dips	No./ dip	No. Af	No. dips	No./ dip
Pools	I	0	260	0	0	160	0	11	225	0.05	0	101	0
	II	1	260	0.004	0	160	0	12	225	0.05	0	101	0
AF	III	3	260	0.01	0	160	0	13	225	0.06	0	101	0
	IV	2	260	0.01	0	160	0	5	225	0.02	0	101	0
	P	0	260	0	0	160	0	0	225	0	0	101	0
PONDS	I	1	110	0.01	0	20	0	0	160	0	0	71	0
	II	1	110	0.01	0	20	0	1	160	0.01	0	71	0
AF	III	1	110	0.01	1	20	0.05	8	160	0.05	0	71	0
	IV	3	110	0.03	0	20	0	0	160	0	0	71	0
	P	0	110	0	0	20	0	0	160	0	0	71	0

AF = *An. funestus*

Table 6.2.3.1c: Density of *An. funestus* in swamps and streams in 1990 and 1991 in Mngaza

Site and Instar		1990 (Before Bti)			1991 (After Bti)			1990 (Before Abate)			1991 (After Abate)		
		No. Af	No. dips	No./ dip	No. Af	No. dips	No./ dip	No. Af	No. dips	No./ dip	No. Af	No. dips	No./ dip
Streams	I	11	605	0.02	3	5316	0.0005	3	325	0.009	0	1478	0
	II	25	605	0.04	10	5316	0.002	6	325	0.02	0	1478	0
AF	III	14	605	0.02	6	5316	0.001	5	325	0.02	0	1478	0
	IV	10	605	0.02	0	5316	0	4	325	0.01	0	1478	0
	P	1	605	0.001	0	5316	0	0	325	0	0	1478	0
Swamps	I	1	270	0.004	0	1696	0	2	310	0.01	0	904	0
	II	0	270	0	0	1696	0	10	310	0.03	0	904	0
AF	III	3	270	0.01	4	1696	0.002	9	310	0.03	0	904	0
	IV	0	270	0	0	1696	0	1	310	0.003	0	904	0
	P	0	270	0	0	1696	0	0	310	0	0	904	0

AF = *An. funestus*

Table 6.2.3.1d: Density of *An. funestus* in footprints and puddles n 1990 and 1991 in **Kumbamtoni** (untreated village)

Site and Instar		1990			1991		
		No. Af	No. dips	No./ dip	No. Af	No. dips	No./ dip
Footprints	I	1	60	0.02	0	440	0
	II	1	60	0.02	0	440	0
Af	III	0	60	0	2	440	0.004
	IV	0	60	0	1	440	0.002
	P	0	60	0	0	440	0
Puddles	I	148	858	0.2	4	4096	0.001
	II	142	858	0.2	31	4096	0.01
Af	III	83	858	0.1	48	4096	0.01
	IV	29	858	0.03	22	4096	0.01
	P	0	858	0	3	4096	0.001

Af = *An. funestus*

Table 6.2.3.1e: Density of *An. funestus* in pools and ponds in 1990 and 1991 in **Kumbamtoni** (untreated village)

Site and Instar		1990			1991		
		No. Af	No. dips	No./ dip	No. Af	No. dips	No./ dip
Pools	I	5	335	0.01	0	260	0
	II	14	335	0.04	1	260	0.004
Af	III	12	335	0.04	9	260	0.03
	IV	2	335	0.01	2	260	0.01
	P	0	335	0	2	260	0.01
PONDS	I	0	335	0	0	85	0
	II	9	335	0.03	0	85	0
Af	III	4	335	0.01	1	85	0.01
	IV	9	335	0.03	0	85	0
	P	0	335	0	0	85	0

Af = *An. funestus*

Table 6.2.3.1f: Density of *An. funestus* in swamps and stream in 1990 and 1991 in **Kumbamtoni** (untreated village)

Site and Instar		1990			1991		
		No. Af	No. dips	No./ dip	No. Af	No. dips	No./ dip
Streams	I	5	325	0.02	7	1018	0.01
	II	18	325	0.06	9	1018	0.01
Af	III	18	325	0.06	16	1018	0.01
	IV	4	325	0.01	7	1018	0.01
	P	0	325	0	0	1018	0
Swamps	I	7	190	0.04	0	240	0
	II	13	190	0.07	0	240	0
Af	III	4	190	0.02	5	240	0.02
	IV	2	190	0.01	3	240	0.01
	P	2	190	0.01	0	240	0

Af = *An. funestus*

The number of immature stages of culicines per dip in the footprints was generally less after Bti and Abate application than before, although the effect of Abate against culicines was less marked than that of Bti (Figure 6.2.3.1b1 and 2). In Kumbamtoni the numbers caught in footprints were too small for comparison purposes. In puddles and pools there was virtual disappearance of the pupae but the other stages were little affected in the treated sites (Figure 6.2.3.1b3 and 4). In ponds Abate reduced the numbers of all stages considerably but Bti did not do so. Although the numbers caught in the streams and swamps were very small the data suggest that, in contrast to other types of site, there was a decline in the density of larvae after the streams and swamps were treated with Bti but not with Abate (Figure 6.2.3.1b: 9-12). In Kumbamtoni there was an increase in the numbers of the final stages caught in the treatment period in Mngaza (Figure 6.2.3.1d)

(c) Effect on the final aquatic stages in terms of percent reduction

Further comparison was made using only the final aquatic stages (4th and pupae) of *An. gambiae s.l* and culicines in terms of percent reduction in the number of final stages per dip in Mngaza, using the formula of Mulla *et al* (1971) which attempts to correct for year to year variations by use of the control village as an indicator of what would have been expected in the experimental village if treatment had not been applied:-

(%) reduction = 100 - (K1/M1 x M2/K2) x 100

Where K1 = no. final stages per dip in Kumbamtoni village (untreated) in 1990.

K2 = no. final stages per dip in Kumbamtoni village (untreated) in 1991.

M1 = no. final stages per dip in Mngaza village before treatment.

M2 = no. final stages per dip in Mngaza village after treatment.

The results are shown in Table 6.2.3.1g. In the sites where the numbers of final stages were sufficient for this type of comparison Abate caused between 80% (ponds)

Table 6.2.3.1g: Percent reduction in the density of *An. gambiae s.l* and culicine final immature stages after larviciding with Bti and Abate.

Mosquito species	Type of breeding site									
	Fp		Pd		Pl		Pn		Sw	
	Bt	Ab	Bt	Ab	Bt	Ab	Bt	Ab	Bt	Ab
Ag	81	83	68	84	96	99	-	80	88	99
Cx	99.8	53	38	81	52	81	-108	58	-	-

Fp, Pd, Pl, Pn, and Sw = Footprints, Puddles, Pools, Ponds and Swamps respectively.

Bt, Ab = Bti or Abate treated sites

Ag, Cx = *An. gambiae s.l* or culicines

- = Numbers too small for reliable estimation

and 99% (pools and swamps) reduction of *An. gambiae s.l.* There was 83% and 84% reduction in the density of *An. gambiae s.l* in Abate treated footprints and puddles respectively. Also, 68% and 81% reduction in the density of *An. gambiae s.l* in footprints and puddles were achieved . With Bti, there was well over 80% reduction in density of *An. gambiae s.l* in ponds and swamps. Abate did better in puddles (81%) and pools (81%) while Bti did better only in footprints with a control factor of 96%. The results obtained after year to year variations were put into account using the formula of Mulla *et al.* (1971) appear to give a more realistic estimate than direct comparison of density of larvae before and after application of insecticide. For example, before year to year changes in the density of *An. gambiae s.l* Bti appeared not to have had marked effect on the larvae. But after such corrections were made it was found that Bti, caused a reduction factor of 96% in pools.

An overall estimate was made of the percent reduction of the final stages of *An. gambiae s.l* per square meter (discussed in section 6.1) for all sites combined using the same formula as above:

(%) reduction = 100 - (3.4/6.8 x 1.7/6.5 x 100)

= 100 - 13

= 87

It was concluded that there was an overall control factor of about 87%. The implication of the 87% reduction factor for the final stages of *An. gambiae s.l* with respect to adult mosquito density in Mngaza is discussed in section 6.2.4.

6.2.3.2 Effect of Bti and Abate on predators of mosquitoes

As mentioned in the literature review (Chapter 2) Bti is considered to be specific against mosquito larvae and is not harmful to other aquatic invertebrates which predate on the mosquito larvae. This contrasts with Abate which is considered to be a broad spectrum insecticide. Nevertheless, Bti and Abate had not been extensively applied against *An. gambiae s.l* to see if such predator populations really were differentially affected in the present study. For comparative purposes, the combined presence or

absence of Notonectids, dragon fly larvae and *Culex tigripes* as one group, were used rather than the numbers per dip, the distributions of which were highly skewed.

The results of the effect of Bti and Abate on predators are shown in Table 6.2.3.2. Bti had no significant effect on the presence of predators (Odds ratio = 0.87, df= 1, $P > 0.63$, while Abate had a significant effect on predators (Odds ratio = 0.04, df= 1, $P < 0.001$).

Another study on the effect of Abate and Bti on Notonectid and dragon fly larvae was carried out in Muheza Town in a large puddle near a construction site. This was the site where Ragoonanansingh *et al.* (1992) compared the effect of *B. sphaericus* and Abate on Notonectids (*Anisops* and /or *Enthares* spp) density (see Appendix 6.2.3.2). The water in this puddle was permanent. As a result it was found to have high densities of Notonectid and dragon fly larvae. Owing to its proximity to Ubwari Field Station it was chosen for this study so that daily follow-up could be feasible for prolonged period to assess the effect of the two types of insecticide on Notonecta and dragonfly larvae.

In the first two days the puddle was monitored for the density (no./dip) of early stages (stage I-III), late stages (IV and pupae), Notonectids and Dragonfly larvae. Then, on day 0, the breeding site was treated with a standard dose of Bti. The density of larvae, Notonectids and dragonfly larvae continued to be monitored on daily a basis. On day 5 the breeding site was retreated with Bti with a dose six times higher than the standard dose, after the numbers of early stage larvae were found to have increased (Table 6.2.3.2a). Retreatment with insecticide was done after dipping had been carried out on that day. Similar retreatment was done on day 8 and monitoring continued as described above.

On day 11, the breeding site was treated with a standard dose of Abate (i.e. 1 ppm), and monitoring for mosquito and dragonfly larvae and Notonectids continued to day 31 (Table 6.2.3.2a).

Table 6.2.3.2: The effect of larvicides on predators
- number of site visits when predators were found to be present or absent

(a) Bti

Group	Predators			Odds Ratio	95% CL	p-value χ^2
	present	absent	total			
Before	26	83	109	0.87	0.53 & 1.43	0.63 NS
After	212	781	993			
Total	238	864	1102			

(b) Abate

	Predators			Odds Ratio	95% CL	p-value χ^2
	present	absent	total			
Before	48	110	158	0.04	0.02 & 0.08	< 0.001
After	12	723	735			
Total	60	833	893			

Table 6.2.3.2a: The effect of Bti and Abate on *An. gambiae s.l*, culicines, dragonfly immature stages, and on notonectids (No./50dips)

Treatment Day		Ag		Cx		Noto nectids	Dragon flies
		I-III	IV & P	I-III	IV & P		
Before treatment	-2	202	29	50	5	28	3
	-1	258	37	20	6	51	6
After standard Bti on day	0						
	1	1	1	0	0	10	1
	2	54	0	0	1	28	0
	3	43	0	5	0	30	2
	4	42	0	8	0	9	0
	5	30	7	1	4	6	3
After 6x standard Bti dose on day 5							
	6	4	0	2	1	16	1
	7	32	0	19	1	5	2
	8	38	0	2	4	12	2
After 6x standard dose Bti on day 8							
	9	0	0	1	5	23	0
	10	6	0	4	4	16	1
	11	17	0	17	1	13	1

Table 6.2.3.2a: The effect of Bti and Abate on *An. gambiae s.l*, culicines, dragonfly immature stages, and on notonectids (No./50dips): **continued**

Treatment day	Ag		Cx		Noto nectids	Dragon flies
	I-III	IV & P	I-III	IV & P		
After standard **Abate** treatment on day 11						
12	0	0	0	0	1	0
13	1	0	0	0	0	0
14	0	0	0	0	0	0
15	1	0	0	0	0	0
16	2	0	0	0	0	0
17	15	0	1	0	6	0
18	22	0	5	0	1	0
19	30	0	3	0	3	0
20	0	0	0	0	2	0

Table 6.2.3.2a: The effect of Bti and Abate on *An. gambiae s.l*, culicines, dragonfly immature stages, and on notonectids (No./50dips): **(Abate continued)**

Treatment day After standard **Abate** treatment on day 11 **(continued)**	Ag		Cx		Noto nectids	Dragon flies
	I-III	IV & P	I-III	IV & P		
21	22	0	1	0	8	0
22	3	0	0	0	4	0
23	23	0	0	0	7	0
24	13	0	12	0	6	0
25	10	0	4	0	1	0
26	13	0	5	1	2	0
27	20	0	4	0	6	0
28	17	0	12	0	7	0
29	39	0	2	0	9	0
30	56	1	9	0	1	2
31	1	0	1	0	2	0

The results of this study shows that both Bti and Abate reduced the numbers of both early and the final stages of *An. gambiae s.l* and culicines, the effect being more marked after Abate application. When the site was treated with Bti, small numbers of anopheline and culicine 4th instar larvae and pupae continued to appear in successive days after treatment. This is contrary to what is reported below (section 6.2.3.3.b), that *An. gambiae s.l* 4th instar larvae and pupae were not found in four small puddles in a 10 day follow-up period after a single treatment with Bti. This can be attributed to the relatively large size of the puddle treated with Bti and Abate in the present study. Bti was been shown to be more effective in footprints and probably in smaller than in larger puddles (see section 6.2.3.1). However, when the site was treated with Abate no final stage appeared until 19 days later. This shows that Abate persisted longer in such breeding sites than Bti at a concentration which is sufficient to control the appearance of the final stages. This partly explains why Abate caused more marked reduction in the density of the final stages of *An. gambiae s.l*, in small as well as large puddles, than Bti (see section 6.2.3.1).

Treatment of the breeding site with Bti did not appear to cause marked reduction in the number of Notonectid and dragonfly larvae even at higher concentrations. This observation is similar to that of Balaraman *et al.* (1983) that at 100 times the dose required to kill *Cx. quinquefasciatus*, *B. thuringiensis* did not adversely affect Notonectids, crustaceans or larvivorous fish. However, a single treatment with a standard dose of Abate caused the disappearance of Notonectids and dragonfly larvae for 19 days in the present study. Similar observation was reported by Ragoonanansingh *et al.* (1992) with another type of bacterial insecticide, *B. sphaericus*, in which Abate unlike *B. sphaericus*, caused marked reduction in the populations of Notonectids (see Appendix 6.2.3.2.). Although the importance of Notonectids and dragon fly larvae has not been quantitatively established, their predatory activity on mosquito larvae may contribute significantly to the reduction of mosquito larval densities in addition to the effect of larvicides. By selectively larviciding in the breeding sites that are found to contain larvae, mortality in the Notonectid and dragon fly larvae would be minimized.

6.2.3.3 Residual period of Bti and Abate in the water

(a) Bti residual period in the breeding site

The residual effect of Bti and Abate in the breeding sites was assessed differently . Since Bti treated sites were found to be positive with stage I-III, a day or two after being treated with higher concentrations, it was suspected that the insecticide that remained in the breeding sites after some water had flowed away, was not sufficient to kill all existing larvae. Therefore the residual period of Bti was assessed after applying twice the concentration that was routinely used in larviciding (i.e. 1 ppm) in four breeding sites and following them up every day to see when first instars of *An. gambiae s.l* appeared and where possible to determine whether the larvae developed into the final stages.

In the three stagnant puddles, the average number of days before the first instars appeared after treatment with twice the routinely used concentration of Bti was found to be 2.3 days (Table 6.2.3.3b). One puddle with flowing water was followed up and the first instars of *An. gambiae s.l* appeared 2 days after treatment. In none of these puddles did larvae develop into the final stages during the time of follow up which lasted up to eight days since the first instars appeared. It appears that in smaller breeding sites Bti controlled effectively the final stages for a long period than in medium sized puddles as reported above in section 6.2.3.2.

(b) Assessment of Abate residual period in the breeding site by bioassay.

Bioassay tests were carried out to assess the residual period of Abate and associated percent mortality in the immature stages of mosquitoes during that period. Water was collected from treated breeding sites in the study villages. Third stage larvae of *Culex quinquefasciatus* were used since they are considered to be more tolerant to organophosphate insecticide than *An. gambiae s.l.*

The results of regular bioassay of samples of water from Abate treated sites are

Table 6.2.3.3b: Bti residual period in four sample puddles and reappearence of *An. gambiae s.l* after treatment (no./13 dips)

		Puddle (water flow Y/N)			
		1(N)	2(N)	3(N)	4(Y)
Before treat.	I - III	3	39	40	32
	IV & P	2	7	9	1
Days After treatment 1	I - III	0	7	0	6
	IV & P	0	0	0	0
2	I - III	0	0	0	0
	IV & P	0	0	0	0
3	I - III	0	3	0	0
	IV & P	0	0	0	0
4	I - III	17	1	11	6
	IV & P	0	0	0	0
5	I - III	4	6	12	8
	IV & P	0	0	0	0
6	I - III	11	4	10	16
	IV & P	0	0	0	0
7	I - III	1	7	5	8
	IV & P	0	0	0	0
8	I - III	0	0	1	5
	IV & P	0	0	0	0
9	I - III	3	2	0	4
	IV & P	0	0	0	0
10	I - III	0	1	0	5
	IV & P	0	0	0	0

shown in Table 6.2.3.3c including correction for % mortality in controls using Abbott's formula (W.H.O, 1975):

$$\text{Corrected \% mortality} = \frac{\text{\% test mortality - \% control mortality}}{\text{100 - \% control mortality}}$$

The results of bioassay tests show that in stagnant water up to 94% of *Cx. quinquefasciatus* larvae were killed after one week and 50% after two weeks since the last treatment of the site with 1ppm of Abate. But after that the numbers killed were much less. On the other hand, in sites with flowing water, 70% was the highest mortality achieved in 10 days, declining to less than 50% in three weeks. From these results it can be concluded as long as the interval between two cycles of larviciding did not exceed one week, the insecticide in the breeding sites would be sufficient to kill most of the larvae and pupae.

6.2.4 Discussion

Larviciding with Abate and Bti caused an overall reduction of 87% in the density of the final stages of *An. gambiae s.l* per square meter in Mngaza. This level of decrease in the number of larvae was achieved without drainage works which, to some extent, could have contributed to further reduction in larval numbers by reducing the area of some of the breeding sites.

Although Bti applied at least once a week was effective against the final stages of *An. gambiae s.l* in most breeding sites, it did not perform well in the puddles. A possible explanation for this could be that the puddles were often in stream beds and even in dry weather there was some flow into and out of them so reducing the concentration of the insecticide. On the hand, interchange of water between footprints was likely to have left sufficient insecticide in them to kill the larvae. Further- more, any loss of insecticide from footprints may have been compensated for by overestimation of the size and number of pinches of saw dust that should be thrown

Table 6.2.3.3(c): Corrected percent mortality of *Cx. quinquefaciatus* from bioassay done using water treated with Abate during larviciding.

Puddle	Water flow	Last treated	Interval (days)	Mortality (%)	95% CL of mortality (%)
1	Yes	14.06.91	17	33.3	20 - 40
	Yes	14.06.91	23	0	-
	No	05.09.91	20	95	88 - 102
2	Yes	14.06.91	11	70.8	61.4-80.2
	Yes	14.06.91	19	50.1	39.9-60.3
	Yes	14.06.91	23	5.8	1.1 - 10.5
3	Yes	14.06.91	11	66.7	56.9-76.5
	Yes	14.06.91	19	29.7	20.5-38.9
	Yes	14.06.91	23	18.9	10.9-26.9
4	No	14.06.91	11	94.4	89.7-99.1
	No	14.06.91	17	69.7	59.7-79.7
	No	14.06.91	23	43.2	33.0-53.4
5	No	28.05.91	28	100	93 - 107
	No	03.05.91	41	100	89.8-110.2
6	No	14.06.91	11	83.3	75.5-90.9
	No	14.06.91	17	42.4	31.8-53.0
	No	14.06.91	23	27	18 - 36

into the footprints. The water in pools and ponds was more stable than in puddles and the concentration of insecticide established by throwing in impregnated corn cob slices presumably did not change much and hence higher levels of control could be achieved. Good control of *An. gambiae s.l* by Abate in most breeding sites suggests that the species is susceptible to the insecticide and despite low rates of water flow into and out of some sites sufficient insecticide is left for killing the immature stages for about a week after treatment.

Bti had little or no effect in controlling culicines in most breeding sites except in footprints probably for the same reason given for *An. gambiae s.l* above, and in streams and swamps where the densities were much lower. This is contrary to the observation of Sharma *et al.* (1983), Kramer (1984) and Balaraman *et al.* (1983) that the mortality of both anophelines and culicines from Bti was similar. The observed difference on the effect of Bti on anophelines and culicines can probably be attributed to the difference in the levels of organic pollution which, as described in chapter 4, tended to be associated more with culicines than anophelines. Organic pollution has been found to reduce the efficacy of Bti (Mulla, *et al.* 1990).

The effect of Abate against culicines differed from that of Bti in that it gave adequate control in puddles, pools and ponds with reduction factors of 81%, 81%, and 58% (Table 6.2.3.1g). The final stages of culicines were found to be significantly associated with pools and pond (see chapter 4). However, the poor performance of Abate in footprints streams and swamps, may be attributed to resistance in *Cx. quinquefasciatus* to organophosphate insecticides which has been reported by Curtis and Pasteur (1981) from Tanga (40km from the site of the present work). R. Wood (unpublished) has also found a high level of organophosphate resistance in *Cx. quinquefasciatus* from Muheza, about 10km from Mngaza. In large breeding sites such as some puddles, pools and ponds it is possible that the insecticide accumulated so causing much higher mortality even in partially resistant populations.

The observed killing of predators by Abate at a standard mosquito larvicidal concentration is a matter of concern since it would interfere with the natural balance between the predators and the mosquito immature stages. It may be desirable to avoid

using Abate and replace it with Bti in footprints where the microbial agent performed well. This may help to maintain some predator populations in the area. This might avoid an "explosion" in numbers of immature stages of mosquitoes in the event of interruption of control programme totally dependent on Abate.

Bti treated sites had only a day or two of total absence of immature stages. This is in agreement to the observation of Sharma *et al.* (1983) and Balaraman *et al.* (1983). Karch *et al.* (1992), achieved a 98% reduction in the density of *An. gambiae s.l* in suburban Kinshasa within 48 hours with *B. sphaericus*. However, frequent applications of insecticide at intervals of 15 days were required for to achieve this level of control. Unfortunately, the dramatic reduction in the density of immature stages of *An. gambiae s.l* was accompanied by only a 13.6% reduction in adult densities. Although no explanation was given for the disappointing results on adult control it appears that a 15 days interval between successive applications of insecticide was long enough for larvae to develop to the adult stage. Most probably immigration of adult mosquioes might have contributed to a disappointingly low level of reduction in adult densities.

In the present study, it was encouraging to note that during the 10 day follow-up period no fourth instar larvae or pupae were formed. This suggests that retreatment of the sites every four days at higher concentration than at the dose used in this project could probably prevent the production of the final immature stages of mosquitoes.

The results of the tasting trial with Abate treated implied that, where the water was stagnant, caution was necessary not to treat the sites continuously to avoid excessive accumulation of insecticide. In view of the observation that the level of control was as high as 94% one week after the previous application of insecticide it was considered this was an appropriate time interval for the treatment cycle for the whole area.

CHAPTER 7:

EFFECT OF LARVICIDING WITH ABATE AND BTI ON ADULT *AN. GAMBIAE S.L*

7.1 INTRODUCTION

Assessing larviciding for its effect on mosquito larval densities is only the first stage of evaluation. A more important criterion for a successful larval control programme is the associated reduction in the number of adult mosquitoes in the treated area. Various larvicidal agents have been used by different workers against immature stages of different culicine species of mosquitoes resulting in dramatic decline in the adult mosquito densities (Curtis, *et al.* 1990). However, the effect of larviciding on the density of adult *An. gambiae s.l* has seldom been measured. As mentioned in chapter 2, the most significant attempt at controlling *An. gambiae s.l* by antilarval measures is probably that of Watson (1953) in the Zambian copper-belt. In this mining community massive drainage and larviciding operations were carried out, resulting in significant reduction in the density of *An. gambiae s.l* as well as *An. funestus* This led to a decline in malaria cases.

In many rural communities of Tanzania such costly operations could not possibly be to carried out. What is required is a simple method of larviciding, as described in chapter 6, which could be adopted by the community if proven to be effective against adult mosquitoes. In this chapter the results of the larviciding work, described in chapter 6, on adult *An. gambiae s.l* will be presented.

7.2 MATERIALS AND METHODS

7.2.1 Adult mosquito sampling

Adult mosquitoes were sampled in the three study villages of Mngaza, Kumbamtoni and Mlingano described in chapter 3. These villages were visited fortnightly for that purpose. Three houses had been selected for use as catching stations in each village for a 3½ year trial of impregnated bednets (Magesa *et al.*, 1991). A light trap operated by a rechargeable six volt battery was hung beside an untreated bednet that was in regular use by members of each house. In this way catches are obtained which are proportional to human biting catches (Lines *et al.*, 1991). For the bednet trial each village had light traps installed in 3 houses once a fortnight. This system was continued for the present project.

The morning after a light trap had been operated the mosquitoes were removed from the light traps into paper cups where they were provided with a sugar solution to keep them alive until they were identified and counted at Ubwari Field Station laboratory. A small proportion of fed or unfed female mosquitoes were dissected and scored for parity by the method of Detinova (1962)

7.3 RESULTS AND DISCUSSION

7.3.1 Estimation of the effect of larvicides on the density adult *An. gambiae s.l*

Figures 7.1(a,b & c) show the quarterly mean number of female *An. gambiae s.l* caught (no./light trap) in Mngaza Mlingano and Kumbamtoni from 1986 to 1991 reflecting large seasonal fluctuations in both villages and the mass killing effect of introduction of impregnated bednets into these villages in 1987 (Mngaza and Mlingano) and 1989 (Kumbamtoni) respectively. The suppression of the adult population was better maintained in Mngaza (where net re-impregnation was carefully continued at six monthly intervals) than in Mlingano (where it was carried on rather

Fig. 7.1a: An. gambiae s.l quarterly mean (no./light trap) catches: Mngaza

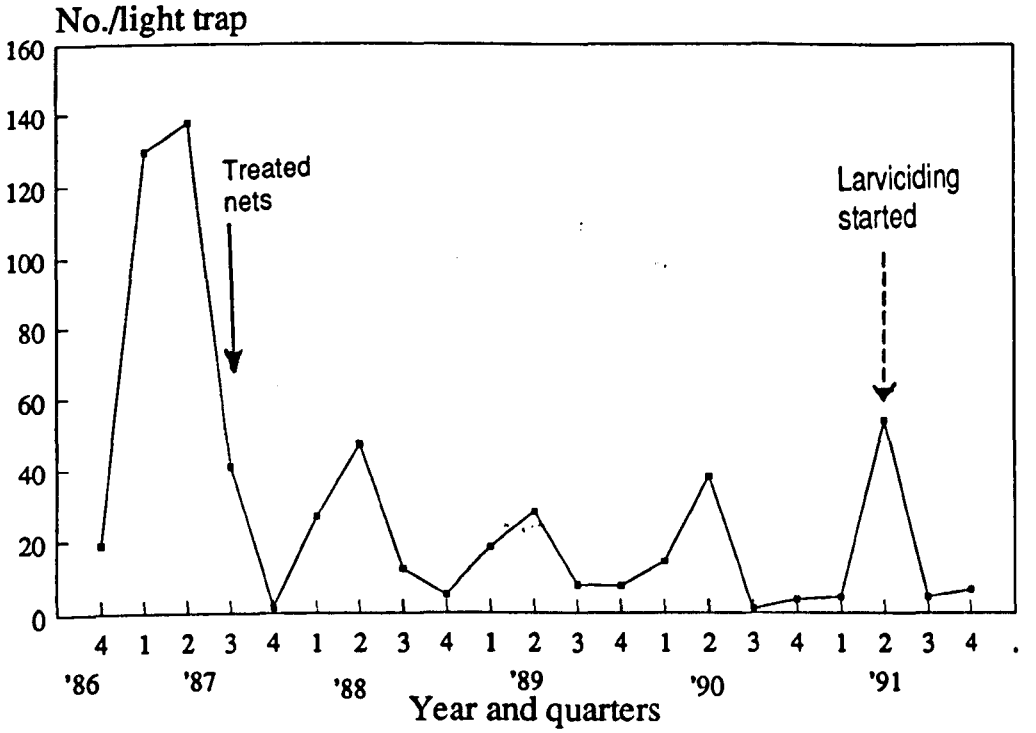


Fig. 7.1b: An. gambiae s.l quarterly mean (no./light trap) catches: Mlingano

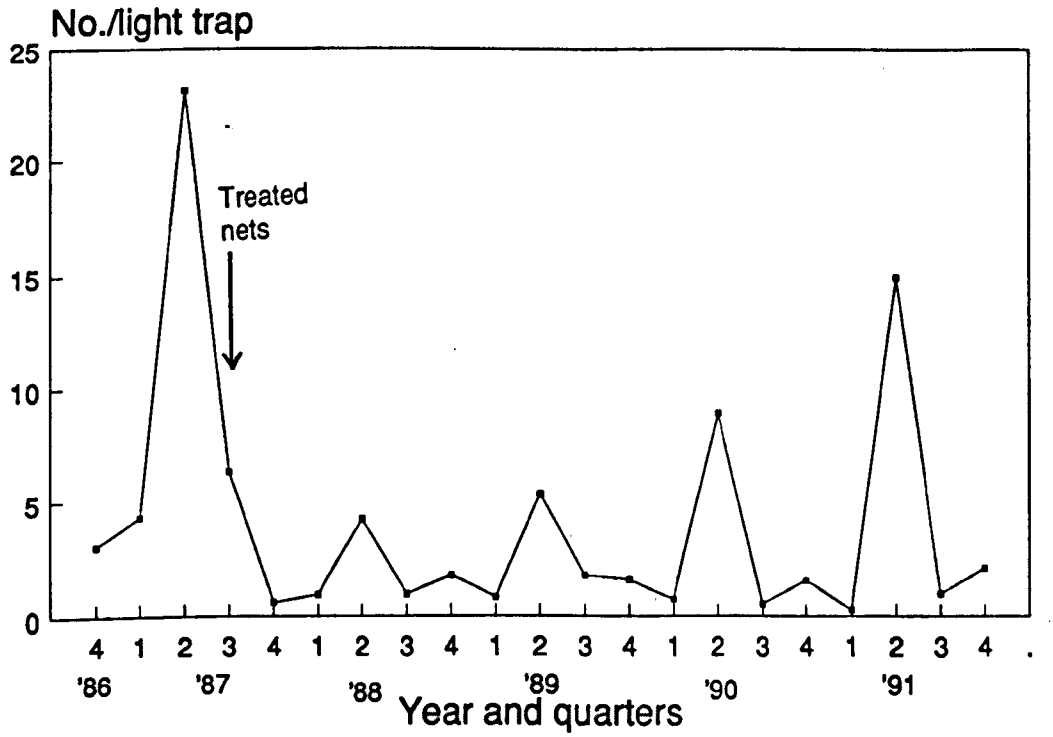
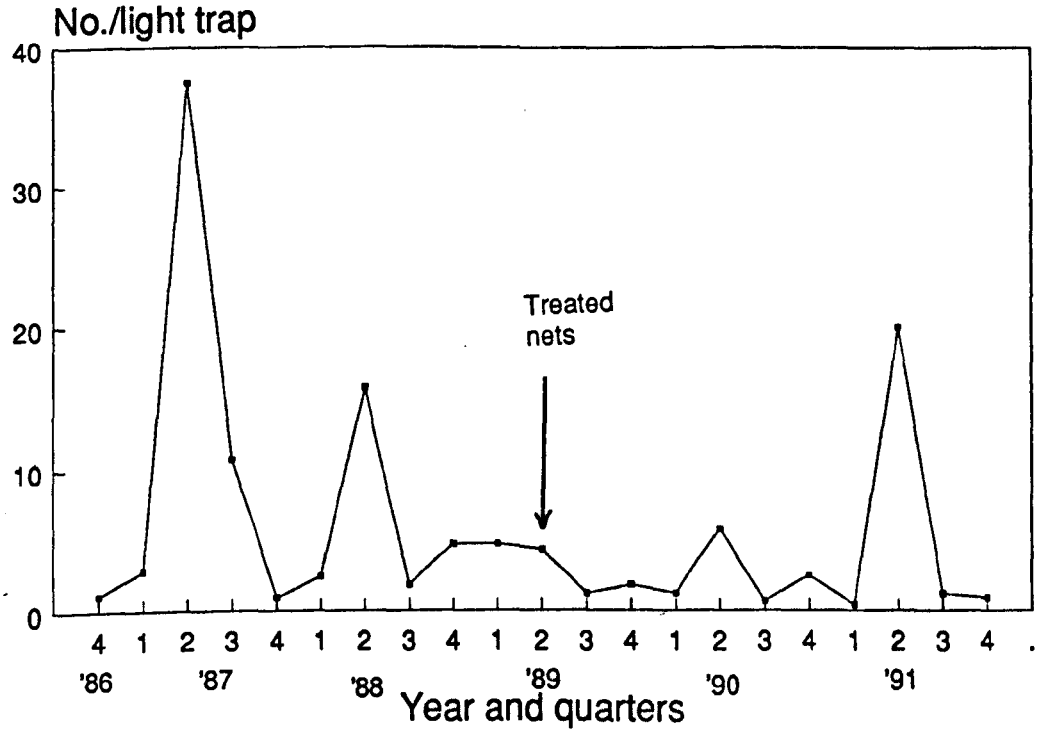


Figure 7.1c: *An. gambiae s.l* quaterly mean (no./ light trap) catches: Kumbamtoni



haphazardly by the estate management) and Kumbamtoni where it was discontinued in 1990.

In each village there was maximum production of adult mosquitoes between April and June (Figures 7.1a-c) which coincided with the long rains (Fig 7.2). The numbers of *An. gambiae s.l* in Mngaza were consistently higher than Kumbamtoni or Mlingano. Table 7.1 shows data for 1990 and 1991 in more detail to allow an assessment of impact (if any) of the larviciding operations on adult densities. The monthly collections of adults were pooled and the average used for estimating percent reduction in the adult population in Mngaza after larviciding, corrected for natural year-to-year changes using the data for Kumbamtoni where there was no larviciding. The pooled data included the months of April to December to coincide with larviciding which started in April 1991.

The effect of the larvicides on the adult mosquitoes was assessed using the formula of Mulla, *et al.*, (1971) described in section 6.2.3.1c:

Corrected % Reduction $= 100 - (K_1/M_1 \times M_2/K_2 \times 100)$

Where:
K_1 and K_2 are 1990 and 1991 mean densities respectively for Kumbamtoni
M_1 and M_2 are 1990 and 1991 mean densities respectively for Mngaza.

Substituting the pooled data from Table 7.1 the corrected % reduction in the adult population was estimated as:-

$= 100 - (3/13.9 \times 21.4/7.4 \times 100)$

$= 100 - 63$

$= 37\%$

The reduction of only 37% in the population of adult *An. gambiae s.l* is much lower than the 87% in reduction of the final immature stages of this species as described in chapter 6. This discrepancy might be explained by immigration from outside the area

Figure 7.2: Mean monthly rainfall for Mlingano, Mngaza and Kumbamtoni 1990 and 1991

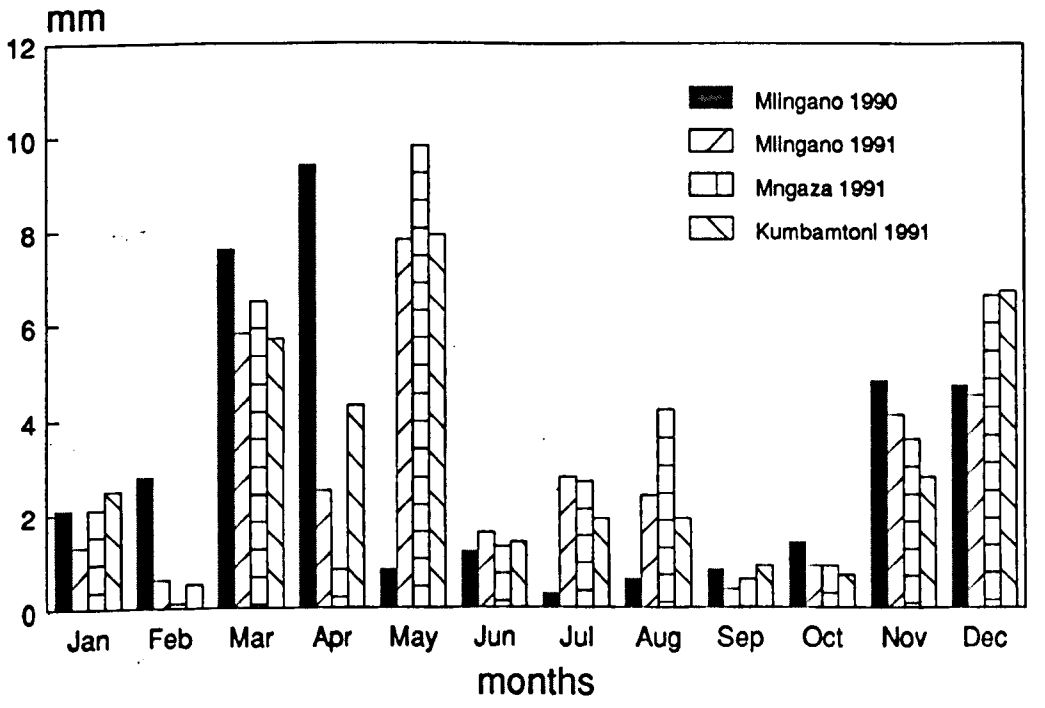


Table 7.1: Monthly mean (no./light trap) catches of *An. gambiae s.l* in Mngaza, Kumbamtoni and Mlingano[a]

	Mngaza		Kumbamtoni		Mlingano	
	1990	1991	1990	1991	1990	1991
Jan	12.2	4.8	2.2	0.0	0.8	0.53
Feb	10.2	3.2	0.0	0.65	0.7	0.0
March	19.4	4.6	1.5	0.65	0.7	0.3
April	74.0	29.1	11.5	12.0	16.7	8.1
May	28.1	98.6	4.7	14.5	9.2	22.7
June	9.7	33.4	1.3	33.5	0.9	13.8
July	1.0	9.0	0.5	2.24	0.16	1.70
August	1.5	2.6	0.9	1.4	0.0	0.95
Sept.	0.8	1.3	0.65	0.0	0.0	0.24
October	0.0	0.6	0.1	0.1	0.2	0.08
November	5.4	1.5	4.5	0.6	1.0	0.7
December	4.8	16.8	3.0	1.9	3.4	5.5

[a] Assessment of the effect of larviciding on adult density was based on the period starting on 1st April 1991 to 31st December 1991

of larviciding. The percentage of mosquitoes that would have to be assumed to be immigrants into this village after the control operation can be approximately estimated from the residual populations of adults and larvae after the control operations as follows:

$$(A - L) \times 100 / A$$

where A = residual adult mosquitoes as corrected %
(100 - corrected % reduction).

L = residual population of final aquatic stages as corrected % (100 - corrected % reduction).

From the present study the percentage of the adult mosquitoes in Mngaza which would have to be assumed to be immigrants into the village was:

$$\{(63\% - 13\%) \times 100\} / 63\% = 79\%$$

To test whether such a high percentage of immigrants is plausible a mark-release-recapture experiment was undertaken as described in the next chapter.

7.3.2 Parous rates

Adult mosquitoes were dissected and scored for parity by the method of Detinova (1962).

Any rise in parity rates as a result of larviciding was expected to be temporary owing to the stabilization of age structure after the residual older mosquitoes died off after a few weeks of larviciding. The parity rates were therefore plotted monthly for the few months before and after larviciding started and the corresponding months of 1990 (figures 7.3a and b). Figure 7.3(a and b) show that there was a significant temporary increase in the parity rate following the introduction of larviciding in April, 1991, and this contrasts with declining trend of parity rate in 1990.

Fig. 7.3.a: An. gambiae s.l monthly parity rates (February to May): Mngaza

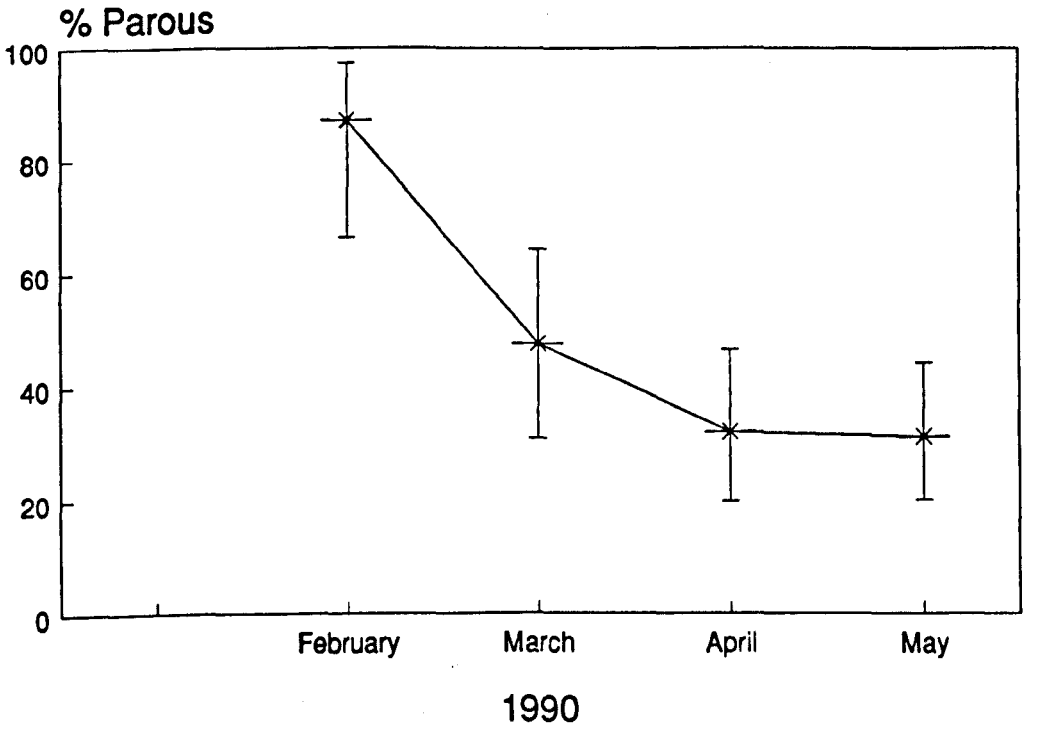
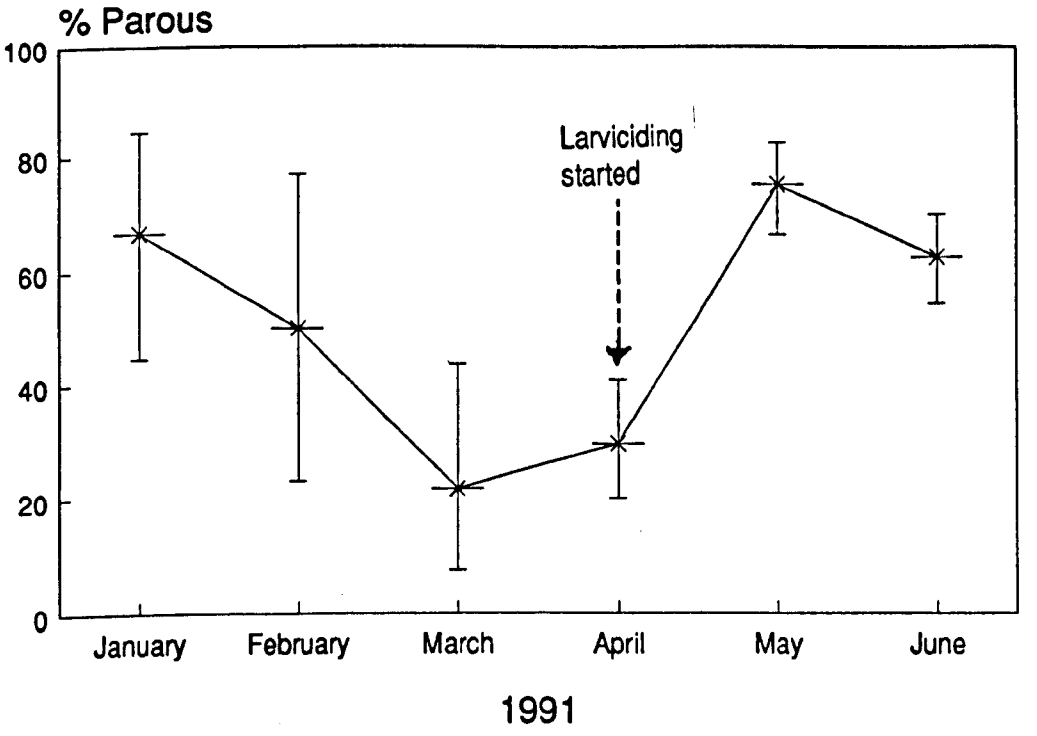


Fig. 7.3b: An. gambiae s.l monthly parity rates (January to June): Mngaza



The rise in parity in May 1991 was presumably due to sudden reduction in pupal emergence in breeding sites close to Mngaza due to larviciding. On the hypothesis that an increasing proportion of the population were immigrants after local breeding was largely eliminated, the rise in parity suggests that immigrants do not enter a village population until they have already become parous.

CHAPTER 8:

EXCHANGE OF *ANOPHELES GAMBIAE S.L* BETWEEN VILLAGES WITH LARVAL AND ADULT MOSQUITO CONTROL ACTIVITIES

8.1 INTRODUCTION

In an attempt to reduce adult mosquito population either by larviciding or adulticiding one of the important factors that needs to be taken into account is the degree of movement of mosquitoes between the controlled and the surrounding areas. In adult mosquito control programmes, the area surrounding the intervention zone has frequently been given insecticidal treatment in order to create a barrier zone to eliminate migrating mosquitoes into the evaluated intervention area (WHO, 1975). However, in a treated bednet trial that was conducted in Muheza for 3½ years (Magesa, *et al.*, 1991) it was found that, even in the absence of such a barrier zone, there was considerable reduction in the mosquito population density, their mean age (as assessed by ovarian age grade), and sporozoite rates after the introduction of treated bednets compared with before introduction or the villages without treated bednets. This led to the hypothesis that each village had its "own" adult mosquito population, despite the observation by Gillies (1961) that mosquitoes could be recaptured at a distance of 2km from their release points. It was thought that such distances may have been covered by only a few mosquitoes.

The question therefore arose whether larviciding in the local breeding sites in treated villages would further reduce the adult mosquito population densities, so that the effect of impregnated bednets could be enhanced. If such efforts did not show significant effect on the adult mosquitoes could it be due to immigration of adult mosquitoes, and what proportion of the mosquitoes in the treated villages were immigrants? Quinones(1991) reported that there was a free interchange of mosquitoes between three small villages in the Gambia, in the West African Sahel region. This

was thought to be a possible explanation for failure of treated bednets to reduce mosquito populations (despite their excellent effect on malaria transmission) in the Gambia (Lindsay, *et al.*, 1989 and in press). As a result of the "villagisation" programme in Tanzania in the 1960s and 1970s villages tend to be larger and more compact than in other African countries and this might affect the proportion of mosquitoes which "belong" to the village or come from other villages. A mark-release-recapture experiment similar to that of Quinones (1991) was conducted in four villages as described below to quantify immigration under Tanzanian conditions. The method of Rawlings *et al.* (1981) was adopted in which there is mark-release and recapture in area A and recapture only in area B (Mngaza). Division of the percent found marked in B by that in A gives an estimate of the proportion in B which came from A, i.e. it allows for unmarked as well as marked immigrants.

8.2 MATERIALS AND METHODS

8.2.1 Study Area

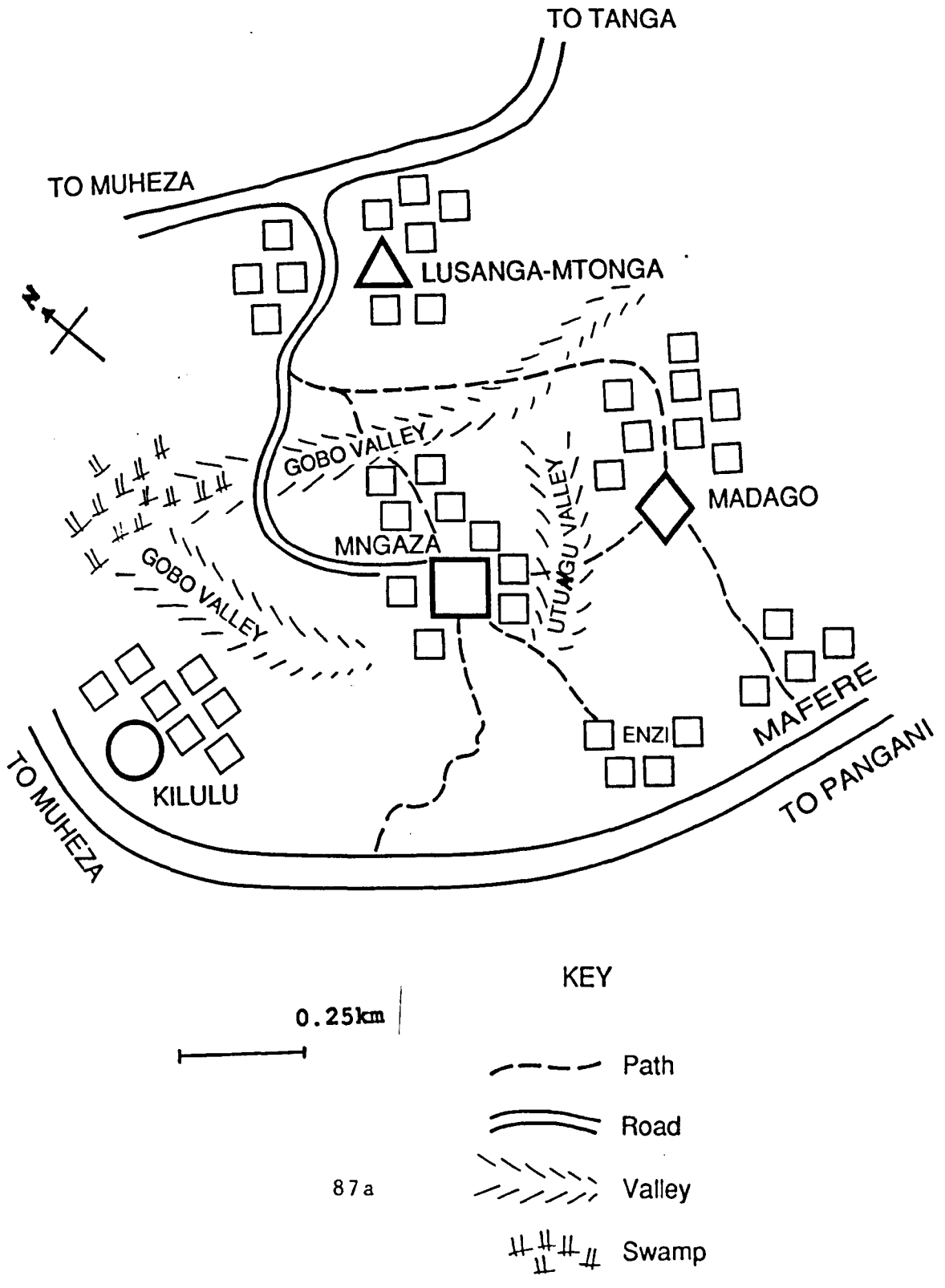
This study was conducted in the villages of Madago, Kilulu, Lusanga-Mtonga, and Mngaza (Figure 8.2.1). As shown in the figure, between Mngaza and the other three villages are two valleys which had all their potential breeding sites for *Anopheles* treated with temephos impregnated maize-cobs during the experiment which was carried out over a period of 30 days from 2/5-31/5/92.

During this time the mean minimum and maximum temperatures recorded at Mlingano Agricultural Research Institute (MARI) were 21.4^{0}C and 29.4^{0}C respectively. The mean rainfall for the same period also recorded at MARI was 7.74mm/day with a maximum on one day of 44.8mm.

8.2.2 Materials

CDC light traps, pyrethrum solution, white cotton sheets, and siphons were used in the collection of mosquitoes (W.H.O, 1975). Paper cups held mosquitoes during

Figure 8.21: VILLAGES WHERE MARK-RELEASE RECAPTURE WAS CARRIED OUT: MADAGO, MNGAZA, KILULU AND LUSANGA-MTONGA.



collection, while plastic cups painted black on the outside were used for holding mosquitoes when they were being chilled to inactivate the mosquitoes and allow them to be scored for colour marks. Chipped ice for chilling mosquitoes was obtained by crushing ice blocks wrapped in a sisal bag and holding it in insulated flasks. An experiment before marking and releasing of mosquitoes started showed that on transparent plastic cups without any paint on the outside condensation formed when they were chilled, and this debilitated the mosquitoes. However, cups painted black on the outside did not suffer from this problem and also allowed markings to be seen more easily. A black cotton cloth with a "roof" (Figure 8.2.5.1b) and a 6 volt ultra violet lamp were used in the search for marked mosquitoes as described below. Magenta, orange and green fluorescent dusts (series 610) from Sterling Industrial Colours Ltd, U.K, were used for marking mosquitoes. A lamp glass was used for holding mosquitoes during the marking and releasing processes. The advantages of using these glasses instead of paper-cups is that they can hold large numbers of mosquitoes and also that mosquitoes being marked can be seen to pick-up the dust. The glasses were placed wider end uppermost with a netting top secured by a rubber band around the rim of the glass.

8.2.3 Experimental design

There were a total 28 handcatch sampling occasions in each of the release villages i.e Madago, Kilulu, and Lusanga (Tables 8.3a-c), while in Mngaza there were 25 pyrethrum spray catch and 9 light trap collections (Table 8.3d). In Madago, Kilulu and Lusanga-Mtonga mosquitoes were caught, chilled until knocked down, inspected for any previous marking and then marked and released. In Mngaza mosquitoes were caught and inspected for any markings but not marked and released.

8.2.4 Mosquito Sampling Procedure

Live mosquitoes were collected by the handcatch method (W.H.O, 1975), between 07.00 hrs and 11.00 hrs in all the villages except Mngaza. In Mngaza

mosquitoes were collected by means of (a) three light traps run overnight beside beds with untreated mosquito nets (Magesa, *et al.*, 1991; Lines *et al.*, 1991), and (b) the pyrethrum-spray-catch method (W.H.O, 1975), which was carried out between 07.00hrs and 11.00hrs. PSC was used instead of HC in Mngaza owing to the fact that this village had permethrin treated bednets which tend to drive mosquitoes out of the houses (Lindsay, *et al.* 1989; Magesa *et al* 1991). Also, since no mosquito releases had been planned for Mngaza, capture of live mosquitoes was not essential. Usually, more mosquitoes were caught during the early morning hours with a decrease in the catch around 09.00hrs as temperatures rose and smoke was created by villagers cooking breakfast.

8.2.5 Search for marked mosquitoes, marking and releasing mosquitoes in Madago, Kilulu and Lusanga-Mtonga

8.2.5.1 Search for marked mosquitoes

The black plastic cups for holding mosquitoes and the mosquito netting to be secured to the mouths of the cups were first checked in the dark for any fluorescent dust particles using the U.V torch.

The captured *An. gambiae s.l* were held in white paper cups. They were removed using siphons, counted, recorded and placed in black plastic cups in groups of about 50-70 mosquitoes. As soon as a cup was full it was quickly transferred into chipped ice which was held in four wide-mouthed insulated flasks (Figure 8.2.5.1a). A cavity to accommodate the cup was made in the chipped ice. The mosquitoes were put into the insulated flask with the lid closed for about two minutes. Then the plastic cup was removed, and inspected with a torch to determined whether all the mosquitoes had been knocked down. In most instances one or two mosquitoes were still clinging to the netting, though stunned. These mosquitoes had not come into direct contact with a cold surface and were likely to revive too soon. The cup was therefore tapped to dislodge the clinging mosquitoes and returned to the ice for about a minute. On average it required about four minutes for all the mosquitoes in a cup to be inactivated

Figure 8.2.5.1a: Chilling mosquitoes with chipped ice in insulated flasks

by cold.

The cup was then passed on to an observer who was sitting under the black cloth described above (Figure 8.2.5.1b) so that his eyes were adapted to the dark. The netting top on the cup had to be removed to allow the inactive mosquitoes to be carefully examined under U.V. light. The black cloth was tucked-in around the observer so that if a mosquito revived while being observed it could easily be recaptured. Inside the black cloth the observer had the U.V. torch, an ordinary torch, forceps, and labelled specimen tubes. The ordinary torch and the siphon were used for collecting escapees and the specimen tubes for keeping marked mosquitoes. By opening and slowly tilting the black cup any marked mosquitoes could be clearly seen under U.V light. Mosquitoes marked with fluorescent dust appear as shining spots on a dark background (Figure 8.2.5.1c). The number and colour of the recaptured mosquitoes were called out to a recorder. The netting was then quickly replaced on the cup and the cup passed on to an assistant. With a siphon, the assistant took mosquitoes from the holding cup and as they rested in the siphon they were quickly counted (about 15 to 20 mosquitoes were conveniently counted), the number recorded, and the mosquitoes transferred to a clean lamp glass for marking (see below) and their subsequent release. All the mosquitoes scored as marked and 10% of those scored as unmarked were kept in labelled specimen tubes for rechecking in the laboratory.

8.2.5.2 Marking mosquitoes with fluorescent dust

Fluorescent dusts (Sterling Industrial Colours Ltd, UK) were used for marking mosquitoes from Madago, Kilulu, and Lusanga-Mtonga. Three distinct colours in the 610 Series were used for the three villages as follows: magenta for Madago, orange for Kilulu and green for Lusanga-Mtonga. For each village the equipment was kept separately in plastic bags to minimize the chance of cross-contamination. Female mosquitoes which were held in the lamp glass as described above were almost all blood fed. The glass was placed on a petri-dish, and fluorescent dust of appropriate colour blown in using an improvised form of insufflator (Figure 8.2.5.2). A sufficient quantity was used for the mosquitoes to show clearly the colour of the dust in

Figure 8.2.5.1b: Scoring mosquitoes for marks under black cloth with a roof

Figure 8.2.5.1c: Yellow and green marked mosquitoes as seen under U.V light

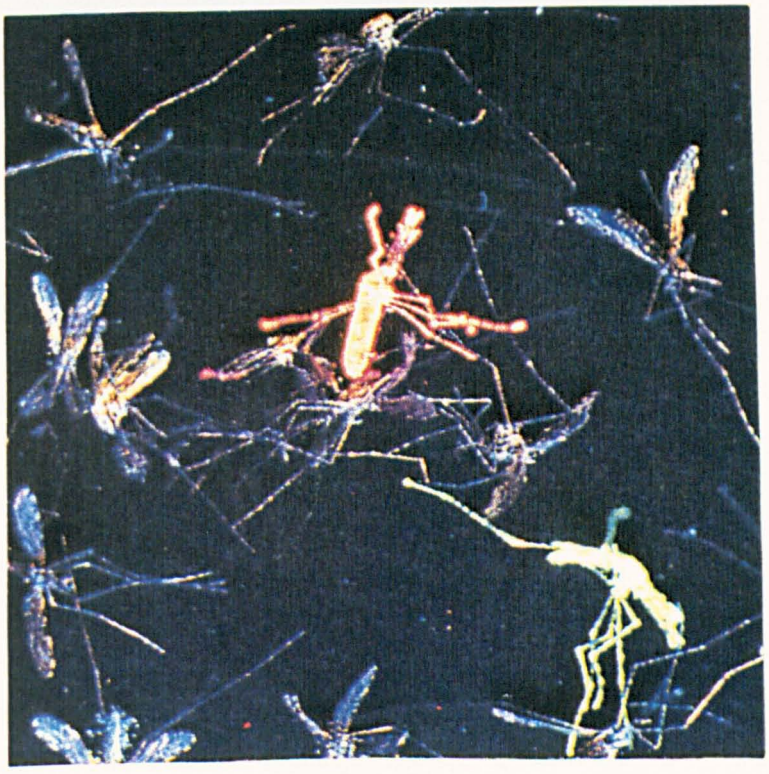
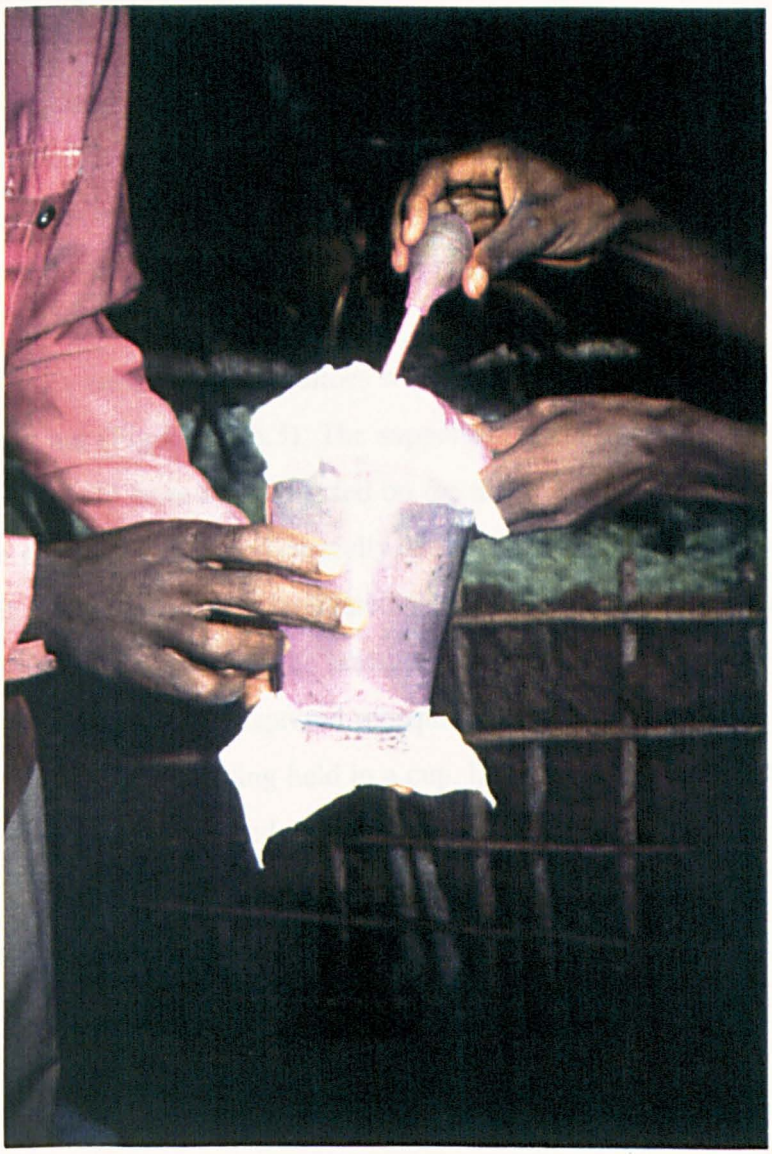


Figure 8.2.5.2: Marking mosquitoes with fluorescent dust



daylight. The marking of mosquitoes was done inside the release huts.

8.2.5.3 Releasing mosquitoes

The mosquitoes were released from a single point in each village. For this purpose, huts made of mud and thatch were built in Madago, Kilulu and Lusanga-Mtonga. Large eave gaps were left in order to leave enough space for the mosquitoes to egress and disperse (Figure 8.2.5.3). The huts were built in the shade of trees.

A glass chimney containing mosquitoes was put in an earthenware-pot hung from the ceiling of the hut (Figure 8.2.5.3). The supporting ropes for the pot were greased to trap ants that would have otherwise fed on the marked mosquitoes. The nets on both sides of the glass were removed carefully to allow mosquitoes to escape. It was found that the exit of mosquitoes was facilitated by tilting the glass. The mosquito nets were thoroughly washed and dried in the laboratory before re-use on another day. Washing was essential to ensure that captured mosquitoes could not pick up fluorescent dust after capture as they were being held in a cup. Dead marked mosquitoes found in the glass and pot were counted and recorded the following morning.

8.2.6 Search for marked mosquitoes in Mngaza

In Mngaza, the black cups were first checked for freedom from fluorescent dust and the dead mosquitoes caught by PSC put inside. These were checked for markings under the black cloth as described above. The marked ones were picked out, placed in specimen tubes and 10% of those judged to be unmarked, were taken to the laboratory for rechecking. Live light trapped mosquitoes were knocked down like those for release in the other villages. They were also checked for markings and 10% of those judged to be unmarked were taken to the laboratory for rechecking.

8.2.7 Rechecking mosquitoes in the laboratory

In the laboratory, live mosquitoes were killed using chloroform and rechecked for

Figure 8.2.5.3: Releasing mosquitoes

any marking colours in a dark insectary using U.V light. Those found marked were recorded. Some female *An. gambiae s.l* from Mngaza were occasionally dissected for parity (Detinova, 1962) and recorded, as part of the adult mosquito monitoring activity in Mngaza, Kumbamtoni and Mlingano as described in the last chapter.

8.3 RESULTS AND DISCUSSION

The daily mosquito catches by HC, PSC and light-trap (LT) are shown in Tables 8.3(a to d). A total of 30,508 mosquitoes were caught in all the villages. Out of these, 10,042 came from Madago, 9,657 from Kilulu, 8,021 from Lusanga-Mtonga and 2,788 from Mngaza. With the exception of Mngaza large numbers of mosquitoes could be caught by 30 man-minutes of searching per house in each of ten houses. Only active individuals that were not moribund were recorded as marked and released. The moribund individuals on the day of marking (DE) plus those that were found the following day, to have failed to fly off after being marked and released (DR) were added together and counted as dead. These were subtracted from the final number of released mosquitoes recorded which also excluded the recaptures (r) and the live ones (LL) taken to the laboratory for rechecking. The number of mosquitoes recorded as released (R) was therefore obtained as follows:

$$R = C - (DE + DR + LL + r)$$

where C = number of female mosquitoes caught

DE = Number of dead female mosquitoes on examination

DR = Number of female mosquitoes that died on release

LL = Number of live female mosquitoes taken to the laboratory for rechecking

r = Number of recaptures

A comparison was made of the field scores and rechecking in the laboratory of recaptured mosquitoes. It was found that in the recapture village of Mngaza, field and laboratory scores did not differ. In the mark and release villages the field scores indicated a total of 199 recaptures but the 10% of supposedly unmarked mosquitoes revealed an extra 3 marked recaptures when examined in the laboratory. This level of

Table 8.3a: Number of caught, marked, released and recaptured *An. gambiae s.l* in Madago in May 1992.

Date	No. Caught (HC)	No. Marked	Relea-sed	Lab. Racapture scores		
				M[a]	O[b]	G[c]
2.5.92	268	238	227	-	-	-
3.5.92	419	442	442	0	0	0
4.5.92	398	359	359	1	0	0
5.5.92	433	390	387	1	0	0
6.5.92	497	448	422	3	0	0
7.5.92	-	-	-	-	-	-
8.5.92	470	423	398	4	0	0
9.5.92	366	330	321	2	0	0
10.5.92	493	444	442	3	0	0
11.5.92	363	327	267	4	0	0
12.5.92	504	454	454	7	0	0
13.5.92	393	354	353	2	0	0
14.5.92	457	412	404	2	0	0
15.5.92	295	266	261	1	0	0
16.5.92	218	197	167	3	0	0
17.5.92	374	337	310	3	0	0
18.5.92	-	-	-	-	-	-
19.5.92	225	203	180	1	0	0
20.5.92	446	402	368	5	0	0

[a]Magenta [b]Orange [c]Green HC = Handcatch

(continued)

Table 8.3a: Number of caught, marked, released and recaptured *An. gambiae s.l* in Madago in May 1992:

(continued)

Date	No. Caught (HC)	No. Marked	Relea-sed	Lab. Racapture scores		
				M[a]	O[b]	G[c]
21.5.92	257	232	205	1	0	0
22.5.92	221	199	167	0	0	0
23.5.92	264	238	187	0	0	0
24.5.92	433	390	355	1	0	0
25.5.92	401	361	303	3	0	0
26.5.92	217	196	182	2	0	0
27.5.92	314	283	253	2	0	0
28.5.92	285	257	197	0	0	0
29.5.92	249	245	193	2	0	0
30.5.92	480	432	402	1	0	0
31.5.92	230	-	-	2	0	0
Total	10042	8859	8206	56	0	0

[a]Magenta [b]Orange [c]Green HC = Handcatch

Table 8.3b: Number of caught, marked, released and recaptured *An. gambiae s.l* in Kilulu in May 1992.

Date	No. caught (HC)	No. Marked	Released	Lab. Racapture scores		
				M[a]	O[b]	G[c]
2.5.92	186	143	143	-	-	-
3.5.92	525	473	437	0	0	0
4.5.92	558	503	443	0	0	0
5.5.92	434	391	370	0	3	0
6.5.92	534	481	420	0	4	0
7.5.92	-	-	-	-	-	-
8.5.92	581	523	469	0	6	0
9.5.92	473	426	355	0	4	0
10.5.92	469	423	364	0	3	0
11.5.92	598	539	517	0	10	0
12.5.92	566	510	503	0	4	0
13.5.92	311	280	241	0	2	0
14.5.92	499	450	385	0	10	0
15.5.92	475	428	386	0	5	0
16.5.92	380	342	317	0	3	0
17.5.92	260	234	191	0	3	0
18.5.92	-	-	-	-	-	-
19.5.92	437	394	363	0	4	0
20.5.92	231	208	170	0	1	0

[a]Magenta [b]Orange [c]Green HC = Handcatch

(continued)

Table 8.3b: Number of caught, marked, released and recaptured *An. gambiae s.l* in Kilulu in May 1992:

(continued)

Date	No. caught (HC)	No. Marked	Released	Lab. Racapture scores		
				M[a]	O[b]	G[c]
21.5.92	205	185	159	0	4	0
22.5.92	321	289	242	0	3	0
23.5.92	143	129	79	0	2	0
24.5.92	257	232	174	0	3	0
25.5.92	203	183	147	0	0	0
26.5.92	189	171	148	0	2	0
27.5.92	173	156	136	0	0	0
28.5.92	168	152	133	0	2	0
29.5.92	137	124	119	0	2	0
30.5.92	185	167	157	0	1	0
31.5.92	159	-	-	0	2	0
Total	9657	8536	7568	0	83	0

[a]Magenta [b]Orange [c]Green HC = Handcatch

Table 8.3c: Number of caught, marked, released and recaptured *An. gambiae s.l* in Lusanga-Mtonga in May 1992

Date	No. Caught (HC)	No. Marked	Released	Lab. Racapture scores		
				M[a]	O[b]	G[c]
2.5.92	189	137	114	-	-	-
3.5.92	227	205	164	0	0	0
4.5.92	354	319	252	0	0	2
5.5.92	226	195	151	0	0	0
6.5.92	229	198	160	0	0	1
7.5.92	-	-	-	-	-	-
8.5.92	206	186	164	0	0	2
9.5.92	228	206	180	0	0	1
10.5.92	162	146	130	0	0	1
11.5.92	288	260	231	0	0	4
12.5.92	401	361	345	0	0	3
13.5.92	555	500	496	0	0	5
14.5.92	361	325	317	0	0	4
15.5.92	388	350	337	0	0	9
16.5.92	363	327	307	0	0	7
17.5.92	311	280	243	0	0	2
18.5.92	-	-	-	-	-	-
19.5.92	418	377	321	0	0	3
20.5.92	299	270	230	0	0	4

[a]Magenta [b]Orange [c]Green HC = Handcatch

(continued)

Table 8.3c: Number of caught, marked, released and recaptured *An. gambiae s.l* in Lusanga-Mtonga in May, 1992

(continued)

Date	No. Caught (HC)	No. Marked	Relea-sed	Lab. Racapture scores		
				M[a]	O[b]	G[c]
21.5.92	192	173	155	0	0	2
22.5.92	271	244	226	0	0	1
23.5.92	198	179	133	0	0	0
24.5.92	354	319	251	0	0	3
25.5.92	329	297	254	0	0	0
26.5.92	308	278	231	0	0	0
27.5.92	281	253	214	0	0	1
28.5.92	138	125	109	0	0	2
29.5.92	216	195	158	1	0	0
30.5.92	275	248	218	0	0	3
31.5.92	254	-	-	0	0	2
Total	8021	6955	6091	1	0	62

[a]Magenta [b]Orange [c]Green HC = Handcatch

Table 8.3d: Number of *An. gambiae s.l* caught and recaptured in Mngaza in May, 1992

Date	PSC		LT	
	No. caught	Recap-tures	No. caught	Recap-tures
4.5.92	106	1 M	-	-
5.5.92	224	0	-	-
6.5.92	131	0	-	-
7.5.92	-	0	251	0
8.5.92	68	0	-	-
9.5.92	65	0	-	-
10.5.92	35	0	-	-
11.5.92	61	0	-	-
12.5.92	54	0	-	-
13.5.92	8	0	93	1 M
14.5.92	98	0	-	-
15.5.92	44	0	-	-
16.5.92	5	0	237	0
17.5.92	65	0	-	-
18.5.92	-	0	217	0
19.5.92	94	0	49	0
20.5.92	-	0	-	-

PSC = Pyrethrum spray catches
LT = Light trap catches
M = Magenta

... continued

Table 8.3d: Number of *An. gambiae s.l* caught and recaptured in Mngaza in May 1992 (Continued)

Date	PSC		LT	
	No. caught	Recap-tures	No. caught	Recap-tures
25.5.92	26	1 M	-	-
26.5.92	26	0	-	-
27.5.92	38	0	-	-
28.5.92	26	0	251	0
29.5.92	59	0	-	-
30.5.92	18	0	-	-
31.5.92	18	0	-	-
Total	1440	1 M	1348	1 M

PSC = Pyrethrum spray catches
LT = Light trap catches
M = Magenta

Table 8.3e: Total number of *An. gambiae s.l* caught and recaptured in the four study villages in May, 1992.

Village	No. caught (a)	No. Recaptured (b)			% recaptured (b/a x 100)		
		M	O	G	M	O	G
Madago	9283	56	0	0	0.6	0.0	0.0
Kilulu	8946	0	83	0	0.0	0.93	0.0
Lusanga-Mtonga	7605	1	0	62	0.013	0.0	0.82
Mngaza	2788	2	0	0	0.072	0.0	0.0
Total	28622	59	83	62			

M = Magenta O = Orange G = Green

error was so small that it was not considered worthwhile to attempt to correct the data for it.

A total of 56 magenta mosquitoes were caught in Madago where they had been marked and released. There were no recaptures in Madago, of mosquitoes marked in Kilulu or Lusanga. In Kilulu, 83 orange mosquitoes were recaptured after having been released there. No incoming marked mosquito was caught in Kilulu. In Lusanga-Mtonga, 62 green mosquitoes were recaptured in this, their village of release, and one magenta mosquito from Madago was recaptured. In Mngaza, which was the only village without mosquito releases, there were 2 magenta mosquitoes recaptured. The overall ratio of recaptures in their village of release to those in other villages (201:3) contrasts with the results of Quinones (1991) in The Gambia of 4:4 (χ^2= 42.6 $p<$ 0.001) which supports the hypothesis proposed above that there is not so much interchange of mosquitoes between the larger Tanzanian villages as between the small Gambian hamlets. Any further analysis of Tanzanian data cannot give accurate estimate as only three marked immigrants were recaptured. However, it was logistically impossible to extend the experiment and these are the only data available to test the plausibility of the immigration hypothesis to explain the disappointing results of larviciding. An attempt at analysis is therefore made.

The intention of marking, as well as recapturing mosquitoes in Madago, Kilulu and Lusanga-Mtonga was to be able to estimate the proportion of unmarked, as well as marked immigrants in Mngaza which had come from one of the other three villages. This was done by dividing the percentage found marked in Mngaza 0.072% (2/2,788) by the percentage found marked in Madago, Kilulu and Lusanga-Mtonga of 0.78% (201/25,834) which gave 9.2%. This follows the method used by Rawlings *et al.*, (1981). 95% confidence limits were attached to the percentage of marked mosquitoes in Mngaza from tables based on the binomial distribution (Fisher and Yates, 1948). These limits of the best estimate were 0.00868% and 0.259%. Dividing each of these limits by the estimate of 0.78% for the three release villages gives 1.1% and 33.2%. This takes no account of the sampling error in the estimate of 0.78% marked in the three release villages. Thus, the true confidence limits for Mngaza

immigrants are even wider than 1-33%.

The three release villages were not the only possible source of immigrants to Mngaza - as shown in figure 8.2.1 the villages of Enzi and Mafere which could not be included in the mark-release-recapture study, were relatively close to Mngaza and might have contributed as many immigrants as the 3 release villages. Thus, it seems possible to stretch the upper confidence limit of the proportion of immigrants in Mngaza from the three villages of more than 33% to make it consistent with the 79% of immigrants which is demanded by the residual larval and adult populations discussed in chapter 7. A very extensive and laborious mark-release-recapture experiment would be required to conclusively confirm or rule out immigration as a cause of the disappointing adult control result.

CHAPTER 9:

EFFECT OF THE DENSITY OF IMMATURE STAGES OF *AN. GAMBIAE S.L* ON THE PRODUCTION OF ADULT MOSQUITOES.

9.1 INTRODUCTION

As shown in chapter 6, footprints and puddles constituted the most important breeding sites of *An. gambiae s.l* in the study area. In these breeding sites the larvae have to withstand constantly fluctuating levels of water and the larvae are sometimes left crowded close to the bottom when the site is nearly dry. A number of views have been proposed as to what might be the major factors in controlling survival of *An. gambiae s.l* under such severe conditions (McCrae, personal comm.) as shown in Figure 9.1. However, as described in chapter 2, little has been done to investigate these basic phenomena for *An. gambiae s.l.* This is becoming particularly important in the light of increasing interest in the use of biological larvicides which require thorough understanding of the biology of the target species. In this chapter an attempt has been made to answer two questions on the survival of *An. gambiae s.l* in the breeding sites. These are: (i) what is the effect of density of immature stages on their rate of development? (ii) how does increase in the density of immature stages affect the rate of survival into adult into stage?

9.2 MATERIALS AND METHODS

The study was conducted in the grounds of the Ubwari Field Station between September and April which is the warmer part of the year. Partially immersed cages measuring 0.25m x 0.25m x 0.2m similar to those described by Rajagopalan *et al*, (1976; 1977) were used to retain larvae through their aquatic life. The material with

which the cages were covered was fine cotton cloth since test runs showed that first instar larvae could escape through sandfly netting. The cages had a short wide sleeve on top through which first instars could be introduced and dips could subsequently be taken. This sleeve also served to exclude egg laying wild mosquitoes and predators. Each cohort was divided between two replicate cages of larvae. The cages were held partially submerged in water by means of two poles running across a pit (1m x 1m x 0.3m) containing water (Fig. 9.1). A rubber band was used to secure the cages to the poles. A plastic lining was provided to the pit to prevent the water from draining away. In order to keep the water as natural as possible, some mud was regularly added and the water stirred.

Samples of 20, 50, 90, 100, 150, 200, and 300 first instars were introduced into the cages on different occasions. The introductions were made into the cages either on a single occasion and the larvae were kept until the adults emerged or first instar larvae were introduced at regular intervals, usually every other day, the same number of larvae being introduced into the cage on each occasion. Eggs from wild caught blood fed female mosquitoes were hatched about twelve hours before the first instars were introduced into the cages. This procedure was followed after observing heavy mortality among the first instars when they were immediately introduced into the cages.

On the day when the first cohort was introduced into the cages no dipping was carried out to allow the larvae to settle. On each of the following days, five dips were taken from each cage, and the larvae were counted and replaced. Large numbers of dips were avoided as, given the frequency of sampling, they might have caused stress and increased mortality among the larvae. After dipping was completed, the sleeve was tied until the following day, in the case of cages with single introductions. In the case of cages with multiple larval introductions these were made after the completion of dipping for that day. It was thought desirable to replace the smaller mesh cloth with wider mesh net after the larvae grew bigger in the case of cages with single introductions. However, this idea was dropped after heavy mortality was found to occur among the larvae whenever such a change was made.

Figure 9.1: Partially submerged cages for density dependent mortality studies

As the larvae continued to develop more care was taken before opening the sleeve in order to catch the emerged mosquitoes. This was achieved by covering the mouth of the sleeve with a small net cage. The sleeve and the sides of the submerged cage were shaken gently to cause most of the resting adult mosquitoes to fly. Any remaining mosquitoes were collected using a siphon.

9.3 RESULTS

The data collected from experiments with single introductions were used to assess if there was any correlation in the density of first instars introduced and the rate of development, as measured in terms of instar duration. Then the data from experiments with both single and multiple introductions were used to assess if there was any correlation between density and survival of immature stages, as assessed from the number of adults that emerged in relation to the total number of first instars introduced.

9.3.1 Estimation of instar duration

The durations of different instars were estimated by subtracting the weighted average duration in days for each instar from that of the preceding instar. In the case of the first instar the time (day) when the eggs were hatched (D_h) was subtracted from the weighted average of this instar. Estimation of the weighted average (W) of each instar can be represented as follows:

$$W_j = \frac{(Day_1 \times N_{1j}) + (Day_2 \times N_{2j}) + \ldots (Day_i \times N_{ij})}{T}$$

where $Days_{(1,2\ldots i)}$ = Days from when the instar appeared to when it disappeared.

N_{ij} = Number of larvae of instar $_j$ found on day $_i$

T = Total number of that instar up to D_i

In the case of the first instar, D_1 was the day when dipping started, (i.e. the day following their being introduced into cage). From the above representation the instar duration for stage one larva is equal to $W_1 - D_h$, while that of other stages follows was $W_2 - W_1$, $W_3 - W_2$, etc.

The instar durations estimated for different stages were plotted against their densities to find if there was any correlation between the two parameters in this species (Figure 9.2). Correlation and regression analysis of instar duration on number of first instars introduced was carried out for each stage to determine whether the observed regression coefficients were significantly different from zero and whether a significant proportion of variance was explained by regression. The following were the results of this analysis for each instar and the combined data of all instars:

Stage I: $Y = 1.692 - 0.0019X$

$r = -0.83$

95% confidence limits for slope = -0.0042 and 0.00044,

$F = 6.657$ df= 1,3 $P = 0.082$ ns

Stage II: $Y = -0.042 + 0.0067X$

$r = 0.91$

95% confidence limits for slope = 0.0011 and

0.012, $F = 14.75$ df= 1,3 $P = 0.032$ *

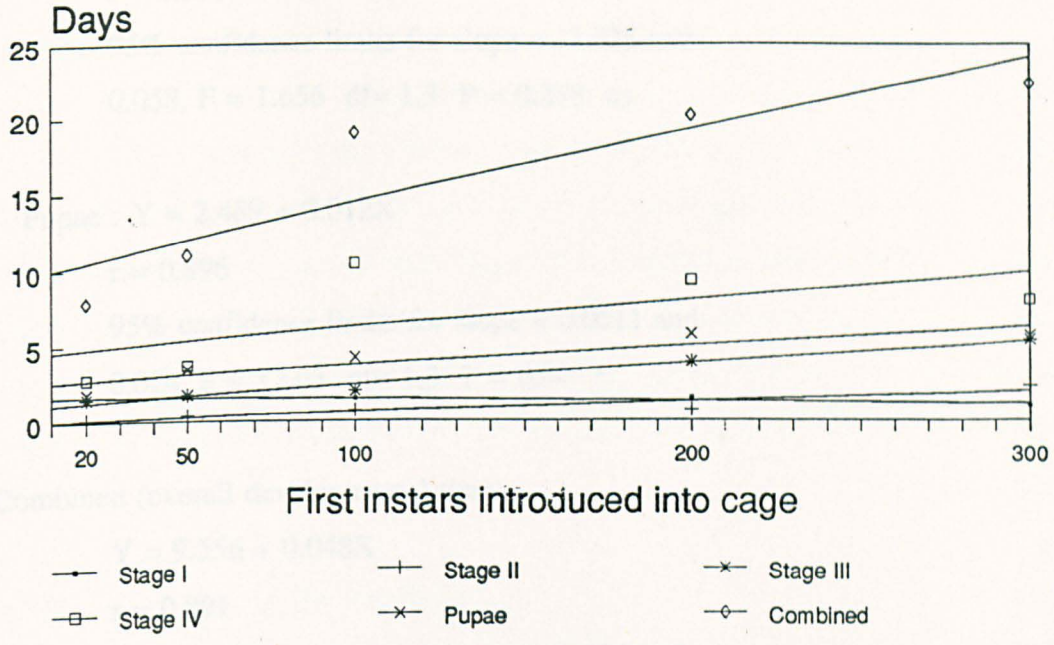
Stage III: $Y = 1.047 + 0.014X$

$r = 0.991$

95% confidence limits for slope = 0.011 and

0.018, $F = 169.55$ df= 1,3 $P = 0.000978$ ***

Figure 9.2: Effect of larval density on instar duration of An. gambiae s.l



Stage IV : Y = 4.389 + 0.017X

r = 0.596

95% confidence limits for slope = -2.025 and 0.058, F = 1.656 df= 1,3 P = 0.288 ns

Pupae : Y = 2.489 + 0.012X

r = 0.896

95% confidence limits for slope = 0.0011 and 0.024, F = 12.03 df= 1,3 P = 0.04 *

Combined (overall developmental time):

Y = 9.556 + 0.048X

r = 0.891

95% confidence limits for slope = 0.003 and 0.094, F = 11.52 df= 1,3 P = 0.043 *

The results of this experiment show that there was no significant relationship between increasing density of first instars introduced in the cage and their instar duration. This could be explained by their small size and small food requirements which would allow large numbers to share the small amount of available food and other resources without much interference. The other stages showed a positive correlation between instar duration and the density of first instars introduced which was significant in all except one case. It can therefore be concluded that although conditions in natural breeding sites are likely to be different from those in the experimental cages, it is likely that higher densities of larvae, particularly in small collections of water, cause increase in instar duration.

9.3.2 Effect of density on survival

Survival was estimated from the percentage of the total number of adults that emerged to the total number of input of first instars. Graphs of these survival rates against the densities of the first instar inputs are shown in Figures 9.3a and 9.3b for experiments with single and multiple introductions results respectively. In both cases the graphs were curvilinear. Careful study was made to investigate the relationship of the observed decrease in survival with input. Following the method used by Varley and Gradwell, (1963) and Rajagopalan *et al*, (1976) this was done by regressing the K values (log 1/survival) on the inputs as shown in Figures 9.4a and 9.4b for single and multiple introductions respectively . The equations for these regression lines were as follows:

Single cohort $Y = -1.739 + 0.0084X$

$r = 0.964$

95% confidence limits for slope = 0.0042 and 0.013, $F = 39.52$ $df = 1,3$ $P = 0.00813$ **

Multiple cohorts

$Y = -1.737 + 0.000506X$

$r = 0.989$

95% confidence limits for slope = 0.00027 and 0.0074, $F = 88.38$ $df = 1,2$ $P = 0.011$ *

In both cases there was a highly significant correlation. This was a further indication of density dependency in *An. gambiae s.l.* However, survival is defined as output/input and correlation of this with input may be spurious. According to Varley and Gradwell (1968) density dependency of survival could be proven to be real only if the regression lines of log of first instar inputs on log adult output, and log adult output on log of first instar inputs were significantly different from a slope of b = 1 and both lie on the same side of b = 1 (Varley and Gradwell, 1968). The regressions of these

Figure 9.3a: Effect of density of larvae on their survival to the adult stage

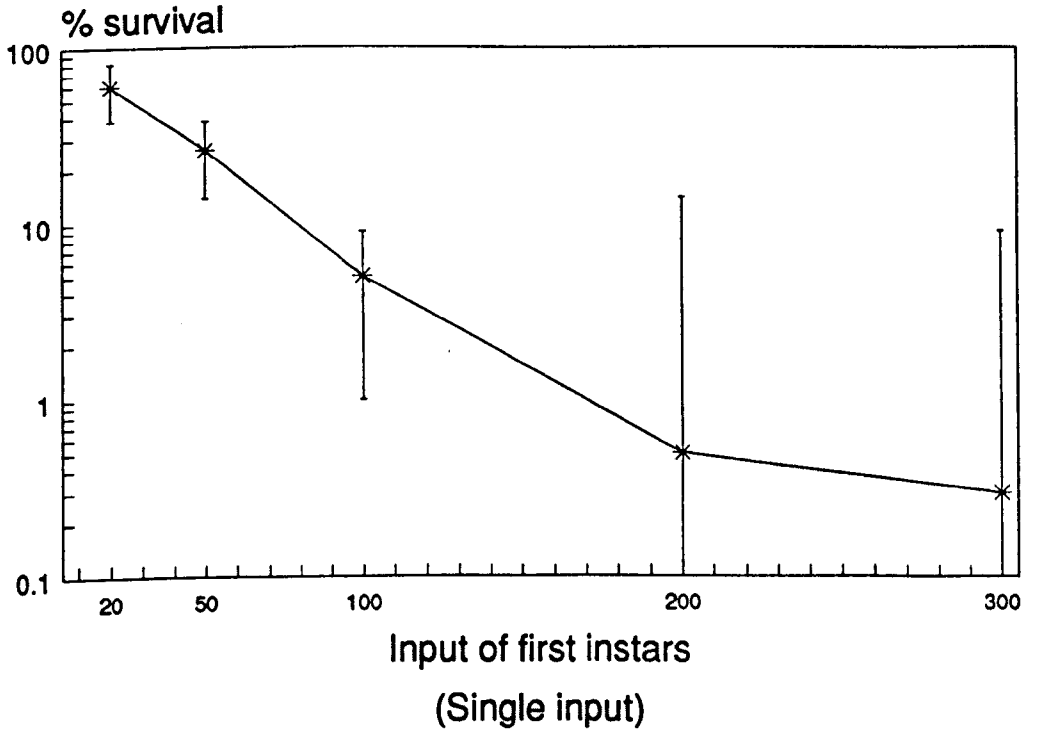


Figure 9.3b: Effect of density of larvae on their survival to adult stage

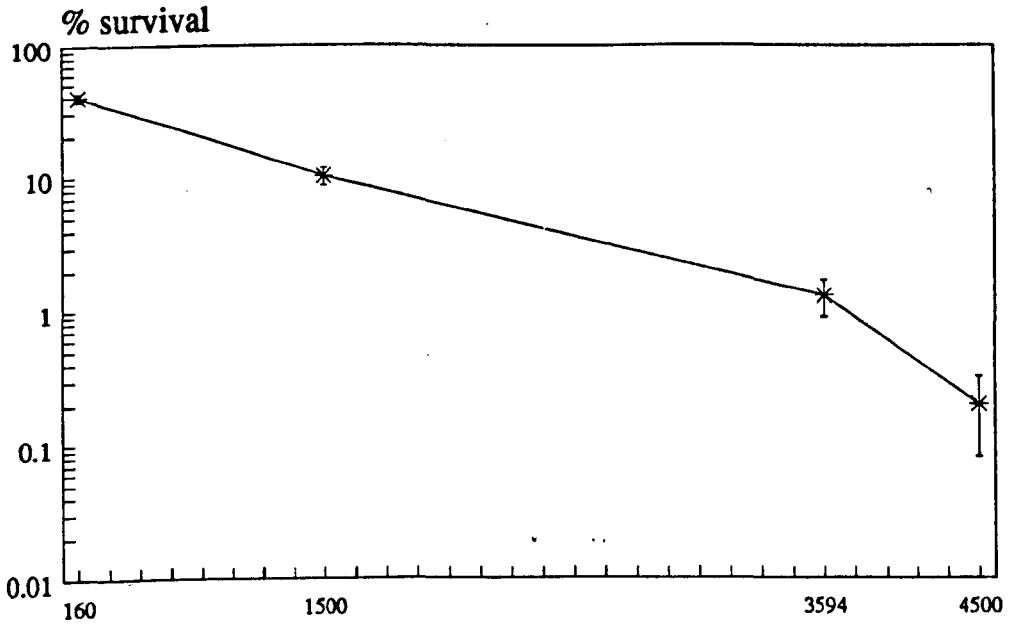


Figure 9.4a: Regression of the values
K (=log 1/survival) on input densities

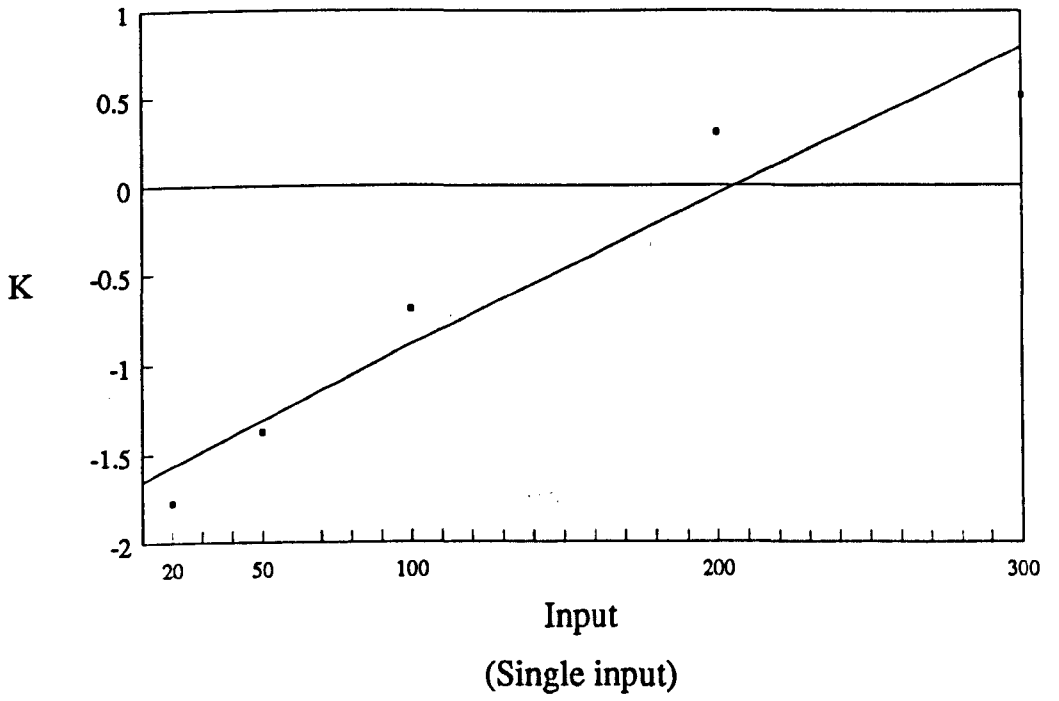
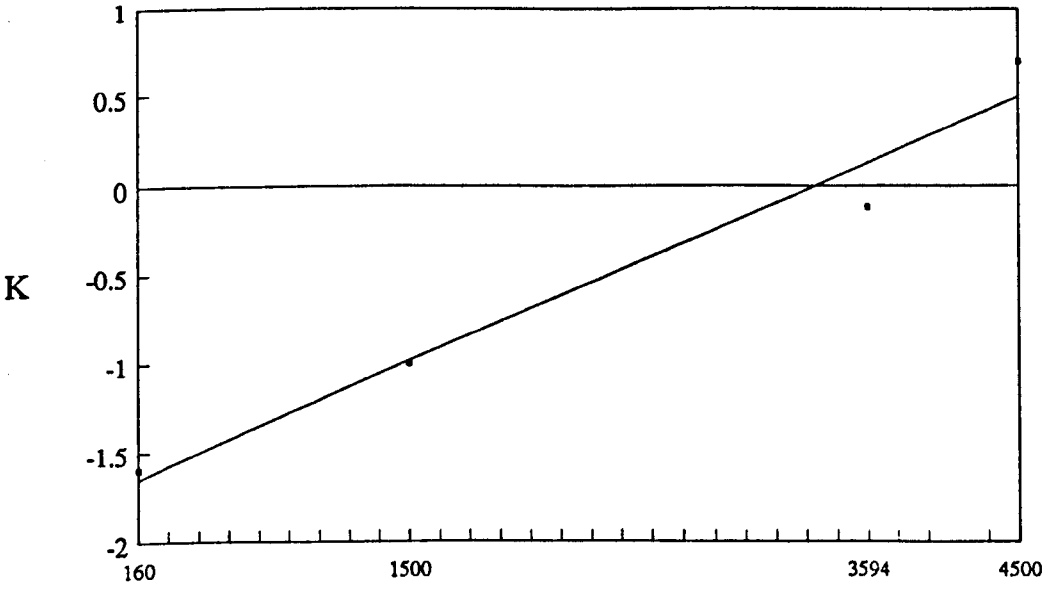


Figure 9.4b: Regression of the values of K (=log 1/survival) on input densities



parameters for the experiment with multiple introductions were as follows:

Regression of log of first instar input on log adult output gave:

$Y = 3.036 + 0.104X$

$r = 0.104$

95% confidence limits for slope = -2.933 and 3.141

$t = 1.27$ $df = 2$ $P = 0.332$ ns.

Regression of log adult output on log first instar input gave:

$Y = 0.868 + 0.103X$

$r = 0.104$

95% confidence limits for slope = -2.913 and 3.12

$t = 1.279$ $df = 2$ $P = 0.329$ ns

Both regressions were found not to be significantly different from $b = 1$. Therefore no attempt was made to determine the type of density dependency in the experiment with multiple introductions.

On the other hand the regression lines of parameters mentioned above for the experiment with single introductions (see Figure 9.5a and 9.5b) were both significantly different from the slope $b = 1$ as shown below:

Regression of log density of first instar on log adult output gave:

$Y = 2.41 - 0.791X$

$r = -0.925$

95% confidence limits of regression coefficient

= -0.195 and -1.386

$t = 9.547$ $df = 3$ $P = 0.00242$ **

Regression of log adult output on log first instar input gave:

$Y = 2.694 - 1.083X$

$r = -0.925$

Figure 9.5a:Regression of log density of first instar on log adult output

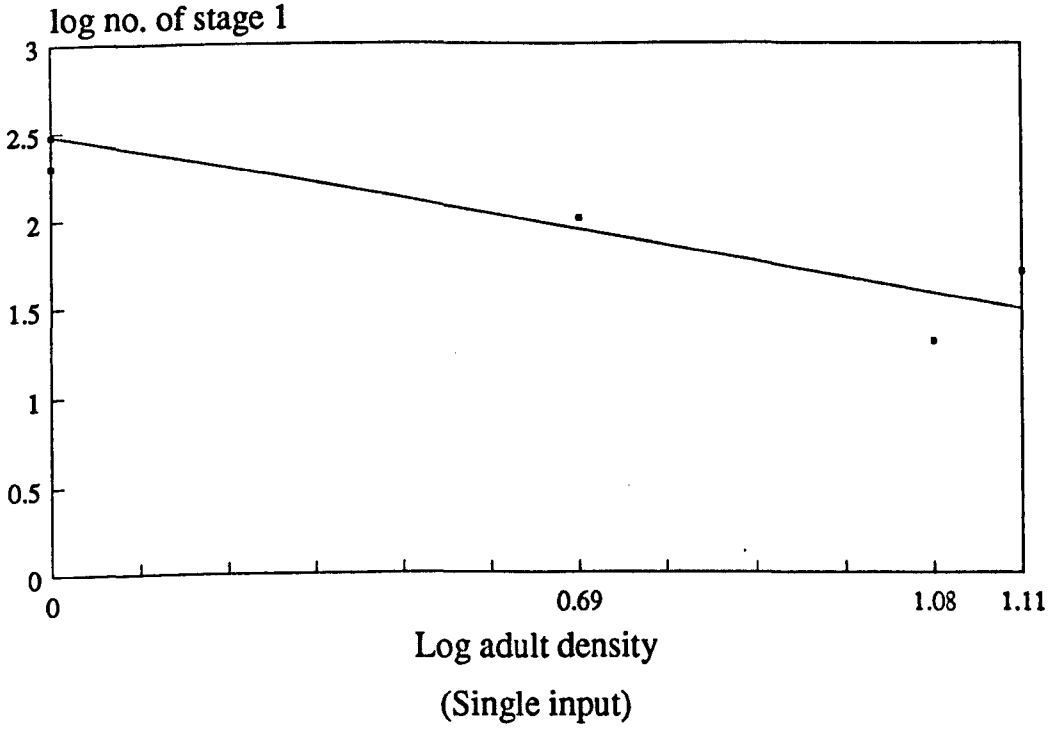
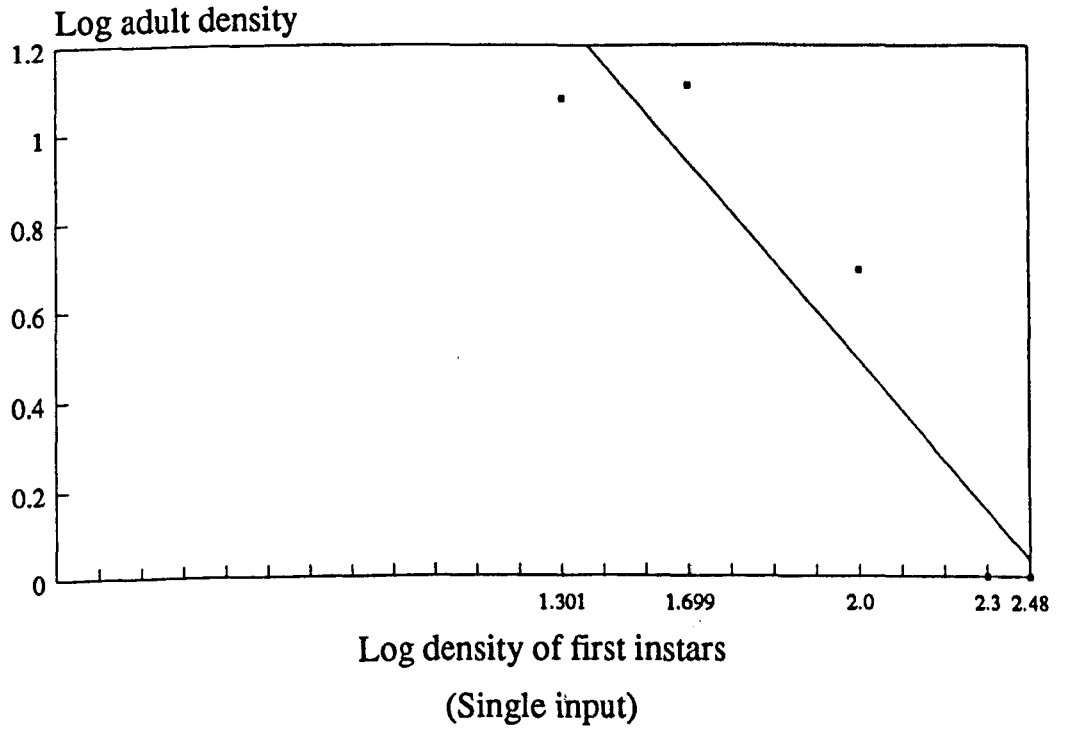


Figure 9.5b: Regression of log adult density on log density of first instars



95% confidence limits of regression coefficient = -1.898 and -0.268

t = 8.13 df = 3 P = 0.00389 **

In this case both regression lines were significantly different from a slope of b = 1 and lay on the same side of b = 1. It can therefore be said that there was a genuine causal relationship of survival on density (Rajagopalan *et al*, 1976).

A further attempt was therefore made to find the type of density dependence that was in operation in these cages with single introductions (Viswanathan, *et al*, 1952). For this purpose, a regression of K values on log density of first instars was carried out and the results are shown below:

Y = -4.718 + 2.103X

r = 0.979

95% confidence limits for slope = 1.303 and 2.904

F = 69.86, df= 1, 3, P = 0.000359 ***

The estimated slope of 2.103 (i.e. greater than 1) suggests that in the course of these experiments with single introductions there was an overcompensating density dependence (Varley and Gradwell, 1968; Rajagopalan *et al*, 1976). This means that reductions in the density of first instar larvae would lead to an increase of larval survival greater than that required to keep the adult output constant.

9.4 DISCUSSION

The results of this study have shown that with the exception of first instars, there was a tendency for the duration of each instar to increase with increasing larval density and for some instars, and for all instars combined, this relationship was significant. This observation is in agreement with common experience in laboratory rearing of mosquitoes and in laboratory based studies in *Aedes (Stegomyia) aegypti* and *Aedes (Stegomyia) albopictus* (Moore and Fisher, 1969), in *Anopheles stephensi* (Reisen, 1975) and in *Culex quinquefaciatus* (Ikeshoji and Mulla, 1970). Larval autotoxins, or growth retardant factors have been suggested as the possible causes of

increased instar durations in these studies. These autotoxins are considered to retard larval development, increase mortality, and result in the production of smaller sized adults.

The results of this study also suggested that increase in density reduced the survival rate of immature stages. In the case of the experiment with single introductions the decline in the survival rate was shown to be genuinely density dependent. The type of density dependence was found to be overcompensatory, suggesting the possibility that incomplete larval control might actually be counter-productive in that it might lead to increase in adult output.

Unfortunately, the more realistic situation of multiple larval inputs did not lead to a clear cut result, possibly because the range of input numbers were too high. Thus it remains uncertain whether one should advise against attempting larval control unless complete elimination of larvae can be assured.

CHAPTER 10

EFFECT OF CHANGING WATER LEVELS ON THE APPEARANCE AND DEVELOPMENT OF FIRST INSTAR LARVAE OF *AN. GAMBIAE S.L*

10.1 Changing water levels and appearance of first instars.

10.1.1 Introduction

One of the factors that is thought to influence the population density of *An. gambiae s.l* in temporary breeding sites is change in water level (McCrae, personal comm). Decrease in water level is thought to cause stranding and drying out of eggs. Conversely, if the level rises, the conditions will become more and more favourable unless there is flushing out of the larvae by flooding. Either of these events are likely to affect the recruitment of first instars and hence the age structure of the immature population in a given breeding site at a particular time. In particular, small rises in water level may stimulate the hatching of stranded eggs (this is often seen in the laboratory). If this happens in nature, it is expected that sudden increases in the number of first instars should be associated with rises in water levels of breeding sites as long as there is no flooding. However, this hypothesis has never been tested in the field.

This section describes a field experiment conducted in Kisiwani village, near Ubwari Field Station, to observe the effect of changing water levels on the appearance of first instars. This work was done between April, 1990 and February, 1991.

10.1.2 Materials and Methods

Four puddles measuring 0.5m x 0.5m x 0.3m (depth) were dug out in a seepage area in a valley between Kisiwani village and Ubwari Field Station. The sides of these puddles were slanted to resemble the natural shoreline of such breeding sites. In each of these puddles a pole marked in millimetres was firmly installed in the middle (Figure 10.1). These pits filled with water from seepage and rain water.

The larvae were sampled using a ladle (W.H.O, 1975) and recorded every morning during the study period of 309 days (see Appendix 10 for a sample of recording form). The number of dips to be taken was estimated daily using the method described in chapter 3 and they ranged between 10 and 25. The depth of the water to the nearest millimetre in the puddle was also recorded daily before carrying out the dipping.

Irregular recruitment

The observed differences in water levels between successive days for each pit were placed in three categories. These were: increase (+), no change (0), and decrease (-) in water level. All occasions when there was at least doubling over successive days in the numbers per dip of first instars were noted. Each occasion was then categorised according to whether the water level had risen, fallen, or stayed level during the previous 24 hours (Day -1 to day 0), or during the 24 hours before that (Day -2 to day -1).

10.1.3 Results

The analysed results are shown in Table 10.1.3a and b with separate results for the four puddles and Table 10.1.3c and d with combined data. (More detailed results are displayed later in table 10.2.3). The analysis showed that there was no significant

Figure 10.1: Puddle with a pole in the middle for measuring water levels.

Table 10.1.3a: The relationship between changes in water levels in the last 24 hours and occasions when the density of first instars of *An. gambiae s.l* at least doubled

Puddle No.	Doubling of of density of first instars	No. of occasions when water level				χ^2	P	Signif.
		increased	stayed level	decreased	Total			
1	Yes	15	4	32	51	0.0038	0.9981	ns
	No	45	12	98	155	df= 2		
	Total	60	16	130	206			
2	Yes	14	6	23	43	3.53	0.1712	ns
	No	40	9	95	144	df= 2		
	Total	54	15	118	187			
3	Yes	23	15	29	67	3.3	0.2087	ns
	No	62	26	99	187	df= 2		
	Total	85	41	128	254			
4	Yes	15	14	20	49	0.99	0.6106	ns
	No	57	37	76	170	df= 2		
	Total	72	51	96	219			

Table 10.1.3b: The relationship between changes in water levels between 48 and 24 hours previously and occasions when the density of first instars of *An. gambiae s.l* at least doubled

Puddle No.	Density of first instar doubled	No. occasions when water level				χ^2	P	Signif.
		increased	stayed level	decreased	Total			
1	Yes	12	1	38	51	5.07	0.0794	ns
	No	48	15	92	155	df= 2		
	Total	60	16	130	206			
2	Yes	8	3	32	43	3.25	0.1969	ns
	No	46	12	86	144	df= 2		
	Total	54	15	118	187			
3	Yes	23	8	36	67	1.22	0.5443	ns
	No	62	33	92	187	df= 2		
	Total	85	41	128	254			
4	Yes	18	17	14	49	7.17	0.0278	*
	No	54	34	82	170	df= 2		
	Total	72	51	96	219			

Table 10.1.3c: The combined data of the four puddles for day -1 to day 0 and at least doubling of first instars of *An. gambiae s.l*

Doubling of density of first of larvae	Change from day -1 to 0				χ^2	P	Signif.
	+	0	-	Total			
Yes	67	39	104	210	5.02	0.0813	ns
No	204	84	368	656	df= 2		
Total	271	123	472	866			

Table 10.1.3d: The combined data of the four puddles for day -2 to day -1 and at least doubling of first instars of *An. gambiae s.l*

Doubling of density of first instar larvae	Change from day -2 to -1				χ^2	P	Signif
	+	0	-	Total			
Yes	61	29	120	210	0.83	0.6598	ns
No	210	94	352	656	df= 2		
Total	271	123	472	866			

association between the changes in water levels in the previous 24 hours (day -1 to day 0) and doubling in the number of the first instars in any of the puddles. The same applied to water level changes in the 24 hours before that (day -2 to -1) except for the puddle no. 4 in which there was negative association (χ^2= 7.17, df= 2, P < 0.05). This suggested that when the water level remained unchanged there was more egg hatching than when it increased or decreased. However, when columns "+" and "-" or "0" and "-" were combined this association disappeared. There was therefore no evidence to suggest that recruitment of first instars, which was certainly very irregular, was more associated with unchanging water levels or increases in them.

Likewise, the 2 x 2 Mantel-Haenszel summary χ^2 did not show any significant association between an increase in water level and doubling of the numbers of first instars (M-H χ^2 = 0.01, P > 0.05 for day -1 to 0), and for day -2 to -1 M-H χ^2 = 0.49, P > 0.4852).

10.1.4 Discussion

These results show that in the four puddles studied over a period of about ten months on a daily basis, rising water levels did not seem to produce sudden increases in the numbers of the first instars of *An. gambiae s.l* on successive days or in the last two days. While the observed significant association in only one of the four puddles can be considered not to be a general pattern, the results seem to suggest that there was a negative association between changes in water levels and sudden increases in the number of first instars. However, when "0" and "-" or "+" and "-" were combined the association disappeared. Although no attempt was made to look for stranded eggs, it is possible that a day of exposure for eggs was long enough for them to be killed by drying or predation. Therefore, rising water level would not lead to any significant changes in the numbers of first instars. The sudden changes in the density of first instars were perhaps caused by rapid development of the embryos in the eggs due to increase in temperature or other unknown environmental factors leading to more output of first instars. More probably, they were caused by irregular oviposition, related to short term changes, due to unknown factors, in the attractiveness of the puddles to gravid females.

10.2 Estimation of mortalities of immature stages of *An. gambiae s.l*

10.2.1 Introduction

The factors regulating mortality in mosquito populations are discussed by Service (1976, 1985). Estimation of mosquito mortality and survival rates has been attempted by Service (1971, 1973), Lakhani and Service (1974), Southwood *et al* (1972) and Reisen and Siddiqui, 1979; Reisen *et al.* 1982, 1989). The knowledge of mortality and survival rates is important particularly in the planning of biological control measures. This is because if a mosquito population is regulated by strong density dependent mortality, the control measures may lead to an adult population which is equal to or even larger than it would have been without control (Service, 1985b).

The only reported work on the study of mortality rates of *An. gambiae s.l* immature stages is that of Service, (1971, 1973, 1977). In this set of studies "life-tables" were used (Varley and Gradwell, 1970) to summarise both mortality and survival rates. A life-table should be based on a continuous and intensive study of a population in one place (Varley and Gradwell, 1970) and not sampling a number of similar habitats in different years. Time-specific or vertical life-tables are used for populations with overlapping generations while age-specific or horizontal life-tables are used to study populations with discrete non-overlapping generations, by tracing the fate of individual cohorts (Service, 1976). The type of life-table that was used for *An. gambiae s.l* was the time-specific life-table.

There are limitations to the reliability of the results analysed by Service (1971, 1973, 1977). First, the results were based on a few days of observation (usually not more than 11). Indeed, it was stated in the discussion that time specific life-tables calculated from such representative samples are usually less reliable (Service, 1977).

Secondly, although the estimation of instar durations was done both in the laboratory and in the field (Service, 1973), they add up to a surprisingly long duration of development of immature stages (11-12 days) for *An. gambiae s.l.* These cannot be representative of instar durations of this species. Likewise, the field method that was used for estimation of instar duration is not described by Service (1971, 1973, 1977). If estimations of instar durations were done by holding larvae in a container beside a breeding site as described by Service (1993), that could be an explanation for long estimates of instar durations.

While no attempt was made to estimate instar durations or instar mortality in this study, a description is given of the pattern of age distribution of the immature stages in the breeding sites, in four different puddles between April, 1990 and February 1991. The overall age distributions obtained are used to show that, owing to short term fluctuations in age-distribution, instar mortalities estimated using data collected over short periods may be misleading.

10.2.2 Materials and Methods

Sampling for immature stages was done daily during the period of study as described in section 10.1.2 above. The mean number per dip of larvae and pupae combined was calculated for each puddle.

The combined means and variances were used in the estimation of the degree of dispersion of the larval and pupal populations in the breeding site as that was one of the essential underlying factors for explaining the observed distribution. This was achieved by calculating the coefficient of dispersion (CD) as the ratio of the variance (s^2) (of the calculated overall means of larvae and pupae per dip) to the mean (m)ie. $CD = s^2 / m$ (Service, 1971; Reisen, 1982; Thompson, 1983). If the CD is greater, equal to or less than the mean then the population is said to have a contagious, random or regular distribution respectively (Thompson, 1983). A regular distribution, with even-spacing of individuals is considered to be a rare phenomenon among insects. Most studied pests and vectors have been found to have a contagious type of distribution.

An attempt was made to relate the observed overall age distribution of the immature stages in the four puddles to their distribution over short periods. This was done by plotting histograms of the number per dip of the immature stages collected on each day of a set of selected successive days.

10.2.3 Results

(a) Dispersion characteristics

The overall coefficients of dispersion of *An. gambiae s.l* for the four puddles were as follows:

Puddle 1: mean = 1.21
s^2 = 2.56
CD = 2.12

Puddle 2: mean = 1.51
s^2 = 5.06
CD = 3.35

Puddle 3: mean = 1.22
s^2 = 1.96
CD = 1.61

Puddle 4: mean = 0.7
s^2 = 0.94
CD = 1.34

These results show that the CD values were consistently greater than unity in all the puddles. This suggests that the larval distribution in these puddles was contagious and not a random (Poisson) distribution.

(b) Comparison of overall age structure of *An. gambiae s.l* and age structure over

shorter sampling periods.

The age distributions of the larvae and pupae observed in the different puddles are shown in Table 10.2.3. The overall age structures, shown in figure 10.2, are much the same in all the puddles, although puddle number four had smaller numbers of immature stages collected than the other three. The confidence limits of the means were rather wide, being a result of clumped distribution as seen from the dispersion coefficients discussed above.

The overall age structure shown by histograms of mean number per dip for the different instars gives the impression of a smooth transition from one stage to another. However, Table 10.2.3 indicates that recruitment is very irregular. There are long periods in each puddle when the number of newly appearing first instars is very low or zero, and other periods when large numbers of first instars appear within a few days of each other. It is therefore likely that during any short period of a few days, age-structure of the larvae sampled will not resemble that of samples pooled over a long period, such as those in figure 10.2.

Table 10.2.3 also shows that some of these cohorts of newly recruited first instars survived much better than others. Examples of this are indicated in the table within boxes; those that survived well to late-stage larvae and pupae are shown in bold, and those that survived less well in italics. It is also noticeable that when first instars appeared after a long period of absence (in some cases due to the puddle drying up), pupae were sometimes observed surprisingly soon - 6 days or even less - afterwards. Examples can be seen on days 111-115 (puddle 2), days 131-135 (puddle 4), and days 206-210 (puddle 3).

10.3 Discussion

The observation that *An. gambiae s.l* had a clumped distribution agreed with that of Service (1971). This contagious type of distribution emphasises the need for either sampling numerous breeding sites or, if the sites are few, to take many samples from

TABLE 10.2.3 Longitudinal observations of the age distribution of *An. gambiae s.l.* larvae in four puddles. Sampling took place daily over 300 days, but about half the data have been omitted (indicated by rows of dots). For each puddle the table shows the water level in centimetres and the relative density of each instar. On most occasions 10 dips were taken. For clarity the figures have been standardised and rounded to numbers of each instar per 10 dips, although on some occasions 15 or 20 dips were actually taken. Figures bracketed by asterisks highlight in bold type occasions when a high proportion of newly recruited first instars survived to become fourth instars, and in italic type when a low proportion of them did so.

	PUDDLE 1						PUDDLE 2						PUDDLE 3						PUDDLE 4					
DAY	WATER LEVEL	INSTAR 1	2	3	4	P	WATER LEVEL	INSTAR 1	2	3	4	P	WATER LEVEL	INSTAR 1	2	3	4	P	WATER LEVEL	INSTAR 1	2	3	4	P
18	28.6	20	1	1	0	0	26.4	3	0	1	0	0	25	9	2	0	0	0	12.6	4	1	3	1	0
19	28.6	2	1	0	0	0	25.8	1	2	0	0	0	26.8	4	4	0	0	0	12.8	2	1	0	0	0
20	28.6	0	1	0	0	0	25.6	0	1	1	1	0	27	1	1	0	1	0	13	0	1	0	0	0
21	28.6	0	0	3	0	0	25.6	1	1	1	0	0	26.8	0	0	0	0	0	13.2	0	0	0	0	0
22	28.6	0	0	0	0	0	25.8	1	0	0	0	0	26.8	* 3	0	0	0	0*	13.4	2	0	0	0	0
23	28.4	1	1	1	0	0	25.8	1	0	0	0	0	27	* 7	2	0	0	0*	12.8	2	1	0	0	0
24	28.4	0	0	0	0	0	25.2	1	0	0	0	0	27.2	* 7	7	5	1	0*	13.1	1	1	0	0	0
25	28.4	3	1	0	0	0	25.4	4	1	0	0	0	27	*11	7	1	0	0*	12.8	2	1	0	0	0
26	28.2	0	1	0	0	0	25.2	1	0	0	0	0	27.2	* 4	2	1	1	0*	13	1	1	0	0	0
27	28.6	5	2	0	0	0	25.4	1	0	0	0	0	27.2	* 8	12	30	1	0*	13.8	6	6	3	0	0
28	28.8	9	2	1	0	0	25.2	3	0	0	0	0	27	*21	6	13	9	0*	13.5	7	3	2	1	0
29	27.6	3	8	2	2	0	25.2	5	1	2	0	0	27.2	*11	2	1	2	0*	13.2	5	3	0	0	0
30	26	*15	5	0	0	0*	24.6	13	3	0	0	0	27.2	* 4	7	0	7	0*	13.8	0	5	6	0	0
31	24	*26	2	2	0	0*	22.2	24	5	1	0	0	27.2	* 8	2	0	0	0*	13.8	1	1	1	1	0
32	21.6	* 7	0	0	0	0*	20	11	3	0	0	0	27.6	* 3	0	1	0	0*	13.8	1	4	0	0	0
33	21.1	*12	9	0	0	0*	20	11	1	0	1	0	27.2	* 1	0	0	0	0*	13.2	5	0	0	0	0
34	20	* 6	5	2	0	0*	18.2	5	6	3	1	0	27.2	3	2	0	1	0	13.2	9	8	2	0	0
35	19.8	* 5	5	1	0	0*	18	6	0	0	0	0	27	0	1	0	1	0	13.4	2	6	0	0	0
36	19.2	*28	1	5	0	0*	17.6	15	0	1	1	0	27.2	5	6	5	4	0	14.2	4	1	1	2	0
37	18	* 4	2	0	0	0*	16	6	1	0	0	0	27.8	3	6	4	3	0	15	0	3	9	2	0
38	17	* 3	3	2	0	0*	14	2	3	0	0	0	27.8	22	5	0	0	0	14	2	1	2	1	0
39	25.8	*22	7	11	1	0*	23.8	20	6	4	0	0	27.4	12	3	4	0	0	14.4	1	2	1	2	0
40	22	* 6	6	1	1	0*	20.6	2	13	1	2	2	27.8	0	6	0	1	0	14.6	1	2	1	2	0
41	19.6	*17	6	1	0	0*	18.3	6	11	6	0	0	27.8	6	1	0	0	0	14.8	2	1	0	0	0
42	22.2	*41	4	5	2	0*	21.4	54	1	3	1	0	27.8	8	0	3	1	0	14.6	4	0	1	0	0
43	19.4	*23	3	3	0	0*	17.8	27	3	2	0	0	27.6	3	3	0	0	0	14.4	3	1	0	0	0
44	16.8	* 6	13	1	0	0*	13.4	5	31	3	0	0	27.4	3	1	2	0	0	14.6	5	0	0	0	0
45	14.8	*11	10	0	0	0*	12.6	11	27	7	1	0	27.6	9	7	1	0	0	14.6	1	6	0	0	0
46	14.6	*10	1	4	0	0*	12	8	1	5	1	0	27.4	2	4	2	1	0	14.6	11	7	2	0	0
47	14	16	11	3	0	0	11.8	9	3	5	1	0	27.4	11	3	0	0	0	14.6	8	5	2	0	0
48	11.6	4	2	1	0	0	11.6	10	18	0	0	0	27.4	0	3	5	6	0	14.6	2	4	1	0	0
49	14	6	7	3	1	0	11.2	38	4	7	0	0	27.4	10	0	5	3	0	14.6	12	7	3	1	0
50	14	14	3	2	0	0	12.2	13	1	0	0	0	27.6	15	0	0	1	0	14.4	4	3	1	0	0
51	13.6	12	2	0	0	0	10.6	38	17	0	3	0	27.8	26	11	0	2	0	14.6	7	4	1	0	0
52	13.2	1	4	0	0	0	9.6	5	12	3	0	0	27.6	5	9	2	0	0	14.8	2	5	1	1	0
53	14.8	*43	4	4	0	0*	12.4	43	7	13	0	0	26.4	9	7	12	0	0	14.4	11	5	0	2	0
54	23.8	*24	29	6	3	0*	22.2	64	67	25	5	0	27.2	12	7	4	2	0	14.6	2	1	2	0	0
55	24.2	*12	15	27	1	0*	27.2	33	29	22	3	0	27.4	9	6	11	8	0	14.6	4	3	6	5	0
56	21.8	*20	47	42	15	0*	21.8						27.6	3	2	1	4	0	14.8	0	0	0	1	0
57	19.8	* 8	10	13	12	4*	16.2	4	10	15	4	0	27.8	6	4	3	5	0	14.6	6	3	1	1	0
58	18.6	* 6	1	6	13	0*	15.8	0	11	10	1	1	27.8	0	3	2	0	0	14.8	2	1	0	0	0
59	28.6	*35	3	0	1	0*	24.8	5	0	0	0	0	27.8	1	0	0	0	0	14.8	0	0	0	0	0
60	28.8	* 2	14	2	1	2*	24.8	7	4	7	5	0	27.4	0	0	0	0	0	14.8	1	1	2	0	0
61	29.8	* 0	5	7	0	0*	24.8	1	14	5	6	11	27.6	1	5	0	0	0	14.8	1	0	1	0	0
62	26.8	* 0	2	16	6	1*							28.8	0	1	1	0	0	14.9	0	0	0	0	0
63	25.8	* 4	9	19	22	0*	21	14	14	54	13	3	28.8	3	5	0	0	0	14.8	0	0	0	0	0
64	23.8	*11	0	2	6	0*	19.8	3	8	31	16	0	28	4	7	0	0	0	13.8	0	1	0	0	0
65	22.4	6	3	1	5	0	18.2	6	3	1	8	0	28	0	1	0	0	0	13.8	0	0	0	0	0
66	21.2	3	5	1	5	0	16.2	15	10	3	6	0	29	4	0	0	2	0	13.8	2	1	0	0	0
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

TABLE 10.2.3 continued

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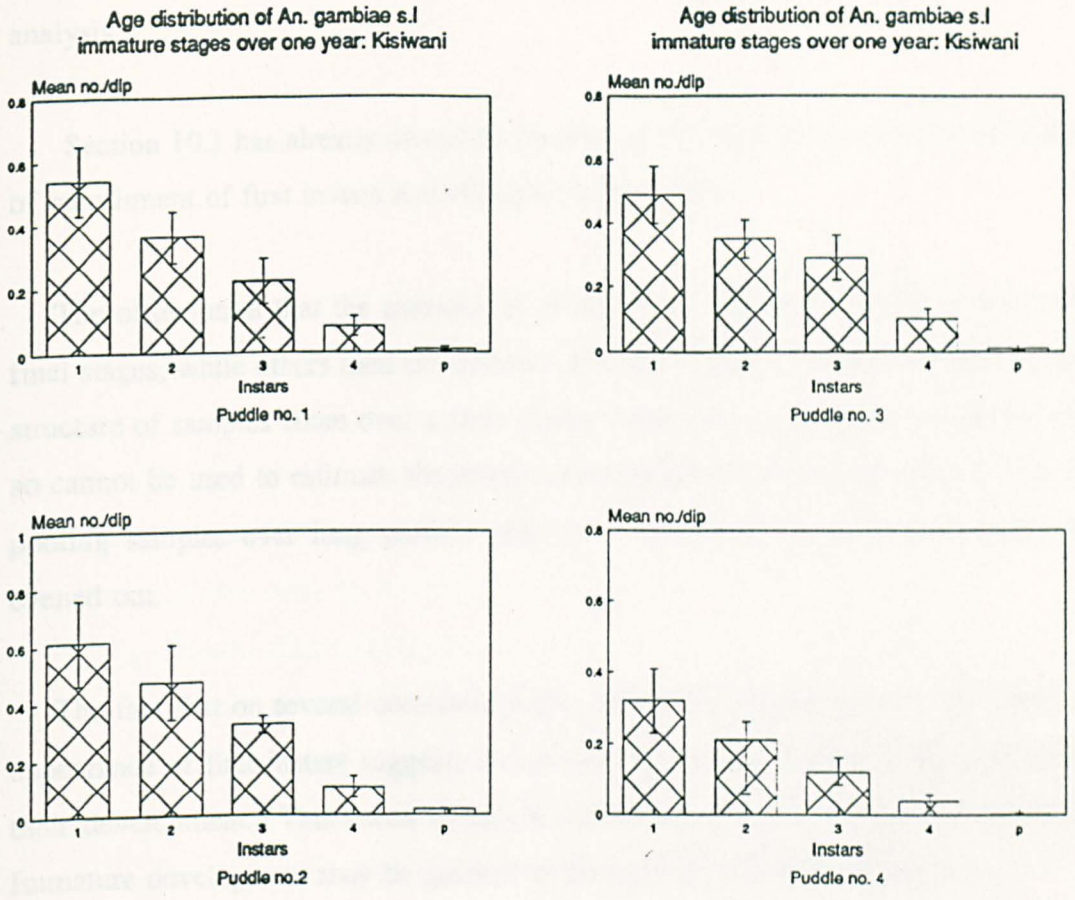
74	19.8	6	2	2	0	0	14	6	6	9	1	0	27.8	1	1	2	2	0	12.6	1	0	3	1	0
75	18.4	3	2	0	0	0	13.8	6	2	0	0	0	28.2	2	0	0	0	0	12.8	1	1	0	0	0
76	16.8	2	2	1	0	0	12.8	0	3	0	0	0	28.8	11	4	2	0	0	12.6 *	0	1	0	0	0*
77	15.6	6	0	0	0	0	12.6	2	1	0	0	0	27.8	22	8	0	0	0	12.6 *	3	2	0	0	0*
78	15.8	1	1	0	0	0	11.8	1	0	0	0	0	27.8	7	3	0	0	0	12.6 *	56	2	0	0	0*
79	15.6	1	1	1	0	0	11.8	4	0	0	0	0	27.8	4	5	0	1	0	12.6 *	38	2	0	1	0*
80	15.6	12	2	0	0	0	12.8	2	0	0	0	0	27.8	0	1	1	0	0	12.6 *	18	13	0	0	0*
81	15.6	6	1	0	0	0	12.8	4	0	0	0	0	27.8	2	1	0	0	0	12.4 *	7	6	12	0	0*
82	15.8	0	3	3	0	0	12.8	6	1	1	0	0	27.2	1	1	3	1	0	12 *	0	4	7	0	0*
83	14.6	9	0	1	0	0	12.6	7	1	4	0	0	26.6	0	4	0	2	0	11.6 *	0	1	1	0	0*
84	14.8	9	0	0	0	0	11.4	0	2	0	0	0	26.8	2	1	1	1	0	11 *	2	1	4	0	0*
85	14.6	1	2	0	0	0	10.6	0	2	0	0	0	26.8	4	0	0	0	0	10.8 *	6	2	0	0	0*
86	14	2	0	1	0	0	10	0	0	0	0	0	25.8	4	2	1	1	0	10.6 *	8	1	0	0	0*
87	15.6	0	0	0	1	0	10.8	0	0	0	0	0	21	3	0	0	0	0	11.8 *	8	0	0	0	0*
88	14	6	3	0	0	0	10.8	0	0	0	0	0	25.8	0	1	1	0	0	10.2 *	7	6	0	0	0*
89	14	1	0	0	0	0	10.8	2	2	0	0	0	25.4	0	0	1	0	0	10.2	3	2	1	0	0
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
102	13.8	0	0	0	0	0							18.4	0	1	0	0	0	2.6	0	0	0	0	0
103	12.6	1	0	0	0	0			dry				18.4	2	0	0	0	0	2.8	2	0	0	0	0
104	8.4	3	0	0	0	0							17.4	4	0	0	0	0	0.6	0	0	0	0	0
105	25.8	8	1	0	0	0	0.8	0	0	0	0	0	20.2	13	5	0	0	0	6.8	1	1	0	0	0
106	17.9	0	2	0	0	0	1	0	0	0	0	0	22.2	6	2	0	0	0	7.4	1	1	0	0	0
107	16.8	1	3	0	0	0	1	0	0	0	0	0	22.8	4	7	2	0	0	5.8	3	1	0	0	0
108	18.3	0	0	0	0	0	1.6 *	0	0	0	0	0*	24	1	6	1	0	0	8.3	0	2	0	1	0
109	16.2	0	0	0	0	0	6.2 *	0	0	0	0	0*	23.4	0	1	5	3	0	6	0	0	0	0	0
110	6.2	2	0	0	0	0	4.2 *	0	0	0	0	0*	21.8	0	3	0	4	0	2.8	2	0	0	0	0
111	12.8	4	2	0	1	0	12.2 *	9	0	0	0	0*	25.8	0	0	2	6	0	9.8	5	0	1	0	0
112	15.8	16	7	0	5	2	14.8 *	8	1	0	0	0*	28.2	16	0	3	6	0	12.6	2	0	0	0	0
113	14.3	2	7	5	0	0	23 *	0	2	2	0	0*	28.4	8	0	0	2	0	13	2	3	1	0	0
114	12.6	0	4	2	0	0	11.2 *	1	1	5	0	0*	28.4	12	3	0	1	0	11.8	1	1	2	0	0
115	9.6	0	0	1	2	0	7.4 *	1	1	1	4	0*	27.8	13	0	0	3	0	9	2	2	0	5	0
116	8.8	3	2	2	2	0	7.2 *	0	0	2	5	1*	26.8	8	13	1	0	0	7	2	0	2	0	0
117	8	4	0	1	2	0	5.6 *	1	0	1	0	0*	24.8	3	13	2	0	0	5.2	0	0	0	0	0
118	6.6	9	0	0	0	0	4.8 *	0	1	1	1	0*	23.8	6	9	1	2	0	4.4	0	0	0	0	0
119	7	2	0	0	0	0	5.4 *	0	0	0	0	0*	22	2	4	3	1	1	4	0	1	0	0	0
120	5.8	5	1	0	0	0	2.8 *	1	0	1	0	0*	18.8	0	0	1	0	1	2	1	0	1	0	0
121	5	2	0	0	0	0	2.5	0	1	0	0	1	19	1	1	3	1	0	3	0	0	1	0	0
122	3.8	1	1	0	0	0							17.7	1	2	1	0	0	2	0	0	0	0	0
123	1	0	0	1	0	0							16	0	0	0	0	0	1.6	0	0	0	0	0
124	0.8	2	7	5	0	0							10	2	0	0	0	0	0.6	0	0	0	0	0
125	0.6	2	1	0	1	0			dry				9.7	3	0	1	0	0	0.8	0	0	1	1	0
126	1.8	3	0	0	0	0							9.7	1	0	0	0	0	0.6	0	0	0	0	0
127	3	1	0	0	0	0							10.2	3	0	0	0	0	0.6	0	0	0	0	0
128	5.7	1	0	0	0	0							11	3	0	0	0	0	2	0	0	0	0	0
129	5	2	1	1	0	0	1	0	0	0	0	0	10.8	2	0	0	0	0	1	0	0	0	0	0
130	10	0	0	1	0	0	5.2	0	0	0	0	0	14.2	0	1	0	0	0	4.4	0	0	0	0	0
131	9.8	0	2	0	1	0	5.4	0	0	0	0	0	15.6	0	3	0	0	0	4.6	3	0	0	0	0
132	10.6	1	0	0	0	0	5	2	0	0	0	0	16.4	2	0	0	0	0	4.6	1	0	0	0	0
133	9.4	5	1	0	0	0	4.8	1	0	0	0	0	16.8	9	0	3	0	0	4.7	1	2	0	0	0
134	10	7	0	1	0	0	5.2	4	0	2	0	0	17 *	13	0	1	0	0*	5	1	0	0	0	0
135	12	16	3	1	3	0	11.2	15	3	0	2	0	21.6 *	25	6	0	1	0*	11	9	4	0	1	0
136	10.8	8	8	0	1	0	9	13	1	0	1	2	22 *	20	9	0	0	1*	10.8	13	6	1	1	1
137	10	9	3	6	1	0	7	2	4	0	0	0	21.9 *	18	7	0	0	1*	9.2	0	1	0	0	1
138	9	1	2	2	0	1	6	3	1	0	1	0	20 *	12	14	13	0	0*	7.7	1	2	6	1	0
139	8.4	1	1	0	0	0	4.4	1	2	2	1	0	28.2 *	11	7	7	0	0*	4.5	3	2	3	0	0
140	3.6	2	0	1	0	0	0.7	13	3	0	0	0	14.7 *	9	2	3	0	1*	2.4	1	0	0	0	0
141	0.4	3	2	2	2	0							11.3 *	6	0	1	0	0*	0.8	0	0	0	0	0
142	7.6	2	4	0	0	1	2.6	1	2	0	0	0	13 *	10	6	5	0	0*	2.7	0	0	0	0	0
143	5.1	0	0	0	0	0	1	0	0	1	0	0	11.8 *	3	2	0	0	0*	2.3	1	0	0	0	0
144	2	0	0	0	0	0							10.1	2	12	7	3	0	1.8	0	0	0	0	0
145	0.8	2	21	2	0	0							9.7	9	1	2	0	0	1.6	1	0	0	0	0
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
153		dry											2.8 *	0	2	0	0	0*						
154													6 *	1	0	0	0	0*						
155	2	0	0	0	0	0			dry				10.8 *	21	2	0	0	0*						
156													9.2 *	1	20	10	0	0*						
157													8 *	7	10	49	0	0*						
158		dry											6.8 *	3	2	16	0	0*						
159													5 *	0	2	10	0	0*						
160													5.8 *	0	1	0	0	1*						
161	6.6	0	0	0	0	0	4	0	0	0	0	0	10.2	0	1	2	0	0	4	0	0	0	0	0

TABLE 10.2.3 continued

162	11	0	0	0	0	0	6.6	0	0	0	0	0	16	1	3	1	3	0	7.4	0	0	0	0	0
163	5.6	0	0	0	0	0	2.8	0	0	0	0	0	13.6	0	0	4	2	0	4	0	0	0	0	0
164													10	0	0	1	1	1						
165													7.6	0	2	1	1	1						
166																								
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
204		dry						dry						dry						dry				
205																								
206	10.7	0	0	0	0	0							13 *	1	0	0	0	0*	9	0	0	0	0	0
207	8.4	0	0	0	0	0	6	0	0	0	0	0	10.4 *	0	0	0	0	0*	2.8	0	0	0	0	0
208	5	1	0	0	0	0							8.2 *	3	0	0	0	0*	2.1	0	0	0	0	0
209	13	8	0	0	0	0	2.4	0	0	0	0	0	14.8 *	35	6	1	0	0*	11	21	0	0	0	0
210	12.2	1	6	1	0	0	8	0	0	0	0	0	14.4 *	1	23	2	1	0*	9	0	12	0	0	0
211	7.8	0	4	16	0	0	7	0	0	0	0	0	11.6 *	0	2	16	2	0*	4	0	0	16	0	0
212													8 *	0	0	7	2	0*						
213													6 *	0	1	4	10	0*						
214													2.8 *	0	0	0	4	1*						
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
236	18	25	1	1	2	0	14	13	5	6	3	0	21	18	13	1	5	0	16	17	33	1	0	0
237	17	12	19	10	3	0	13	34	1	5	1	0	21.4	9	6	4	1	0	15	18	4	0	5	0
238	14	1	1	0	0	0	13	15	11	9	1	0	21	0	6	3	2	0	15	5	15	10	1	0
239	12.4	1	2	1	0	0	11	4	3	1	0	0	20.4	4	13	4	3	0	15	1	11	10	2	0
240	11	* 5	8	0	1	0*	9	1	3	0	0	0	20	15	15	7	7	0	15.2	10	10	7	1	0
241	9.8	* 0	5	0	0	0*	7.8	2	4	0	2	0	19.8	9	7	9	4	0	15.2	3	1	3	1	0
242	9.2	*10	15	1	0	0*	6.2	0	1	1	0	0	19.1	61	27	17	3	0	15	7	7	3	5	0
243	18.8	*39	16	1	1	0*	6	9	12	0	0	0	21.6	11	9	5	1	0	15.6	3	8	2	1	0
244	21.4	* 7	33	9	0	0*	14	17	25	7	1	0	21.7	3	12	4	0	0	15.6	2	2	1	1	0
245	22.4	* 4	13	27	0	0*	17.6	7	43	23	1	0	21.6	0	3	9	0	0	15.2	0	5	20	7	0
246	22.7	* 5	2	7	0	0*	17.1	6	18	41	3	0	21.7	21	7	9	7	0	15.4	9	5	6	11	0
247	19	* 3	17	5	1	0*	18	9	2	11	3	0	21.4	2	1	1	5	0	15	0	1	3	0	0
248	15	* 1	13	0	0	0*	14.8	3	8	6	0	0	20.6	1	1	2	1	0	14.1	0	0	0	0	0
249	12.6	* 0	3	3	0	0*	11.8	1	3	1	1	0	20.4	5	3	4	1	0	15	4	0	0	1	0
250	9.6	* 1	1	1	0	0*	9.8	1	0	1	0	0	18.6	1	27	5	0	0	15	5	3	0	3	0
251	12.1	* 1	0	0	0	0*	6	0	0	0	0	0	17.4	3	8	23	6	1	15.2	2	11	0	0	2
252	14.4	*28	1	0	0	0*	2	0	1	2	0	0	19.2	24	1	5	2	0	15.6	3	1	1	0	0
253	11	* 3	3	0	0	0*	5.8	7	0	0	0	0	18.4	5	26	11	0	1	15.4	0	0	0	0	0
254	23.8	* 2	1	0	0	0*	3.6	11	5	1	0	0	22	1	1	0	0	0	15.8	5	0	0	0	0
255	23.2	5	6	5	1	0	17.2	1	5	1	0	0	21.6	3	1	1	0	0	15.8	1	1	0	0	0
256	22.4	9	16	2	2	0	15	1	5	7	0	0	21.8	6	7	3	1	0	15.8	3	4	1	0	1
257	19	27	15	23	5	0	14.2	7	7	5	2	0	21.6	11	5	0	1	0						
258	16	5	12	5	0	1	11	27	11	3	13	0	21	5	9	0	0	0	16	1	8	0	0	0
259	19	19	3	15	1	2	7.8	9	11	2	1	3	21.8	1	1	1	0	0	16.2	2	2	3	0	0
260	16	1	9	4	1	0	11.4	33	17	13	1	0	21.5	11	1	0	0	0	16.2	2	0	1	0	0

Figure 10.2: Overall age structure of *An. gambiae s.l* immature stages in four simulated puddles in kisiwani.



different points in the sites in order to get more representative samples for further analysis.

Section 10.1 has already discussed the absence of association between the bursts of recruitment of first instars and changing water levels.

The observation that the majority of cohorts took less than a week to reach the final stages, while others died off midway through development shows that the age-structure of samples taken over a short period cannot be taken as representative, and so cannot be used to estimate the relative survivorship of different instars. Only by pooling samples over long periods will short term fluctuations in recruitment be evened out.

The fact that on several occasions pupae appeared only six days or less after the appearance of first instars suggests that confining larvae in cages artificially delays their development. This could be due to restriction of access to food or to shade. Immature development may be quicker in the field than in the laboratory.

The observation that some cohorts of newly recruited larvae gave rise to the subsequent appearance of many fourth instars and pupae, whilst other cohorts died off completely before this stage, suggests that survival varies greatly between cohorts. It would be of some interest to know what the factors determining this might be.

CHAPTER 11

GENERAL DISCUSSION

11.1 The need to re-consider larval biology in relation to mosquito control

Since the advent of residual house spraying with DDT against adult anopheline mosquitoes in the mid-nineteen forties and organophosphate compounds in the 1950s very little has been done on the biology and ecology of anopheline immature stages. Increasing refusals of householders to allow spraying, increasing costs of insecticides, campaigns by environmentalists against the use of insecticides, and resistance of vectors to some insecticides have all led to the conclusion that chemical insecticides can no longer be solely relied upon for vector control (WHO, 1982, 1983). Among the strategies that may be integrated with chemical control is biological control of the immature stages of mosquitoes (WHO, 1982). However, since the relationship between biological control agents and the target vector population may be complex, thorough understanding of the population dynamics of the immature stages of the vectors is now considered to be a prerequisite for successful biological vector control programmes.

In the following sections the results of the present study on the ecology and control of the immature stages of anopheline and culicine mosquitoes are discussed. Also discussed is the relationship between density of immature stages of *An. gambiae s.l* and abundance of the adult population.

11.2 The effect of changing water levels on the appearance and development of first instar larvae of *An. gambiae s.l*

As shown in chapter 6 the most productive breeding sites of *An. gambiae s.l* are footprints and puddles. These relatively small water bodies are more temporary than the other sites. As a result, predators such as Notonectids and dragonfly larvae do not become successfully established in such sites. Service (1971, 1973) identified a numbers of predators and was of the opinion that they could be the most important limiting factor in the survival of mosquito immature stages, although no quantitative data were given to relate percent survival and density of predators. A long list of pathogens, parasites and predators of medically important arthropods has been given by Jenkins (1964). Although it is accepted that the presence of predators causes significant reduction in the number of immature stages of mosquitoes in more permanent sites, this does not apply to footprints and puddles and mortality in these small temporary sites must be caused by other mortality factor.

Puddles and footprints tend to dry out very often, and where they do not dry out, their water levels change considerably. This has led to an hypothesis that mortality by stranding of immature stages could be among the major causes of mortality in *An. gambiae s.l.* (A. McCrae, personal comm.). Such mortality, would be density independent (i.e. could not regulate the population). Conversely, it has been suggested that sudden increases in the number of first instars is associated with hatching of stranded eggs as the water level rises (Lines, personal comm.). A longitudinal study was conducted in Muheza to determine whether there was an association between increase in water levels and sudden increase in the number of first instars. This is reported in chapter 10.

The results show that there was no association between increase in water level and increase in numbers of the first instars. It is therefore suggested that absence of significant association is due to the early death of stranded eggs before the rising

water level reaches them. Eggs of *An. gambiae s.l* are known to be susceptible to desiccation (Gillies and De Meillon, 1968). Likewise, the hatching of eggs on damp surfaces, would expose the first instars to the risk of desiccation and predation. Probably sudden changes in the observed density of larvae are due to rapid development of eggs due to increases in water temperature and perhaps other unknown factors. The absence of association between rising water levels and the appearance of first instars, tends to support the view of McCrae, that stranding of eggs is one of the principal causes of mortality in *An. gambiae s.l.* Therefore, it is suggested that a significant proportion of eggs are killed before hatching, and that drought is one of the most important causes of mortality in *An. gambiae s.l* breeding sites. To test whether stranded eggs die before they are reached by a rising water level, it would be interesting collect the eggs at shorter intervals and monitoring their viability in the laboratory.

In the same experiment it was found that the age structure of immature stages vary so much that estimating mortalities of these immature stages over a short period and attempting to generalize about the population dynamics of *An. gambiae s.l* can be quite misleading, contrary to the view of Service (1971, 1973, 1977). That is, in the puddles used for this study, the majority of cohorts of immature stages completed development in about six days, others took up to 8 days, and others never went beyond the first or second instar. It should be stressed that pupal stages of *An. gambiae s.l* appear to be under-represented in samples, probably due to their evasive behaviour. It could therefore be misleading to deduce the mortality of fourth instars from the number of pupae collected as Service attempted to do (1971, 1973, and 1977). Service (1971, 1973, 1977), reported that the total mean duration of all the instars was 11.77 days. It is not clear exactly how this was estimated, but it was shown in the present study that in the field *An. gambiae s.l* can take a shorter time to develop from first instars to emergence, sometimes as little as 6 to 8 days. This finding is important in terms of *An. gambiae s.l* larval control since leaving intervals of 6-8 days between larviciding with non residual agents would allow development of immature stages to adults.

11.3 Quantitative estimates

11.3.1 Can larval control measures be evaluated quantitatively?

Proper identification of the mosquito breeding sites by the personnel working on control programmes of the immature stages is one of the most essential requirements in such operations. However, classification of breeding sites has for many years been done arbitrarily thus requiring subjective interpretation of previously published descriptions. As explained in chapter 2 various workers have classified anopheline mosquito breeding sites differently on the basis of various environmental features that were considered important in a given location. For example, Boyd (1930) categorised anopheline breeding sites into three major groups for use in the southern United States. These were as follows: (a) Rain water or surface drainage (b) Sites receiving both surface and ground water (d) Seepage or underground water. In this case emphasis was put on the source of the water in the breeding sites. While this type of classification may have been useful in the place it was designed for, the criteria used to distinguish individual breeding sites such as puddles, pools, ponds and lakes, etc remain unclear. In common usage these terms are not precisely defined. Nonetheless, this and other classification systems (e.g those of Shannon 1931; Bates 1949; Hopkins 1952; Laird 1956; Laird 1988 and others mentioned by Laird 1988) rely on subjective interpretation of the meanings of these terms. To try to overcome this problem, proposed objective definitions were given for various types of breeding sites in Chapter 3 of the present study. Only six categories of breeding sites were distinguished. These were footprints, puddles, pools, ponds,streams and swamps. These categories of breeding sites were arrived at by characterising the water bodies on the basis of their depth and to some degree, their area and the presence of vegetation. Other environmental features found in these mosquito breeding sites were noted and used as a guide, but not for the classification of the mosquito breeding sites. This type of breeding site categorisation was found to be simple and easily understood by

the field assistants. This conforms with Laird (1988), who emphasized that fewer categories are likely to be remembered more easily and are less likely to be confused by field assistants. It is believed that a reasonable degree of consistency in the categorization of individual breeding sites was achieved in the present study.

Sampling for immature stages of anopheline and culicine mosquitoes has generally employed a ladle for dipping (WHO, 1975). One problem that is associated with dipping is to decide on the number of dips to be taken from a particular type of breeding site. The numbers of dips to be taken from a breeding site has usually remained at a discretion of the personnel involved in sampling. Multiples of 10 dips have been suggested (WHO, 1975), but the criteria for deciding how many multiples of 10 dips has been left obscure. While the more samples taken the more reliable the estimated population density becomes, personal observation has shown that as more samples are taken the probability of misidentifying and miscounting the immature stages by field workers increases because they become bored. It is therefore necessary to establish the required number of dips for a specified degree of statistical confidence about larval density in each category of breeding site. On some occasions, fewer dips are required for certain breeding sites and that saves time without seriously affecting accuracy. The results obtained in the estimation of mean number of dips which need to be taken from each category of breeding site show that on the criteria specified in chapter 3, ten to twenty dips were the mean minimum and maximum mean numbers of dips required. It is desirable to re-estimate the numbers to be taken from different types of breeding site in each season. In this respect the opinion of Loomis (1959) that "even the best larval sampling techniques lack the standardization and reliability necessary to make such an effort meaningful on a large scale" is too pessimistic. What is required in practical control operations is a standard procedure that can be adapted to different situations.

Dipping for the immature stages entails dipping from different points in the case of larger breeding sites, but repeated dipping at the same place in smaller breeding sites. The results of an experiment to assess if repeated dipping in smaller breeding sites resulted in increased or decreased catchability of the immature stages showed that

neither of these was the case. Also, in another experiment, it was shown that the time spent under water by the immature stages on being disturbed by dipping was less than the time taken to identify the larvae and record them. Thus, the use of a series of quadrats when estimating the absolute numbers of immatures stages through repeated dipping from the quadrats may not be essential. This conclusion applies to both shallow (up to 0.5m) or deeper breeding sites. These results were obtained with larval and pupal replacement after each dip in the breeding site. Whether the same results would be obtained when larvae are not replaced still needs to be investigated. However, replacement of the immature stages as opposed to removal should normally be used as it keeps the size of the population constant so that,for example, in a larviciding program, changes in population density could be attributed to the effect of the insecticide. Complete removal of immature stages has been shown to reduce considerably the numbers caught in subsequent dips as reported by Le Pelley (1935).

The results reported and discussed in this chapter 3 emphasize that dipping can be relied upon for quantitative estimation of density of immature stages of mosquitoes. The relationship of number of larvae and pupae per dip in terms of absolute population density of immature stages is discussed in the next section.

11.3.2 Estimation of productivity of *An. gambiae s.l* per unit area of water.

As explained above, the abundance of immature stages in the breeding sites is generally measured in terms of number of immature stages per dip (Service, 1976). The information so obtained in successive surveys can indicate whether breeding is increasing or declining in particular habitats. What it does not tell us is which habitat is contributing most in terms of the final aquatic stages or adults of a given mosquito species (Zahar, 1975). An observed low density of larvae, in terms of numbers per dip, does not necessarily imply that that type of breeding site is not important in production of the vector population. The area of the breeding site must be taken into consideration and the estimated number of larvae per dip must be re-interpreted in terms of number of immature stages per unit area.

The results reported in chapter 5 on the absolute estimation of the number of immature stages per unit area of breeding using a quadrat have shown that, although these estimations may be time consuming and therefore not recommended for routine use, occasional estimations are feasible and the results used for estimation of the abundance of immature stages per unit area of different types of breeding site. The estimation of a common calibration factor for all the breeding sites was considered justifiable since the regression coefficients of absolute numbers, on density per dip, of all the sites were significantly positive and had overlapping confidence limits. This was an indication that the estimation of density per unit area of immature stages from numbers per dip in different types of breeding sites was reasonably satisfactory and it remained so even at low densities of immature stages such as those encountered in streams. The use of a common calibration factor is not only simpler but also more reliable, than use of a different one for each type of site because it incorporates the estimations from many sites and so reduces the standard error of the estimates.

The possibility of the estimation of a common calibration factor confirms that dipping for larvae is a simple but relatively accurate estimate of density. Relying on quadrats alone for routine sampling of immature stages of mosquitoes, was suggested by Horsfall (1946), because he found variation in efficiency of collectors and it was difficult to quantify the results obtained by dipping. However, for practical control operations this is not a feasible option. What should be emphasised is good supervision of the field assistants.

An attempt to relate the yield of adult mosquitoes from a unit area of water surface was not successful. Correlation between the number of final stages and the emerged adult mosquitoes was found to be significant except in the case of puddles. However, the problem of mosquitos dying before collection and sinking in the water remained unsolved. It may be that a light trap hung inside a quadrat to draw emergent mosquitoes upward into a collecting bag may reduce the chances of losing mosquitoes by predation or sinking into the water if they die before collection. Service (1976), described a number of aquatic light traps. Chandler and Highton (1975) efficiently collected emerging mosquitoes in a ricefield in Kenya with a light trap. If necessary,

an appropriate attractant such as carbon dioxide to act as a bait might be added to the trap to attract more mosquitoes.

Because of lack of success in accurately quantifying adult output the estimated numbers of fourth and pupal stages from evacuation of quadrats were used for estimating the abundance of *Anopheles gambiae s.l* in different breeding sites as described in chapter 6. The use of these counts is considered to be adequate for planning and assessing the larvicidal activities. The estimation of a calibration factor is desirable for most studies of larval ecology and control and may need to be occasionally checked to see if the estimated values vary from place to place or over time.

The above discussion leads to the conclusion that it is possible to evaluate the effect of larval and pupal control measures more quantitatively than hitherto. Owing to variation in the types of breeding sites of mosquitoes, however, flexibility is essential in the procedures that have been discussed above for use in various situations.

11.4 Effect of density of the immature stages of *An. gambiae s.l* on the production of adult mosquitoes.

Larviciding is one of a series of mortality factors that operate in natural breeding sites against the immature stages of mosquitoes including the predators (Service, 1976; Jenkins (1964). Studies of larval survival in floating cages showed, at least in the single introduction experiment, that there was genuine density dependent mortality in the immature stages of *An. gambiae s.l* (Varley and Gradwell, 1968). That is, as the density of larvae declined there was a decline in mortality. However, the experiment also indicated that density dependence was of the over-compensatory type, i.e. when the larval population is reduced the survival of the remaining immature stages could lead to a population explosion of pupae and adults, greater than in the uncontrolled population (Rajagopalan, *et al.*, 1976). Although, this experiment was rather artificial it gave clearer results than the multiple introduction experiment which was closer to continuous recruitment observed in the field as described in chapter 9. Accepting the

artificiality of the single introduction experiment, the results seem to suggest that if the immature stages are not eliminated completely from the breeding sites there is a risk of an adult mosquito population explosion.

11.5 Can environmental features be used as markers for *An. gambiae, An. funestus, An. coustani* or culicines so that the most productive breeding sites could be identified without specialist advice?

An assessment was made of the association between four mosquito taxa (i.e. *An. gambiae s.l, An. funestus, An. coustani* and Culicines) and each type of breeding site. It was found that any stage of each of these taxa could be readily found in any of the breeding sites, except for the final stages of *An. funestus* (see Table 4.3.1.1.1a) which were seldom found in any site. The shortage of the final stages of *An. funestus* in any of the breeding sites is thought to be due to high mortality in the early larval stages. This contradicts the view of Gillies and De Meillon (1968) who thought that the rarity with which the immature stages of *An. funestus* are caught was due to their tendency to stay submerged for long periods. If this was the case, the low frequency of finding the immature stages would not have been associated with low densities of the adult population. This was not the case. Instead, the adult population densities of *An. funestus* were reported by Magesa *et al.* (1991) to have been smaller than that of *An. gambiae s.l* throughout the 3½ years of the trial of treated bednets in the villages in which the present study was conducted. Indeed, after the introduction of the treated bednets in the villages even fewer *An. funestus* could be caught than in the pre-intervention period (Magesa *et al.*, 1991).

Although it was established that several mosquito species could be found in large numbers in the breeding sites in the village of Mngaza, it was equally important to find out if there was a significant degree of variation in the distribution of the species of mosquitoes among the different types of the breeding site. The breeding sites in which *An. funestus* was most likely to be found breeding, albeit in small numbers, were also discovered. As described in chapter 4, the probability of occurrence of each of the mosquito species in the different breeding sites found in this study varied

significantly (see Tables 4.3a-g).

(a) ***An. gambiae s.l***

The results show that although *An. gambiae s.l* could be found breeding in any category of breeding site, footprints were the most important breeding sites of this species, and these were followed in importance by pools. It appears that in the pools there was a higher mortality of the early stages of this species than in the footprints where the final aquatic stages were also found in significant numbers. The streams and ponds were less likely to contain *An. gambiae s.l.* Assuming that the probability of finding the final stages can be taken to reflect the chances of producing adult *An. gambiae s.l*, then the footprints were by far the most important site for producing the adult mosquitoes particularly in the wet season. The data suggests that the number of breeding sites which are likely to produce any adult *An. gambiae s.l* is considerably less than those found to contain early stage larvae.

Although no clear definition was given of "shallow" and "deep" water bodies, Gillies and De Meillon (1968) stated that *An. gambiae s.l* preferred to breed in shallow breeding sites, which in the present study fit the categories of footprints and puddles. Puddles were found not to support breeding of *An. gambiae s.l* as often as footprints owing to the likelihood of drying out. However, when they appeared in large numbers during the rainy season, they contributed more in terms of the final stages of this species than pools and ranked second to footprints. This was both because puddles contained higher densities of the final stages, and because their total area was more than that of pools. Therefore the importance of a breeding site in terms of supporting the final stages of this mosquito species must take into consideration both the frequency of supporting breeding, the density and the area of the breeding site. In this case footprints and puddles were the most important breeding sites for *An. gambiae s.l* in the rainy season when breeding of mosquitoes was at its peak.

Further evaluation was performed on various environmental features to find which ones had a significant association with the observed abundance of *An. gambiae s.l* in

footprints (Tables 4.3.1.1.2a-o and 4.3.1.1.3a-k). Among the plants, only rice, *Oryza sativa,* and rushes, *Jancus* spp., had a positive association with the final stages of *An. gambiae s.l.* Likewise the absence of vegetation had a significant positive association with the final stages of *An. gambiae s.l,* as opposed to "cover" which showed a negative association with any stage, and in particular with the final stages of *An. gambiae s.l.* Mud, still water, and presence of a sloping muddy margin, all had a significant positive association with *An. gambiae s.l.* On the contrary, shade and speed of water flow, had a significantly negative association with this mosquito species. Although surface dust had a significant association with *An. gambiae s.l,* there was no significant trend observed with increasing quantities of dust.

In the breeding sites the features mentioned above may have interacted with one another. Therefore there may have been a certain degree of confounding effect in the data on the breeding of *An. gambiae s.l.* To investigate this possibility, as described in chapter 4, analysis of data using multiple logistic regression showed that the following features had independent positive influence on the final stages of *An. gambiae s.l:* footprints, suspended mud and sloping muddy margin (see Table 4.3.1.1.4b). Those with negative influence on the final stages were: shade, red scum and streams. This is the first time the sloping muddy margin and red scum have been reported to have an association with the breeding of *An. gambiae s.l.* The observation that other features had a strong independent effect is a confirmation of their role in influencing the breeding of *An. gambiae s.l.* Also it suggests that these features can be more confidently used for locating the places where *An. gambiae s.l* is, or is not, likely to breed.

The association of *An. gambiae s.l* and the features mentioned above may be summarised by stating that, in the study area, this mosquito species breeds to the maximum extent in rice fields where there are many footprints left behind by cultivators. Also *An. gambiae s.l* frequently breeds around the edges of swamps where *Jancus* grows and footprints are normally found. The footprints usually have sloping muddy margins and in most cases were found exposed to sunlight. The water in the footprints was usually stagnant and a footprint usually did not have any

vegetation cover. *An. gambiae s.l*, has long been considered to show great diversity in the types of breeding sites it occupied. For example, Holstein (1954) considered sun-lit pools not to be preferred for *An. gambiae s.l* breeding while Vincke and Parent (1944), and Parent and Demoulin (1945) considered permanent marshes to be the dominant habitats along the Congo River. However, the results of the present study with the aid of a computerised statistical form of analysis clearly show that despite the presence of this species in various breeding sites, considerable numbers of the final stages are restricted to just a few breeding sites as also stated by Gillies and De Meillon (1968). This view is further supported by the observation that *An. gambiae s.l* occupied similar type of breeding sites when it was accidentally introduced into Brazil in 1930s (Soper and Wilson, 1943).

In a larval control programme, it is highly desirable to direct larval control to the most productive breeding sites. From the present work it is clear that footprints and puddles should be the targets. As long as these sites are properly defined they can be easily identified with little expert supervision.

(b) *An. funestus*

As mentioned above, *An. funestus* final stages were the least common category of anophelines in any of the breeding sites. An attempt was made to find which among the breeding sites was associated with *An. funestus* final stages on the few occasions it was encounted. It was found to prefer breeding in pools (see Table 4.1.1.1e). The only vegetation which showed a significant association with this species was *Cynodon*.The general category "plant cover" did not have significant association with *An. funestus*. The other features which had significant positive association with the final stages of *An. funestus* include: water depth, Notonectids and Dragon flies. The features which showed a significant negative association with the final stages include: odour of water, floating dust on the water surface and still water. Mud showed significant association with *An. funestus* but with no detectable trend with increasing quantities of mud, while shade did not have significant association with this species. Multiple logistic regression showed that surface dust, depth and odour had

independent significant positive association with the final stages of *An. funestus* (see Table 4.3.2.1.4b).

The tendency of the final stages of this mosquito species to be found in pools and for any stage to be found in pools and ponds appears to be connected with the permanent nature of these breeding sites. For example, it was most likely that the pools and ponds, which are deeper than the rest of the breeding sites, would stay longer before they dried out. In the course of that period, some vegetation grows in these sites, and Notonectids and dragonfly larvae establish themselves. It appears that *An. funestus* larvae can cope with the predatory behaviour of Notonectids and dragonfly larvae probably by hiding in the vegetation i.e vegetation acts as a refuge (Hopkins, 1952, Service, 1976) although Christie (1959) was of the opinion that breeding sites persisting for several months after rains have stopped, did not favour the breeding of *An. funestus*. While there was no quantitative evidence quoted by Christie (1959) in support of his opinion, the findings of the present statistical study seem to support his view. This is because, despite the association of *An. funestus* with pools and predators (Notonectids and dragon fly larvae), its numbers were very low compared with other species found in similar sites. The growing populations of predators may have been inimical to *An. funestus* survival. The apparent significant negative association of the final stages of *An. funestus* with odour and floating dust appears to be a misleading confounding effect with other features because the results of multiple logistic regression showed that they had significant *positive* association with this species. Nonetheless, odour is a very subjective feature particularly when the water is not too foul. The fact that still water is not particularly preferred by this species suggests that the degree of fouling might be reduced by constant flow of water and so odour appears to have a positive influence. Flowing water has a tendency of being clear. Therefore, as was reported by Evans (1938), *An. funestus* can be said to breed in bodies of clear water.

Shade is not easy to assess objectively. It appears that what matters is not the presence of overhead shade but shade very close to the water, as is produced by grass. Gillies and De Meillon (1968) made similar observations and reported that

overhanging shade from trees or bushes was not as important as that of emergent vegetation. It would therefore be justifiable to regard breeding sites with grass growing in or around them as of a type suitable for *An. funestus*. Categorising such shade quantitatively is difficult, but *An. funestus* may be considered to prefer breeding in shaded sites which contain vegetation, with some degree of water flow, with or without surface dust particles and, due to stagnation of the water, a moderate amount of odour may be present. It is interesting to note that odour has not been reported before as having a significant positive association with any stage, or final stages, of *An. funestus*. This independent association was determined by the use of multiple logistic regression with a computer. It is worthwhile to make use of computer facilities to reassess long held views on mosquito ecology which were based on impressions from long experience but, with modern data processing, may in some cases be shown to be incorrect.

(c) *An. coustani*

The final stages of *An. coustani* were found to have significant positive association with pools and ponds (Table 4.3.1.1.1b-g). Among the species of vegetation considered, the final stages were significantly positively associated with *Cynodon* spp. (see Tables 4.3.1.1.2a-o). The presence of any stage of this species was significantly positively associated with *Jancus* spp., but negatively associated with *Paspalum* spp. Shading of the water was significantly positively associated with any stage of this species but this association was not significant for the final stages. On the other hand, the absence of vegetation was negatively associated with any stage, but not significantly so with the final stages. The other features which had a significant positive association with *An. coustani* are shown on Tables 4.3.1.1.3a-l. These Tables show that the final stages of *An. coustani* were significantly positively associated with depth of the breeding site, sloping muddy margin and presence of Notonectids, but not dragonfly larvae. The feature that showed a significant negative association with the final stages of this species was floating surface dust. Suspended mud was not significantly associated with *An. coustani*.

From these findings and as described in chapter four, *An. coustani* can be said to prefer more permanent clear water bodies which include ponds and some pools. These breeding sites are deeper as described above, likely to persist for a long time and contain vegetation. Gillies and De Meillon (1968) stated that this species preferred breeding sites with aquatic and semi-aquatic vegetation including swamps, ponds, streams, ditches, and rice fields. In the present study rice, streams and swamps were not found to be significantly associated with this species. Association of the final stages of this species with *Cynodon* spp. suggests that this species prefers shade like *An. funestus*. Although Gillies and De Meillon (1968) considered shade to be unimportant to the larvae of *An. coustani* as long as it was supplied with floating vegetation, it should be pointed out that shade and vegetation growing in the water are difficult to separate. With hindsight, it is regrettable that overhead shade was given more attention than water level shade in the current study. This is probably the reason for shade not emerging as an important feature for those species associated with some vegetation.

Sloping muddy margins are a feature which is likely to occur in many water bodies especially considering the fact that water levels fluctuates and in so doing erode away steep sides to create sloping shorelines. However, while this feature was not significantly associated with *An. funestus* it is positively associated with the final stages of *An. coustani*. Therefore, while these two species may be found in the same breeding sites, it appears they occupy different microhabitats. As in the case of *An. funestus* the presence of Notonectids is a sign of long persistence of a water body. Apparently because of the growth of grass in such water collections, *An. coustani* can use these plants as refuges and so co-exist with the predators. The observed highly positive significant association of *An. funestus* with Notonectids and dragonfly larvae in comparison with *An. coustani*, may be due to behavioral differences between the two species in avoiding predators. Observation during the present study clearly showed that larvae of *An. coustani* are more sluggish than those of *An. funestus*.

Assessments of the independent effect of environmental features on *An. coustani* are shown in Tables 4.3.3.1a and b. In the case of the final stages, floating surface

dust, pools and ponds appear to have an independent significant positive association with *An. coustani.* Since, with confounding effect of other features, floating surface dust was found to have a significant negative association, it can be concluded that pools and ponds with clear water, which may or may not have floating surface dust, and with vegetation cover, are typical breeding sites of *An. coustani* in the present study area.

(d) Culicines

Tables 4.3.1.1a-g show the association of culicines with the different types of water body. Considering the final stages of culicines, there was a significant positive association of these mosquitoes with pools and ponds. On the other hand there was a significant negative association between the final stages of culicines and streams. Hopkins (1952) reported that the majority of species of culicines bred in ground pools (including small lakes, swamps, springs, rivers, ditches and hoofprints). Those culicines breeding elsewhere were considered as being specialized (Hopkins, 1952). In the present study, however, streams and footprints were not the preferred breeding sites of the final stages of culicines. The vegetation which showed a significant positive association with the final stages of culicines were as follows: *Oryza sativa, Ipomea aquatica, Spirogyra* spp *Lagarosiphon* spp. and the general category "cover" (Tables 4.3.1.1.2a-o). The only plant that showed a significant negative association with the final stages of culicines was *Cyperus* spp. Most culicines are thought to find shelter among vegetation including masses of the alga *Spirogyra* (Hopkins, 1952). Culicines were found to be positively associated with rice because they bred in flooded rice fields, which were categorised as pools based on the depth of the water. *Ipomea aquatica* grew in some pools and ponds, completely covering the water surface. Such water bodies were found to be highly polluted with rotten leaves and they had an unpleasant odour. Culicines bred profusely in these sites. *Cyperus* was found growing in relatively clear water including wells (i.e. some puddles, pools or ponds) in which culicines were not found in significant numbers. The other features and their association with culicines are shown in Tables 4.3.1.1.3a-l. Those which showed a significant positive association with the final stages of culicines were as

follows: still water, depth, sloping muddy margins, Notonectids and dragonfly larvae all of which are associated with pools and pond as explained above. Significant negative association with the final stages of culicines was shown with odour of water, mud and shade. Odour had a significant positive association with culicines under certain conditions depending on the degree of pollution (see table 4.3.3.2b) but not in others (see Table 4.3.1.1.3b), e.g. flooded rice fields were rarely found with water having unpleasant odour. Among the features which showed some independent effect in culicines, shade was the only feature that had a fairly significant negative association. The effect of shade on the breeding of culicines has not been reported before. Although no experimental investigation was conducted to support the impression acquired in this study, it appears that culicines in general prefers to breed in sites without shade. This is probably because light raises the temperature of the water and so increases the rate of organic decomposition. *Culex quinquefasciatus,* breeds in virtually sealed septic tanks and cesspits (Service, 1985). In these situations, the effect of changing light conditions on the density of immature stages is not known. It is possible that increase in light conditions may increase breeding in pits and septic tanks.

These results show that, in this study, culicines preferred to breed in more permanent deep exposed water bodies, with or without unpleasant smell and with vegetation.

11.6 Seasonal variation in contribution of different types of breeding site to *An. gambiae s.l* abundance

The estimation of a calibration factor was followed by the estimation of prevalence of *An. gambiae s.l* in the breeding sites in both Mngaza and Kumbamtoni (see chapter 6). The overall output of final stages in Kumbamtoni was lower than that for Mngaza (see Figure 6.1.3a and b). This observation provides a reasonable explanation for the relatively low adult catches in Kumbamtoni compared with Mngaza (see chapter 7 and Magesa *et al.*, 1991). This suggested that local breeding sites are more important than immigration in determining the abundance of mosquitoes in the two villages. The

effect of larviciding on adult mosquitoes is described in chapter 7 while the role of mosquito migration is described in chapter 8.

As found in numerous other studies of anopheline larvae, the prevalence of the fourth and pupal stages varied greatly during the year (Figure 6.1.3a and b). The prevalence of the immature stages as well as the adults reached their peak in the second quarter of the year, which coincided with the long rainy season. The peaking of the larval population was observed in Mngaza in 1990 before larviciding started, and in Kumbamtoni (without larviciding) both in 1990 and 1991. This corresponded with the peaks in the adult mosquito population (Fig 7.3.1a and 7.3.1c). The effect of larviciding on the immature stages is described in section 6.2.3.1.

The estimation of density per unit area and the total area of each type of site indicated that in Mngaza and Kumbamtoni the footprints contained the largest number of immature stages in the wet season. Puddles were the second in this respect, with other types of breeding sites contributing relatively little to the peak of vector population despite their important role during certain periods of the year when footprints and puddles are almost completely dry (see Figure, 6.1.3c-f). The importance of these breeding sites in the production of the final stages also varied seasonally. Christie (1959), while working in the same area, also noted that temporary pools (including footprints), where the survival of *An. gambiae s.l* was high, were available for short periods only and mosquitoes had to contend with much less favourable conditions in swamps and wells. Although swamps covered a large area in Mngaza village, their overall contribution to the final stages of *An. gambiae s.l* was found to be much less than those of footprints and puddles. It should be emphasised that, unless such absolute estimates of prevalence are carried out it may be wrongly assumed that, despite the low densities of larvae per dip in the swamps, the very large area that they cover might lead to them making a major contribution to the overall mosquito population. Goma (1958) also reported that, despite the vast area covered by the swamps in Uganda, their role in the breeding of mosquitoes was minimal unless cleared for agricultural purposes. Where, however, very large expanses of swamps contribute significant mosquito production, it may be more economic to drain

them rather than attempting to carry out larviciding, especially as control teams would probably be inclined to concentrate on the more accessible areas, leaving the rest of the site untreated. It would be even better if very productive sites could be filled. Breeding sites such as unused pools and ponds, and where possible some puddles, should be filled in to reduce the area to be treated, particularly during the rainy season. Where filling is not possible or acceptable to the local population, selective treatment of such sites, with efforts concentrated on the most productive ones, during particular seasons is to be encouraged. However, the magnitude of breeding found taking place in footprints, stressed the need for regular larviciding especially of this type of breeding site.

11.7 Effect of larviciding with Abate and *Bacillus thuringiensis israelensis* on the prevalence of fourth instar larvae and pupae in different types of breeding site

Larviciding was carried out using Abate and Bti. It was encouraging to find that, after correcting for natural changes in the untreated village, there was an overall reduction of about 87% in the density of the final stages of *An. gambiae s.l* per square metre of water. Abate as well as Bti impregnated into sawdust were both effective in controlling *An. gambiae s.l* in the highly productive footprints. However, Bti was not as effective in the puddles as Abate, as described in chapter 6. For this reason it may be better to use Abate in those breeding sites where Bti does not perform well but to use Bti in the footprints in order to minimize contamination of the environment with organophosphate insecticide. Likewise, since some ponds are sources of drinking water it may be better to use Bti in such sites so as to minimise human exposure to Abate which, as described in section 6.2.3.4, can often be tasted.

11.8 Effect of larviciding with Abate and Bti on adult *An. gambiae s.l* and the role of immigration of this species into Mngaza.

The use of treated maize cobs and sawdust for larviciding was found to be convenient and it is considered that this method could be perfected for wider application. The use of coconut husks, which Novak, *et al.* (1985) found to be as effective against *Aedes aegypti* breeding in automobile tyres as maize cobs, should also be tried against *Anopheles*. If they are found to be effective they could be used in areas where maize is not widely grown such as along the coast of the Indian ocean where there are plenty of coconuts.

The effect of larviciding on the density of *An. gambiae s.l* adults in Mngaza was much less convincing than it was on the immature stages. While there was approximately an 87% reduction in the density of immature stages, the best estimate that could be made, using baseline data from the intervention and control villages, was that there was only abut a 37% reduction in the density of adult stages. This disappointing result might be attributed to immigration of mosquitoes into the treated village. To explain persistence of 63% of the normal adult population when only 13% of normal larval population could be detected it would have to be assumed that about 50% of the normal adult population were immigrants and that about 79% (50/63) of the population subject to larviciding were immigrants. An experiment was therefore undertaken to test whether there was sufficient immigration of mosquitoes into the treated village for this to be a reasonable explanation. The results showed that 2 out of 2788 of *An. gambiae s.l* caught in Mngaza (the treated village) had been marked in one of the three villages in which marked releases had been made. About 0.78% of the mosquitoes in those villages were found to be unmarked. For Mngaza this led to an approximate estimate that 9.2% (95% c.l = 1.1% - 33.2%) of mosquitoes had moved in from the other three villages. Even the upper end of this very wide confidence interval is insufficient to explain failure to obtain better than 37% reduction in the adult population. The remaining explanations for the small reductions in the adult populations are:

(a) Immigration from some other villages such as Enzi and Mafere which are as near to Mngaza as the villages in which marked releases were made (Figure 8.2.1). In order to confirm this it would be necessary to carry out another mark-release-recapture experiment including all villages around Mngaza and or to observe the effect of much more extensive larviciding.

(b) The possible existence of inaccessible breeding sites close to Mngaza which were missed, despite great efforts to make the larval searches comprehensive.

(c) Overcompensating density dependent regulation of the mosquito population after partial control of immature stages with Bti in puddles. This is not a likely explanation because it was shown that the production of IVth instar larvae was greatly reduced in Mngaza. If, however, density dependent regulation also affected production of pupae from larvae this could have contributed to the disappointing final result.

It was hoped to disprove the conventional view of the infeasibility of larvicidal control of *An. gambiae s.l.* However, it is reluctantly conceded that the conventional view is probably correct.

CHAPTER 12

CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK

12.1 The survival and development of immature stages of *An. gambiae s.l*

Receding water levels in small breeding sites such as footprints and puddles appear to be the most important cause of mortality of the immature stages of *An. gambiae s.l* but rise in water levels did not cause a significant increase in the number of first instars.

This appears to be because stranded eggs die of desiccation. To check this it is suggested that eggs should be collected from natural breeding sites at short intervals and their viability assessed in the laboratory.

Developmental duration of the immature stages of *An. gambiae s.l* in simulated natural breeding sites, was found to be about 6-8 days, in contrast to published estimates of about 12 days. This suggests that non-residual larviciding should be carried out at shorter intervals than the conventional 7 days. A study in floating cages indicated the existence of density dependence of larval mortality of the overcompensating type.

12.2 Quantitative evaluation of larval control measures.

Dipping is a reliable and convenient method for estimation of density of immature stages of *An. gambiae s.l.* Estimates of density of immature stages in terms of numbers per dip can be converted into numbers per square meter using a calibration factor estimated with use of a quadrat. In so doing it is possible to establish which category of breeding sites is most productive of the final aquatic stages, taking into account the relative areas of the different categories of site. A common calibration

factor can justifiably be used for different types of site.

12.3 Use of environmental features as markers for different taxa of mosquitoes

A statistical analysis of data from hundreds of site visits showed that environmental features can be used to specify which types of breeding site are preferred by *An. gambiae s.l*, *An. funestus*, and Culicines. *An. gambiae s.l* prefers to breed in footprints and puddles, which are sunlit, and without vegetation. Although its breeding sites are said to be diverse, in this study it has been found that in the peak breeding season its final stages are found in significant numbers only in footprints and puddles. Therefore it would be possible to concentrate on these in a larviciding programme.

An. funestus and *An. coustani* breed in pools and ponds. Although they occupy the same type of breeding site, *An. funestus* strongly favours vegetated places while *An. coustani* does not do so as strongly. Culicines prefer relatively foul water which is not shaded. Larvae of these mosquitoes can co-exist with predators.

12.4 Effect of larviciding with Abate and Bti against *An. gambiae s.l*

Insecticide treated maize cobs are convenient to use in the application of insecticide to breeding sites. Abate and Bti were both effective against *An. gambiae s.l* in footprints. Bti was not as effective as Abate in puddles. It is recommended that Abate should be used in puddles and Bti in footprints and in sources of drinking water to minimise exposure of humans to organophosphate insecticide.

The effect of larviciding around a village reduced the density of immature stages by 87% but adult catches in light traps were only reduced by an estimated 37% after correcting for year-to-year changes seen in an untreated village. A mark-release-recapture study was designed to determine whether this disappointing result can be explained by immigration from adjacent villages. Although very few immigrants were recaptured, from the available data immigration cannot be excluded as a major

contributor to the adult population after the application of larval control. Other possible influences were inaccessible undiscovered local breeding sites and the possible effects of density dependent regulation later in development of the immature stages.

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APPENDIX 1: LARVAL SAMPLING RECORDING FORM (FORM LP1)

Time, DATE:

Village:

Length:

Width:

Diameter

Total length or area

Muddy:

Smell:

Shade:

No. dps:

No.-vedips:

(all spp.)

Adults emerging:

Activity (tick)

Routine sampling dipping

Calibration dipping

Evacuation

Zone:

Site:

Point:

Rust:

Depth:

Dry:

Veget. type:

Water flow spped:

Sloping muddy margin over 10cm:

Other Observations

Red Soum:

Surface dust/particles

etc.

Field Identification

Ag	Ac	Culicines
I	I	I
II	II	II
III	III	III
IV	IV	IV
P	P	P
A	A	A

Predators etc.

No tonecotids:
Ox tigripes:
Mites:

Dragon/Damselflies

FIELD IDENT: Af/Ar	Lab. Ident Af	Ar:
I	I	I
II	II	II
III	III	III
IV	IV	IV
P	P	P
A	A	A

APPENDIX 2: ADULT MOSQUITO SAMPLING RECORDING FORM (FORM LP3)

Village:

Date:			Ag							Af							Cx							Remarks
			UF	F	HG	G	M	NP	P	UF	F	HG	G	M	NP	P	UF	F	HG	G	M	NP	P	
Survey no. 1	LT	House 1																						
		2																						
		3																						
			Av/trap																					

Date
Survey No. 2

Ag = Anopheles gambiae
Af = An. funestus
Cx = Culex mosquitoes
LT = Light trap

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A FIELD STUDY OF *BACILLUS SPHAERICUS* FOR THE CONTROL OF CULICINE AND ANOPHELINE MOSQUITO LARVAE IN TANZANIA

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ABSTRACT: Liquid and briquet formulations of *Bacillus sphaericus* were tested against larvae of *Culex quinquefasciatus* and *Anopheles funestus*. Laboratory tests showed that the LC_{95} was 240 times higher for anopheline larvae compared with the culicines. Two or more weeks of control were obtained in natural breeding sites with 2.5 ppm of a liquid formulation against *Cx. quinquefasciatus* but 60 ppm were needed for similar prolonged control of *An. funestus*. The briquets were ineffective against both species in natural breeding sites.

INTRODUCTION

The microbial control agent *Bacillus sphaericus* Neide has shown considerable activity against several genera of mosquitoes. This activity has been demonstrated both in the laboratory and under field conditions (Davidson et al. 1981; Lacey and Singer 1982; Mulla et al. 1982, 1984, and 1986).

The larvicidal activity is based on the endotoxin crystals produced by *B. sphaericus* during the sporulation process. Following their ingestion by mosquito larvae, the crystals are broken down within the larval gut into the active toxic compounds, which rapidly disrupt the midgut cells. This leads to paralysis and the death of the larvae. The toxic effect appears to be restricted to larval mosquitoes. It does not affect nontarget organisms sharing the aquatic habitat or other invertebrates or vertebrates (Mulla et al. 1984).

Factors, such as the quality and temperature of the water, the sensitivity of different larval instars of the same species or of different species, the larval density, and the occurrence of filter feeding nontarget organisms, all influence the effect of the microbial agents (Mulla 1990). It has been shown that first instar larvae of *Culex pipiens* are two to five times more susceptible to *B. sphaericus* products than are fourth-instar larvae (Wraight et al. 1981). Finally there are differences in the potency of available formulations of *B. sphaericus*.

The present study was conducted in order to evaluate the larvicidal activity of two *B. sphaericus* formulations: Spherimos FC (a fluid concentrate from Duphar B.V., Weesp, Netherlands) and solid Spherimos briquets (Summit Chemical Co., Baltimore, MD, U.S.A.). The susceptibility of larvae of *Culex quinquefasciatus* (a filariasis vector) and *Anopheles funestus* (a malaria vector) to each preparation was evaluated under laboratory and field conditions in Tanzania.

MATERIALS AND METHODS

Laboratory Assays

Spherimos FC was tested in triplicate laboratory bioassays to assess the lethal concentrations (LC_{50} and LC_{95}). A stock solution was prepared by adding 50 µl of homogenized Spherimos FC to 10 ml of distilled water, and 0.1 ml of this mixture was added to 9.9 ml of distilled water and agitated to give a 50 µl/liter stock solution. Samples of 20 *An. funestus* or *Cx. quinquefasciatus*, collected in the field, were placed in plastic bowls with 100 ml of water. Using an Eppendorf Digital Pipette 4710, 1, 2, 4, 8, 10, and 12 ml volumes of the stock solution were added to the plastic bowls containing *An. funestus* larvae to obtain final concentrations of 0.5, 1, 2, 4, 5, and 6 ppm.

In the bioassay with *Cx. quinquefasciatus*, final concentrations of 0.0005, 0.001, 0.0025, 0.005, and 0.05 ppm were tested by adding 1, 2, 5, 10, and 100 µl of stock solution to the corresponding bowls. In each test series, three bowls were left untreated as controls. Larval mortality was recorded after 24 and 48 hours and the LC_{50} and LC_{95} values were estimated after plotting probit mortality values against dosage on a logarithmic scale.

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Field Tests

Small scale field tests were conducted within a five kilometre radius of the Ubwari Field Station near the town of Muheza in northeastern Tanzania during the short rainy season from mid-October to mid-December, 1990. Well-defined typical breeding sites of *Cx. quinquefasciatus* and *An. funestus*, such as pools in blocked streams, were chosen as test sites. They were more or less polluted and the sizes varied from 0.5 to 25 m^2. The depths were not accurately measured but were assumed to be 10 cm when estimating the dosage to be applied.

Larval density was monitored at four day intervals before treatment, immediately before treatment, and one day, two days, and one week after treatment, and later on a weekly basis up to five weeks. The larval population was determined by taking 10 dips (250 ml each) from different positions and the center of each site. Specimens obtained from each site were classified according to instar (first-fourth). Samples of fourth-instar larvae were identified by species. Two sites had both *Cx. quinquefasciatus* and *An. funestus;* the others had only one of these species. At each breeding site an index of the level of organic pollution, pH, conductivity, chloride concentration, and intensity of sunlight were recorded.

The diluted liquid formulation was applied with a hand-held flit gun (calibrated for volume emitted per pump stroke) so as to completely cover the water surface. The final concentrations were 2x, 100x, and 2400x the LC_{95} in the laboratory for *Cx. quinquefasciatus* and 2x and 10x the LC_{95} in the laboratory for *An. funestus*. The Spherimos briquets were placed on the water surface at a rate of one briquet per square meter.

The toxicity of *B. sphaericus* and temephos (Abate) to notonectid predators of mosquito larvae was studied. A pond was treated with Spherimos FC (dosage: 1 ppm), and two weeks later the same pond was treated with temephos at a dosage of 1.0 ppm.

The density of the notonectids was determined by careful observation of the water, one square meter at a time. This was done one day before, immediately before the treatments, and then daily up to one week afterwards.

RESULTS

Laboratory tests of Spherimos FC showed that after 48 hours of exposure, concentrations of 0.002 and 0.025 ppm caused approximately 50 percent and 95 percent mortality, respectively, of early fourth-instar larvae of *Cx. quinquefasciatus*. *Anopheles funestus* larvae were found to tolerate concentrations 240 to 500 times higher than larvae of *Culex;* the LC_{50} and LC_{95} values for early fourth-instar larvae of *An. funestus* were estimated as 1.0 and 6.0 ppm, respectively.

In field tests, Spherimos FC caused a reduction of the *Culex* larval populations even at a dosage of 0.05 ppm (2 x LC_{95}). At a dosage of 2.5 ppm (100 x LC_{95}), complete control was observed in one site over a period of five weeks (see TABLE 1; site no. 4). In the untreated

TABLE 1. Field evaluation of *Bacillus sphaericus* (Spherimos FC and Spherimos Briquets) against *Culex quinquefasciatus* larvae.

Site No.	Dose	Mean Number of Larvae/Dip											
		Before					After Treatment (Days)						
		16	12	8	4	0	1	2	7	14	21	28	35
(1)	0.05 ppm FC	2	6	5	2	2	<1	<1	0	0	0	4	2
(2)	0.05 ppm FC	1	3	1	3	2	<1	0	0	0	<1	0	<1
(3)	2.5 ppm FC	-	50	25	23	-	0	0	1	0	8	2	7
(4)	2.5 ppm FC	5	3	5	3	4	<1	0	0	0	0	0	0
(5)	60 ppm FC	3	6	1	1	6	<1	0	0	0	0	<1	0
(6)	60 ppm FC	1	1	1	2	1	0	0	0	0	0	0	0
(7)	1 briquet/m^2	17	23	18	18	22	13	6	<1	6	7	13	16
(8)	1 briquet/m^2	7	15	5	6	4	4	4	2	3	-	-	-
(9)	Control	4	4	4	5	4	7	6	4	4	4	5	3
(10)	Control	16	6	12	3	8	22	16	7	5	6	18	18

check sites the larval densities were consistently higher than those in treated sites.

Culex egg rafts were frequently noted during the five weeks of post-treatment examination of breeding sites, long before larvae were observed. Extended control was therefore due to the larvicidal efficacy and persistence of *B. sphaericus* and not to the absence of oviposition by the mosquitoes.

In contrast with Spherimos FC, the briquet formulation was ineffective in the natural breeding sites of *Cx. quinquefasciatus* (TABLE 1, sites 7, and 8).

To fully control *An. funestus* larvae, 10 x LC_{95} (60 ppm) of Spherimos FC was required. Application at this dosage completely controlled anopheline larvae within 24 hours and continued to do so up to 28 days, after which first instars reappeared (TABLE 2, site 15).

TABLE3 shows the environmental profiles of each site averaged from data collected 12 days prior to treatment and 14 days after it.

Notonectid (*Anisops* and/or *Enithares* spp.) populations were not affected by the application of Spherimos FC at 1 ppm. By comparison, the presence of temephos reduced the notonectid density by 80-90 percent with many dead notonectids being observed (TABLE 4).

DISCUSSION

Microbial larvicides, such as *B. sphaericus*, offer several advantages over traditional synthetic chemical preparations. These include the potential for toxin recycling and extended residual effectiveness, the limited potential for the development of resistance, and the lack of toxicity to the environment and to nontarget organisms, particularly predators of mosquito larvae. This durability and specificity are coupled with rapid effectiveness, even in polluted habitats, and safety during handling by mosquito control operators. They are also safe for human consumption when applied to drinking water and are stable in storage.

This study was conducted in order to determine the relative susceptibility of *Culex* and *Anopheles* larvae to two different formulations of *B. sphaericus* larvicide. Spherimos FC was also compared to an organophosphorus insecticide, temephos, in regard to toxicity to notonectids, predators of mosquito larvae.

The fact that in these studies *An. funestus* exhibited a lower susceptibility to Spherimos FC than *Cx. quinquefasciatus* agrees with the results of other workers and may have several explanations. *Anopheles* larvae feed at the air-water interface, rarely lower than 1-2 mm beneath the surface (Aly et al. 1987). *Bacillus sphaericus* spores are denser than water and sink to the bottom after application. In comparison, *Culex* larvae feed at all depths and are, therefore, more likely to encounter the toxin. Also, while larvae of both genera are filter-feeders, the filtration rates of *Anopheles* larvae are 10 to 20 times less than those of *Culex* larvae (Aly 1988).

The degree to which these behavioral differences may protect *Anopheles* from receiving a fatal dose of

TABLE 2. Field evaluation of *Bacillus sphaericus* (Spherimos FC and Spherimos Briquets) against *Anopheles funestus larvae*. Note: Sites 14 and 15 in this TABLE were the same as sites 6 and 5, respectively, in TABLE 1.

Site No.	Dose	Mean Number of Larvae/Dip											
		Before					After Treatment (Days)						
		16	12	8	4	0	1	2	7	14	21	28	35
(11)	12 ppm FC	3	1	1	2	2	<1	2	0	0	<1	<1	1
(12)	12 ppm FC	2	1	3	1	3	0	0	<1	2	2	1	1
(13)	12 ppm FC	7	1	2	4	2	<1	<1	0	0	0	2	2
(14)	60 ppm FC	3	3	3	3	4	0	0	0	0	0	0	<1
(15)	60 ppm FC	1	2	1	3	3	<1	0	0	0	0	<1	0
(16)	1 briquet/m²	11	10	-	-	-	4	1	3	1	<1	<1	<1
(17)	1 briquet/m²	8	3	2	8	-	4	<1	<1	<1	<1	3	-
(18)	Control	2	5	1	9	6	6	5	4	10	6	4	5
(19)	Control	2	7	2	2	2	2	2	2	1	2	5	2

TABLE 3. Environmental profile of each site (average of readings taken 12 days before and 14 days after treatment).

Site No.	*Index of Organic Pollution	Sunlight (Lux x 100)	pH	Conductivity (TDS)	Chloride (mg/l)
	(a) *Culex* Sites:-				
(1)	+	203	7.4	42	37
(2)	+++	849	7.1	21	54
(3)	++++	407	5.1	44	61
(4)	+++	385	9.0	39	103
(5)	++	83	7.4	45	76
(6)	+	363	8.4	76	126
(7)	+	1028	7.4	41	36
(8)	+++	0.67	10.1	75	176
	(b) *Anopheles* Sites:-				
(11)	++	240	8.0	37	32
(12)	+	774	7.0	30	61
(13)	+	275	9.2	73	181
(14)	+	363	8.4	76	126
(15)	++	83	7.4	45	76
(16)	+	422	8.1	23	35
(17)	+	132	7.7	14	23

* On a scale from ++++ = highly polluted to + = clean.

TABLE 4: Comparison of the effects of *Bacillus sphaericus* and temephos (both at dosages of 1 ppm) on notonectid (*Anisops* and/or *Enithares* spp.) density.

	Number of Notonectids per Square Meter							
	Before		After Treatment (Days)					
Larvicide	1	0	1	2	3	4	5	6
Spherimos FC	13	10	9	7	8	9	7	9
Temephos	9	-	2	1	2	1	1	1

toxin is not known. However, one would expect that the Spherimos briquets, which float and release the active material at the water surface, would exhibit comparatively higher efficacy against *Anopheles* larvae than the rapidly sinking liquid formulation. The findings of this study do not support this expectation for reasons that are not clear. Distribution of the toxin may be more restricted when the briquet formulation was used. At each of the four sites treated with a single Spherimos briquet (sites between 2.5 and 10 square meters), no larvae were detectable in the vicinity of the briquet but they were readily found elsewhere within the site. In breeding areas where the free drift of the briquet would be limited by vegetation or debris, the liquid formulation should offer greater control. Alternatively, one could envisage a role for a formulation in which the toxin is bound to small buoyant particles, thereby incorporating the advantages of wide distribution and concentration at the water surface and thus offering more effective control of *Anopheles*.

Of the two, the briquet formulation may provide greater efficacy when used in small breeding sites, which are subject to repeated cycles of flooding and drying. The floating briquet would continue to release toxin in the treated site with each successive flooding while the liquid formulation might become bound to bottom sediments. This possibility was not tested in this study.

The efficacy and longevity of the larvicide may be influenced by the degree of water pollution. Higher levels of control in clean, as opposed to polluted, waters were demonstrated by Mian et al. (1983). However, longer residual effects were obtained at higher levels of pollution in a study by Nicolas et al. (1987). The degree of pollution was noted for our sites (TABLE 3) and the range of this variable for either genus alone was limited. As expected, *Culex* were found to tolerate higher levels of pollution than *Anopheles* larvae.

An important advantage of *B. sphaericus is* that nontarget species, and particularly predators of mosquito larvae, remain unharmed by the toxin. Notonectids represent one such group of predators and *B. sphaericus* was compared to temephos with respect to adverse effects on notonectid numbers (TABLE 4). *Bacillus sphaericus* did not reduce notonectid populations significantly. Temephos application, however, resulted in a notable reduction of their numbers for up to one week with numerous dead notonectids observed. Just how important notonectid predation is in the control of mosquito larvae has not been established, but it seems likely that their feeding activity would supplement larval control by a larvicide.

This study led to the following conclusions: 1) In the laboratory the LC_{95} values were found to be 0.025 ppm for *Cx. quinquefasciatus* and 6 ppm for *An. funestus*. 2) The liquid formulation yielded mediocre control in the field at 2x these LC_{95} values for both *Culex* and *Anopheles* larvae. 3) Control of *Culex* was obtained at 2.5 ppm and it persisted for two weeks or more. 4) At the highest concentration of 60 ppm, excellent control of sites containing populations of both larvae was sustained for four weeks or longer. 5) The briquet formulation (10% active ingredient) compared unfavorably with the liquid formulation. This may be because of insufficient distribution of larvicide from briquets. 6) Notonectid species were observed as a representative nontarget species and a known predator of mosquito larvae and were found not to be adversely affected by the larvicide Spherimos FC.

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APPENDIX: 10

LARVAL ECOLOGY PROJECT Form-MR 1

Mosquito Recruitment and water Levels
Village: KISUMANI
Month: JUNE 1990
Puddle no: 3

	Date																
Stage	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
I		1		1		2,1	1,1,1		1,2,1		1	1,1,1,1,1		1,1,2,1	1	1,1	1
II	1,1,1			1,1,1,2	1	2,1,1,1	3,1,1,1	1		1	1,2,2	2,1	1,1,1		1,1	1,3	1,1
III	1,1				1						1		1				1,1,1
IV									1,1								2,2
P																	
No. of -ve dips	4	7	8	6	2	4	3	5	5	7	6	12	3	6	7	6	9
Total no. of dips	5	8	8	7	3	6	4	5	6	8	11	13	4	7	9	7	10
Water level (cm)	60.8	60.8	60.4	60.6	61.8	61.8	61.6	61.0	62	60.2	60.2	60.1	60.0	61.6	61.8	61.0	60.8
Difference in water levels (cm)																	