# Impact of irrigated rice culture on the production of *Anopheles* mosquitoes (Diptera: Culicidae) in the Niono region, Mali.

by

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## Abstract

The study assessed the impact of rice paddy water management practices and related environmental conditions that prevail in Niono, Mali, on the size of larval populations of *Anopheles gambiae* s. l., a major malaria vector. The longer the period of uninterrupted flooding, the greater was the larval population size. As the density of aquatic weeds increased, the size of the mosquito larval population declined, whereas the presence of rice plants enhanced the size of mosquito larval populations. Numbers of mosquito larvae initially increased as rice plant density rose, but then decreased marginally as densities reached their peak. These results confirm that water management plays a major role in the production of mosquito larvae. Nevertheless, comparison with studies conducted in other areas suggest that the impact of water management on mosquitoes varies regionally. In the Niono region, controlled irrigation and drainage should favour the reduction of *Anopheles* mosquito larval populations.

# Impact of rice culture on mosquito production in Mali

## Résumé

Cette étude a vérifié si, dans la région de Niono, au Mali, les pratiques de contrôle de l'eau en rizicultures, et leurs conditions environnementales associées, ont un impact significatif sur la production larvaire de *An. gambiae s.l.*. Plus la période sans interruption d'eau est longue, plus le nombre de larves d'*Anopheles* produites était grand. Plus la densité de plantes flottantes augmentait plus la population larvaire anophelienne diminuait, alors que le nombre de larves augmentait avec la densité du riz jusqu'à un certain point après lequel le nombre de larves diminuait. Ces résultats confirment que le contrôle de l'eau joue un rôle majeur dans la production larvaire de moustiques. Malgré tout, la comparaison avec d'autres études faite dans d'autres régions, suggère que l'impact des pratiques de gestion de l'eau varie régionalement. À Niono, le contrôle de l'irrigation et du drainage devraient être favorisé pour réduire le nombre de larves d'*Anopheles* en riziculture.

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## Table of Contents

## List of Tables

## List of Figures

# 1. INTRODUCTION

## 1.1 Rationale

With increases of the world population comes an increase in food demand. Growing rice is an important part of the solution. In developing countries, particularly in the arid and semi-arid regions of Africa, water is a limiting factor to rice growth, and irrigation is essential to ensure adequate rice production (Bingen, 1985). Unfortunately, irrigation also has its disadvantages, since it encourages the proliferation of vectors of diseases of major medical importance, such as schistosomiasis and malaria (Surtees, 1970a; Lacey & Lacey, 1990; Amerasinghe et al., 1991; Lindsay et al., 1991; Bourassa, 2000).

In the Republic of Mali, West Africa, food self-sufficiency is a national priority, and the government encouraged cereal and particularly rice production. Since the beginning of the 1980's, rice consumption has increased both in cities and in rural areas (DGCI, 1992). Rice production demands a considerable amount of water. However, in the sub-Sahelian zone of Mali, rain is largely restricted to the period from June to September. As a result, irrigation is necessary for rice production in the dry season, which extends for almost seven months of the year. In Mali, as in many other part of Africa, irrigation is a widely used solution to maximise rice production (Robert et al., 1992; Tia et al., 1992).

Many studies conducted in Asia and Africa have shown that *Anopheles* populations and the incidence of malaria vary with agricultural irrigation practices. Research conducted in West Africa has focused on the broad impact of irrigation on the production of mosquito larvae and the level of transmission of malaria in surrounding communities. However, some fields may promote *Anopheles* larval development more than others due to idiosyncrasies in their management as well as to their inherently different basic physical characteristics. In Mali, as elsewhere in Africa, regional irrigation practices are far from homogeneous, but present a complex mosaic where water management in some fields is at best, improvised, and in others fully controlled.

### 1.2 Objectives

Few studies have considered the fine-grained effect of irrigation practices on mosquito larval production. The objectives of this study were to determine the species composition of *Anopheles* mosquitoes in the Niono region of West Africa and to compare larval production in the fields under irrigation practices ranging from virtually unmanaged to highly managed. This study also assessed the concomitant impact of rice density, rice development stage and floating aquatic weeds on larval production. This may shed some light on the extent to which irrigation practices in this vast territory may influence the size of larval populations of malaria vectors, and may suggest means to reduce such populations by water management.

# 2. LITERATURE REVIEW

## 2.1 Malaria Transmission

Malaria is a threat for 2.5 billion people, more than 70% of which live on the African continent (Garcia & Bruckner, 1997). In Mali, malaria accounts for 13% of the total mortality of the population, and 25 to 35 % of the deaths are in children under 5 years of age (Haïdara, 1989).

Human malaria is transmitted exclusively by mosquito species of the genus *Anopheles* (Fortin, 1998). Of the approximately 400 species of *Anopheles* identified, only 60 are known to be vectors of human malaria under natural conditions (Markell & Vogue, 1976; Service, 1996). Transfer of the infection between the adult female mosquito and the human host occurs during the blood meal. *Plasmodium* is ingested with blood. In the intestinal tract, gametocytes transform into gametes which fuse to form ookinetes. These cross the gut wall and form oocysts. Sporogony occurs in oocysts, and millions of sporozoïtes emerge and migrate slowly to the mosquito salivary glands. Here they may be reinjected into the human host during the next bloodmeal (Bruce-Chwatt, 1985). Infection is not transferred transovarially.

The complex "Gambiae" (referred to as *Anopheles gambiae* s.l.) includes six species of *Anopheles* mosquitoes, which are morphologically identical, but can be distinguished by DNA comparison. Only two of the six are found in Mali: *Anopheles gambiae* s.s and *Anopheles arabiensis.* These two species are found in rice paddies in the Niono region (Touré, personal communication). In Africa, *Anopheles gambiae* s. s. is considered to be the most prominent species involved in the transmission of malaria (Service, 1996). *An. funestus* which is not part of this complex, was also found to be a good vector of malaria in the dry season (Bockarie et al., 1994).

## 2.2 Biology of Aquatic Stages of *Anopheles* Mosquitoes

The life cycle of *Anopheles* Mosquitoes is represented by six aquatic stages: the egg, four larval instars and the pupa, and one terrestrial stage, the adult or imago. The complete life cycle (from egg to egg) of *Anopheles* mosquitoes requires from 14 to 21 days and is a function of the species and environmental factors such as temperature and food (Bruce-Chwatt, 1985). Oviposition takes place in shallow water. A female may deposit as many as 100 eggs at a time on the water surface. The eggs of most *Anopheles* spp. including *An. gambiae* s.l., are characterized by small, air-filled bladders that keep them afloat and cause them to lie with the flattened surface uppermost (Bruce-Chawtt,1985). Eggs of *An. gambiae* s.l. held on wet soil at room temperature showed a progressive decrease in hatching, from 91% on day 2 to 1% on day 12 (Beier et al., 1990). These authors also reported that very low numbers of *An. gambiae* mosquitoes emerged from samples of re-flooded soil removed from the top 3 cm of dry temporary ponds and cow footprints along a stream bed. The larvae of a majority of *Anopheles* spp. develop well at 15 to 25°C (Bourassa, 2000). Under natural conditions, *Anopheles* larvae need 5 to 8 days to reach the adult stage. Thus, unless standing water persists for at least that period, no adults will be produced.

## 2.3 Irrigated Rice Cultivation and Malaria Occurrence

Happold (1965) suggested that mosquito larvae can develop in most aquatic environments and that the larval distribution is not determined by the quality of the water itself but by the water cycle. Thus, irrigation practices designed to augment agricultural rice production are often closely related to mosquito development and malaria transmission since many of the important human malaria vectors are associated with emergent vegetation in still water (Hall, 1972), conditions which are characteristic of ricelands.

In some instances, irrigated rice cultivation may not pose a major threat to human health because the mosquito species favoured by the associated environmental modifications are of poor vector capacities, or because the impact is considered negligible in view of the small size of the area modified, or in relation to the already extreme gravity of the situation in some areas (Lindsay et al., 1991).

Mouchet et al. (1993a) have shown that although ricelands in southeast Asia were the breeding sites of many *Anopheles* species, among them *An. sinensis, An. subpicus, An. philippinensis* and *An. aconitu,* none of these was an effective vector of malaria, at worst transmitting some *Plasmodium vivax.* At the same time, *Plasmodium falciparum*, which is the aetiological agent of a more pathogenic form of malaria, was propagated by two forest *Anopheles* species, *An. minimus* and *An. dirus*. As a result, the introduction of irrigation as a component of ricelands management did not significantly change the malaria situation.

Similarly, in Burkina Faso, Robert et al. (1988) showed that rice paddies provided excellent mosquito breeding sites, harbouring 70% of the total larval population, followed by irrigation canals and drainage ditches with 29%. However, Fayes et al. (1993) revealed that there were two major species present in rice paddies: *An. gambiae* and *An. funestus. An. gambiae* from the rice fields were less effective vectors of malaria than conspecifics from natural savannah sites. These authors found that even though *An. funestus* is a good malaria vector (Service, 1996), irrigation had a minor impact on malaria, which was already holoendemic in the region.

In Madagascar, the introduction of irrigated rice cultivation had little impact on the incidence of malaria in hyperendemic shoreline areas, mostly because of the small size of the area affected and the high background levels of the disease. However, in unstable hypoendemic zones in the south of the country, irrigation enhanced *An. gambiae* and *An. funestus* production and the number of malaria cases increased significantly (Mouchet et al., 1993b). Similarly, in the Senegal River delta, rice paddies irrigated with brackish water favoured the production of *An. pharaoenis*, a poor malaria vector. As a result, irrigation did not influence the incidence of malaria (Fayes et al., 1995).

The impact of irrigated rice production on malaria transmission is of growing concern. Nevertheless, increased larval production and vector mosquito density do not always give rise to an increase in malaria transmission. Some studies have shown that the installation of irrigation systems may result in a decrease in the number of infections (Robert et al., 1985). This may be due to changes in human behaviour by people living close to rice growing areas, such as the installation of bed nets, that prevent transmission of malaria (Robert et al., 1985).

In other situations, the implementation of rice culture irrigation systems may create extensive, new, permanent mosquito breeding sites, and lead to a significant increase in the incidence of malaria. Thus, on the Ruzizi Plain of Burundi, installation of irrigation mechanisms gave rise to an increase in *An. arabiensis* populations, raising the incidence of malaria to a new, stable, mesoendemic level (Coosemans et al., 1984). Not only may irrigation of rice paddies increase the number of mosquitoes produced on an annual basis, but it may also extend the period over which they transmit malaria by creating permanent breeding sites. Before the installation of irrigation systems in many West African countries, such as Gambia and the Republic of Mali, malaria transmission occurred almost exclusively during the rainy season (Lindsay et al., 1991; Dolo et al., 1999). Post-irrigation frequencies tended to manifest two annual transmission peaks, with the higher peak occurring in the dry season (Dolo et. al., 1999).

### 2.4 Water Management and *Anopheles* Mosquito Development.

In many countries, irrigation may provide suitable breeding sites for *Anopheles* mosquitoes during the dry season (Lindsay et al., 1991; Mogi, 1993; Amerasinghe et al., 1991; Chandler & Highton, 1976). Konradsen et al. (1998a) suggested that irrigation is of great importance for the production of *Anopheles* larvae since it tends to maintain rice field water levels below 50 cm. Mukiama and Mwangi (1989a) studied *Anopheles arabiensis* populations in rice fields in Kenya and confirmed the importance of irrigated rice paddies as breeding sites for this mosquito species; they also stressed the importance of habitat stability for optimal larval production. Drying out of the larval habitat is the primary cause of larval mortality in that study (Mukiama & Mwangi, 1989a,b). As well, Happold (1965) reported that intraspecific competition for space may be a major limiting factor for mosquito larval populations as habitat contracts during severe droughts. Furthermore, unstable habitats may skew sex ratios in favour of males, since males tend to emerge before females and are, therefore, marginally less susceptible to premature drying of the habitat.

In rice irrigation schemes in Kenya, Mutero et al. (2000) found that intermittent irrigation and drainage may provide a good breeding environment for *An. arabiensis,* since this tends to maintain shallow, clear water with an absence of other aquatic arthropods that are potential predators. Similarly, Mogi (1993) demonstrated that temporary drying of the breeding habitat may be beneficial to mosquito larvae if the reintroduced water is relatively free of predators. Since predators take longer to recover than mosquito populations, production of the latter is enhanced. Nevertheless, under certain conditions, a rapid decrease in water level was found to reduce mosquito larval populations due to their stranding in floating vegetation (Bradley, 1932).

Other aspects of water quality may also have an impact on larval development. Robert et al. (1988) stated that *Anopheles* larvae develop optimally in acidic (pH $< 7.0$) and well oxygenated water. However, most other studies disagree, claiming instead that pH may change the volatility of certain compounds and that low pH favours oviposition. It is generally agreed that pH has little effect on developing mosquito larvae (Angerilli & Beirne, 1980). Happold (1965) stated that natural distribution of mosquito larvae is not due to the quality of the water in the habitat. Lack of oxygen, for example, may reduce mosquito predators but will not affect mosquito larvae (Happold, 1965). He concluded that it is more likely that the distribution is a result of the female mosquito behaviour favouring vegetation, shade and the position of the habitat. Nevertheless, other water quality factors, such as water surface temperature, amount of organic debris and presence of aquatic micro-organisms may influence the development of *Anopheles* larvae as they are surface filterfeeders (Angerilli & Beirne, 1980).

### 2.5 Rice, Aquatic Plants and *Anopheles* Mosquito Development.

Robert et al. (1988, 1992) and Surtees (1970b) reported that *An. gambiae* s.l. development is closely related to the first phase of rice cultivation, which involves the transplantation of the rice to the inundated field. This agrees with the findings of Lindsay et al. (1991) in

Gambia and with those of Chandler & Highton (1976) in Kenya. Larvae develop well until rice plants reach a height of 60 to 100 cm, after which larval development is impaired. In Kenyan rice fields, Mutero et al. (2000) recorded the highest larval densities in the three weeks after rice seedling transplantation and a precipitous decline thereafter as the height of rice plants increased. This may be due to physical obstruction of ovipositing female (Orr & Resh, 1992), increased shade lowering the water temperature and/or the establishment of potential predators (Mogi, 1993; Robert et al., 1988). Klinkenberg (2000) suggested that the decrease in the number of mosquito larvae produced during canopy closure and rice maturation may be related to a decline in the use of fertilizers and herbicides; the effect of these chemicals on *Anopheles* larval production is still unclear.

In the Niono region of Mali, the *Anopheles* population is composed of more than 99% *An. gambiae* s.s. and 0.67 % of *An. funestus* (Coulibali, 1996). *An. funestus* does not seem to use rice paddies as ovipositing sites, and is therefore not influenced by rice cultivation (Robert et al., 1988). *Anopheles gambiae s.l.* is present in all types of rice fields, but is at higher densities in fields where the rice has just been harvested and the field is drying, as well as in earth made channels used for field irrigation (Robert et al., 1988).

Many authors suggest that aquatic plants are beneficial to immature mosquitoes. Thus, Orr & Resh (1992) found that surface cover offered by emergent aquatic plants is the primary habitat of *Anopheles* pre-imagos and that larval movements and oviposition are positively related to the amount of surface cover present. Similarly, Furlow & Hays (1972) reported that floating *Elodea* debris and broken stems and leaves of cattails *(Typha* spp.) provide cover for *Anopheles* larvae.

In contrast, Angerilli (1980) observed that mosquito larvae are less abundant in plant-filled ponds than in waters where there are no plants. One proposed reason for this phenomenon was the effect of plants on water temperature; plant cover keeps the water cooler, whereas mosquitoes tend to select warm ponds for oviposition.

The effect of vegetation on the development and survival of *Anopheles* larvae varies with plant species. Plants affect the kind and quantity of particulate matter present in the aquatic environment in different ways, depending on the species. Particulate matter may serve as a larval food source (Angerilli & Beirne, 1980) or may interfere with normal larval behaviour (Bradley, 1932). The presence of particles also influences oviposition, since it may modify the colour of the water and/or its chemical composition (Angerilli & Beirne, 1980).

Thus oviposition and *Anopheles* larvae abundance are positively related to the density of *Myriophyllum aquaticum* stems present over a range of densities from 0 to 1000 stems/m². It then decreases as stem density approaches 2000 stems/m² (Orr & Resh, 1992). Furlow & Hays (1972) found that environments with submerged vegetation produced large populations of Culicidae with the greatest number collected from habitats containing *Elodea*; they also showed that *Anopheles* larvae live well in floating debris of *Elodea* pools and are found around the broken leaves and stems of cattail.

Low densities of some species of floating plants, such as *Lemna* (duckweed) and *Heteranthera* spp. (star-grass), may favour mosquito larval populations, whereas a complete surface mat may inhibit mosquito breeding (Bradley, 1932). Thus, Furlow & Hays (1972) found no *Anopheles* larvae in pools containing duckweed; mosquito breeding was completely inhibited when these plants were dense enough to cover the total surface of the pools. Smith (1910) reported that under favourable conditions, *Azola* can form a mat that totally prevents mosquito breeding but does not adversely affect fish and other aquatic animals; this may happen where water is stagnant and temperatures are high. However, plant density is seldom sufficiently high to form a complete mat over the surface of the water to impair mosquito breeding, particularly in temporary ponds (Twinn, 1931); Hall (1972) confirmed this with other aquatic plant species. The effect on mosquito larvae is indirect and related to reduced planktonic food availability as a result of reduced light penetration (Bradley, 1932).

## 2.6 Predation

Plant community structure may also influence the presence and abundance of predators. Angerilli & Beirne (1980) concluded that more predators are found in bladderwort (*Utricularia)* and waterweed (*Elodea)* ponds than in ponds without vegetation or where *Lemna* is present. Orr & Resh (1989) concluded that macrophytes provide favourable microhabitat and refuge from predators. Dense vegetation may also favour *Anopheles,* because it may provide habitat for potential alternative prey and thus reduce predation pressure on its larval stages (Orr & Resh, 1989).

Various predators may reduce mosquito larval populations; these include various species of fish, amphibians, hydras, other invertebrates as well as some insectivorous plants (Twinn, 1931; Lacey & Lacey, 1990). There is no doubt that aquatic, predacious enemies play an important part in reducing mosquito populations in both temporary and permanent bodies of water. Twinn (1931) reported that water beetles, dragonfly and damselfly nymphs, adult back swimmers, and water scorpions prey on mosquito larvae. In many parts of the world, fish are regarded to be among the primary predators of mosquitoes. In waters where fish occur, mosquito pre-imagos are usually scarce or absent, although factors other than the presence of predators may be responsible for this (Twinn, 1931).

The architecture of aquatic plants is considered to influence the vulnerability of prey species to predation (Orth et al., 1984). The hypothesis of beneficial effects of aquatic plants on mosquito larvae is also supported by Orr & Resh (1992), who suggest that as habitat complexity increases, predator efficiency decreases. Laboratory and field studies by Heck & Thoman (1981) indicate that vegetation provides significant protection from predators for several species of seagrass invertebrates, and support the findings of Bradley (1932) who demonstrated that *Anopheles* larvae are sheltered by the stems of some aquatic plants.

Other studies present evidence that the presence of vegetation increases the probability of predator-prey encounters, since some predators such as fish search more systematically around the stems of plants, whereas they swim more randomly in open water (e.g., Angerilli, 1980). Larvae of Dysticidae and *Hydra* spp. are more likely to colonise plant-containing habitats because they use aquatic plants as a substrate on which to capture mosquito larvae (Angerilli, 1980).

Bradley (1932) concluded that the most important plants for mosquito larval protection are filamentous algae which grow at or just below the surface of the water. Plants such as duckweeds (*Lemna)* or *Azola* are not as effective, since their leaves float on the surface and, therefore, do not offer hiding places from aquatic predators that approach prey from below.

In summary, *Anopheles* mosquitoes that are vectors of malaria breed in large numbers in rice fields and irrigation schemes in many parts of arid and semi-arid regions of the world. It is known that water management, aquatic vegetation, predators and rice plant characteristics play significant roles in the abundance of mosquito larvae, but the importance of these factors on *Anopheles* larval populations is controversial. In order to control human malaria in some rice growing areas of West Africa, it is of major importance to know which of these factors have a significant impact on *Anopheles* mosquito larva production. The present study was undertaken to assess the impact of such environmental conditions on the production of *Anopheles* larval populations in the Niono region, Mali.

# 3. MATERIALS AND METHODS

## 3.1 Description of Study Area

### 3.1.1 Study Site

The study was conducted in the Niono region of central Mali, West Africa. This region is part of a large-scale irrigation scheme known as the Nigerian Office. The territory controlled by the Nigerian Office includes the inner delta of the Niger River surrounding Niono (Touré et al., 1997). The study area encompassed three villages, Tissana, Niono Koroni and Sokourani, located close to the town of Niono (14°17' N; 8°5' W), 350 km NW of Bamako City, the capital of Mali. The villages were selected on the basis of year-round accessibility, leaseholder agreements, location in relation to the principal irrigation canal, representative levels of rice production and general environmental conditions. Tissana cultivates a total area of almost 600 ha and is located in the northern part of the territory controlled by the Nigerian Office, east of the principal canal. Niono Koroni fields are located on the western shore of the principal irrigation canal between it and a major water distributor canal. Sokourani holds fields on the west side of the principal irrigation canal.

Two thirds of the fields associated with the three villages were under water management. The remaining fields lacked both irrigation and drainage channels. In these unmanaged fields, irrigation was accomplished by means of handdug channels or rain runoff. There is no direct connection between these fields and the irrigation system. Since unmanaged fields could not be drained, it was assumed that if water was found over two consecutive sampling periods, water was present in the interval. In managed fields, temporary drought could occur after drainage. Water levels in managed rice fields are controlled by means of a surplus evacuation system.

### 3.1.2 Social Environment

In 1991, the population of the Niono region was estimated at 150 000. Several ethnic groups are represented. Among these are the Minianka, Bambara, Peulh, Songhaï, Sarakolé, Bela, Dogon and Bozo tribes (DGCI, 1992). The large majority of the population is Islamic, with a small number of Christians and Animalists.

The principal economic activity is rice cultivation which, in the early 1970's, replaced cotton, maize and millet production that had prevailed since European colonisation. Since the beginning of the 1990's, double cropping has been practised in the region. Before that, rice was grown in single cropping. After rice growing, livestock farming, market gardening and fishing are important economic activities.

An association of villages originating from the restructuring "Operation for Rural Development" (ORD) is responsible for rice commercialization and for the purchase of all the necessities for its cultivation. In each village a health service is responsible for the distribution of antimalarial drugs.

### 3.1.3 Physical Environment

The Niono region is a vast clay-sand plain. The soils are fluvial deposits of the Niger River and composed of only 0.4 to 1.0 % organic matter. The Niger River provides water for the Nigerian Office irrigation scheme. No other permanent natural bodies of water are present in the region. Irrigation canals, some small, temporary pools, and irrigated rice fields are the only potential *Anopheles* breeding sites during the dry season. Water accumulates during the rainy season and usually persists for no more than 90 days, once the rains stop (Sagara, 1997).

The climate of the country is Sahelian. Winds divide the year into two seasons. The short, rainy season arrives with the Mousson, which brings rain from the south. During the dry season the Harmattan blows from the north-west. This season is further divided into a cold and a warm period. Thus, March to May represents the warm, dry season, June to September the rainy season, and October to February the dry, cold season.

The Nigerian Office irrigation system was first implemented in 1932, creating the largest irrigated rice-growing area of West Africa. Water is carried by gravity from the Markala Dam, on the Niger River, 250 km N.E. of Bamako, to five different rice cultivation zones and a sugar cane plantation. The system carries the water to cultivated lands for a distance of 60 to 120 km to irrigate an area of 60 000 ha and is still under expansion. The maximum capacity of this system is approximately 960 000 ha.

Irrigation fees, paid by the farmer on the basis of field size, are used by the Nigerian Office to cover the cost of primary and secondary system maintenance (channels and drains). Farmers are responsible for field intakes and drains, as well as associated dikes, tertiary channels and their drains.

Rice is the main crop of the area. It is cultivated either in one cycle a year (single-cropping) where there is no irrigation system, since in the dry season there is not enough rain for rice growing, or in two cycles (double cropping). The single-cropped rice is the most prevalent, although double-cropping is increasing in importance (Diop et al., 1995). Fields are usually composed of 4 basins, approximately 20 meters by 20 meters, they are interspersed with human habitations.

**3.2 Sampling**

In each of the three villages, two fields, one managed and the other unmanaged, each composed of four physically separated basins of approximately 20 meters by 20 meters were selected for sampling. Twice monthly, once during the first half and once again during the second half of the month, each of the 24 basins was sampled. Within each village, both types of basin were sampled on the same day.

The presence or absence of standing water and its depth were ascertained. Water was considered to be present as long as there was enough to support the development of larvae. Water depth was categorized as standard or in excess of standard (>25 cm). At the same time, rice density in each basin was classified as absent, sparse (<1/3 coverage), moderately dense (approximately 1/2 coverage) or dense (>2/3 coverage). Rice developmental stage was characterized as vegetative, reproductive, grain filling or mature (Table 1). The presence of aquatic vegetation other than rice was also assessed; densities were categorized, as described for rice.

**Table 1. Rice developmental stages.**

| |
|---|
| 1. **Vegetative stage (seedbed included):** early development of rice seeds from germination to transplantation. |
| 2. **Reproductive stage:** rapid elongation of the stem. |
| 3. **Grain filling stage:** inflorescence growth (in panicle or spikes) and grain formation. |
| 4. **Maturation stage:** ripening of the grain. |

Larval populations were sampled by walking the perimeter of each basin and dipping with a 250 ml long-handed container every 4-5 paces. All *Anopheles* larvae collected on the same day from the same basin were pooled, preserved in Carnoy's fixative and stored at 4°C (Service, 1993). Samples were collected by the same individual in each village for the duration of the study period; three local guides performed data collection under the direction of 2 supervisors who were in charge of operations in the three villages (one for Sokourani and Niono Koroni and the second for Tissana).

According to Coulibali (1996), more than 99 % of *Anopheles* mosquitoes in the region are either *An. gambiae s.s* or *An. arabiensis.* These species were distinguished by DNA extraction and amplification by means of a polymerase chain reaction (PCR) technique as described by Scott et al. (1993). Each of the 1 290 larvae collected within the 9 month study period were identified individually as to these two species. To verify the validity of the procedure, DNA identification was done on insectarium stock.

### 3.3 Statistical Analyses

Comparison of the proportions of the two *Anopheles* species encountered in managed versus unmanaged basins was tested using Chi-square. The homogeneity of larval population variance for all basins was tested to ensure that the data from the different villages could be pooled. Multivariate analysis was performed on the data set in order to assess any impact of water presence, basin type, rice density, rice developmental stage and aquatic weed density on *Anopheles* larval population size. Comparisons of the number of larvae encountered in managed versus unmanaged basins were performed using ANOVA, These statistical analyses were performed using the SAS general linear model procedure (SAS Institute Inc., 1996). SPSS regression curve analysis was performed on each of the other variables found to have a significant effect on larval population size by the multivariate analysis (Sigma Plot Scientific Software, 1995). Best fit curves were computer generated in Excell.

# 4. RESULTS

## 4.1 Vector Species Composition

Each of the 1 290 larvae collected within the 9 month study period were identified individually as *An. arabiensis* and *An. gambiae s.s.*. Only 15 % of all *Anopheles* larvae collected were identifiable by the PCR technique. Of these 195 individuals, more than 90% were identified as *An. gambiae s.s.* and the remainder (less than 10 %) as *An. arabiensis* (Table 2). Proportions of the two species appear to be the same among basins whether managed or unmanaged (Chi-square = 1.168; $p>0.95$).The proportion of species identified were significantly identical from one village to an other, so the data from the three villages were combined for all the analyses. Identification rates on insectarium stock using the same procedure was 100%. PCR bands from the reared specimens were clear, whereas field collected material was largely unreadable.

## 4.2 Excessive Flooding

Water levels in all managed and most unmanaged basins were consistent with rice culture, ranging from 1 to 30 cm. Exceptions were 4 unmanaged basins in the Tissana village. Basins were flooded to a level of 75 cm or more for half the year, and were not cultivated. Using the mean total number of larvae captured during the 9 month sampling period, the analysis showed that unmanaged, deeply flooded basins yielded 30.25 ±17.91 larvae over the nine month period compared with 82.63 ± 29.95 for standard water level unmanaged basins ($p<0.05$). Because of the significant habitat differences, these 4 deeply flooded basins were removed from the analysis of *Anopheles* larval populations and subsequent analysis was restricted to data from the remaining 20 basins.

## 4.3 Multivariate Analysis

Variances were homogeneous ($P= 0.001$) (Table 3). Therefore it is appropriate to analyse the larvae number obtained from the 20 basins all together. The multivariate analysis showed that all the independent variables studied had an influence on *Anopheles* larval population size, except for rice developmental stage.

Table 2. Comparison of *Anopheles* vector population composition between two types of basin over a nine-month period, in the Niono Region, Mali, 1999.

| Type of basin | Total number of *Anopheles* larvae | ID/total | Number of *An. gambiae* | Number of *An. arabiensis* | % *An. gambiae* | % *An. arabiensis* |
|---|---|---|---|---|---|---|
| Unmanaged | 776 | 86 | 81 | 5 | 94 | 6 |
| Managed | 514 | 109 | 98 | 11 | 90 | 10 |

Table 3. Multivariate analysis to assess the impact of water presence, basins type, rice density, rice development stage, rice density and aquatic weed density on *Anopheles* larval population in the region of Niono, Mali, 1999.

| Source | DF | F Value | Pr > F |
|---|---|---|---|
| FIELD | 1 | 14.16 | 0.0002 |
| AZOLA | 3 | 3.99 | 0.0083 |
| RICED | 3 | 22.48 | 0.0001 |
| RICEDST | 4 | 1.36 | 0.2468 |
| WATER | 1 | 44.31 | 0.0001 |
| FIELD*AZOLA | 3 | 1.54 | 0.2045 |
| FIELD*RICED | 3 | 1.61 | 0.1869 |
| FIELD*RICEDST | 4 | 3.40 | 0.0097 |
| FIELD* AZOLA*RICED*RICEDST | 50 | 2.96 | 0.0001 |

Legend:

FIELD: Type of water management (managed or unmanaged basins)

AZOLA: Floating weed densities

RICED: Rice densities

RICEDST: Rice developmental stages

WATER: Presence or absence of water

## 4.4 Water Management

The presence or absence of standing water sufficient for the development of mosquito larvae varied greatly among fields and somewhat less among basins of the same field. Unmanaged basins tended to remain flooded longer than managed ones. The total duration of flooding also affected the number of *Anopheles* larvae harboured.
Since managed and unmanaged basins were at opposite ends of the gradient in water management, a comparison of the number of larvae produced in these two type of basins was done as a first step. Using the mean total number of larvae captured during the 9 month sampling period, the analysis showed that unmanaged basins yielded twice as many larvae (81± 30 larvae) as managed basins (44 ± 28 larvae ), ($p<0.05$).

To refine the analysis of water management impact on *Anopheles* larvae numbers, two other variables were examined: the length of contiguous wet periods and the number of interruptions of standing water. The number of *Anopheles* larvae increased significantly with the number of contiguous periods of water presence, with a very strong correlation coefficient ($R^2$= 0,93; $p< 0.05$) (Fig.1). Similarly, the number of interruptions of water (drainage or drought) affected the size of the larval population. As the number of interruptions increased from 1 to 4, the number of larvae decreased significantly; this relationship had a very strong correlation coefficient ($R^2$= 0.92; $p<0.05$). (Fig 2). These results suggest that basins in which water is drained frequently, produced significantly fewer larvae. The number of larvae produced decreased as the number of interruptions increased.

## 4.5 Rice and *Azola*

There was a strong relationship between the density of rice plants and the yield of *Anopheles* larvae ($R^2$= 0.80; $p<0.05$) (Fig. 3). As rice density increased from low to moderate levels, the number of larvae per sample increased; then, as rice densities rose further, the number of larvae decreased. The highest number of larvae were collected when rice was moderately dense, with an average number of larvae of 7.35 ± 8.25 per sample. The high variability may reflect sample size or seasonal changes in water temperature.

Plants found in rice paddies were primarily *Azola* but also duckweed, water lilies and sedges. A significant relationship was demonstrated between the number of weeks with floating aquatic weed coverage and the number of *Anopheles* larvae ($R^2$= 0.38; $p<0.05$). As the number of weeks of coverage increased from 4 to 30, larval numbers declined by a factor of approximately 4 (Fig 4).

**Figure 1**

**Length of contiguous two-week wet periods versus the production of *Anopheles* larvae, over a nine-month period, in the Niono region, Mali, 1999.**

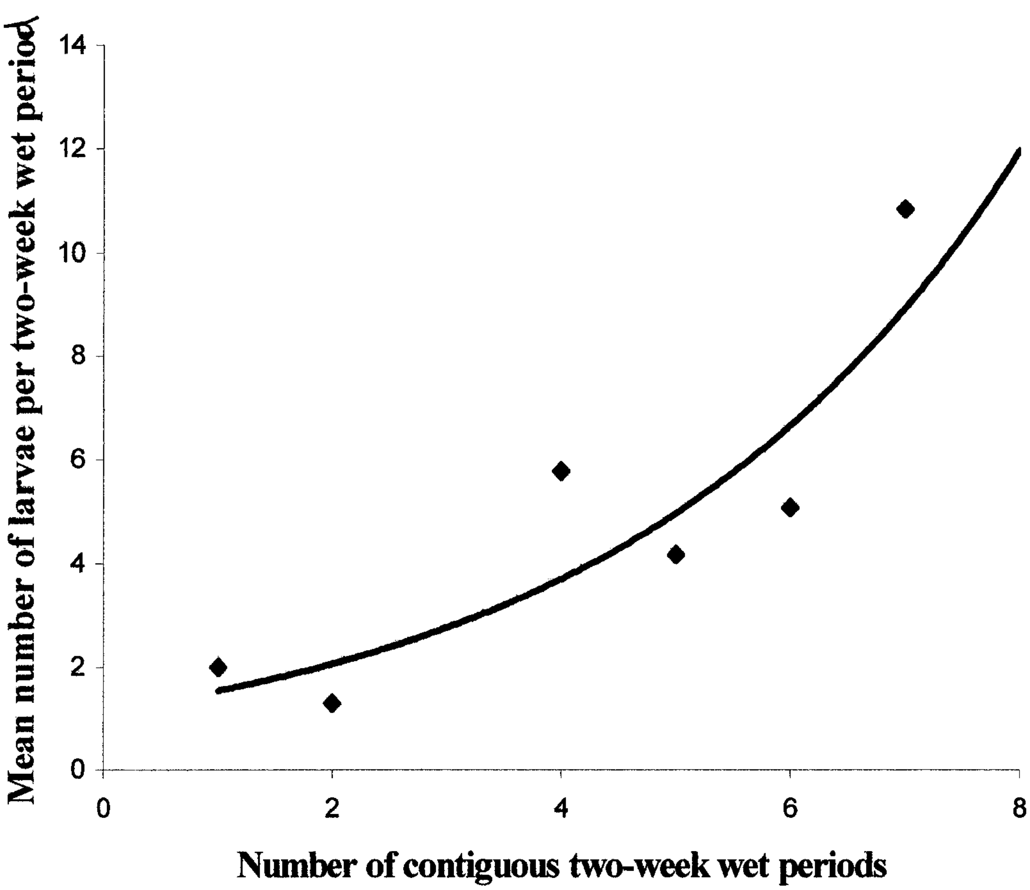
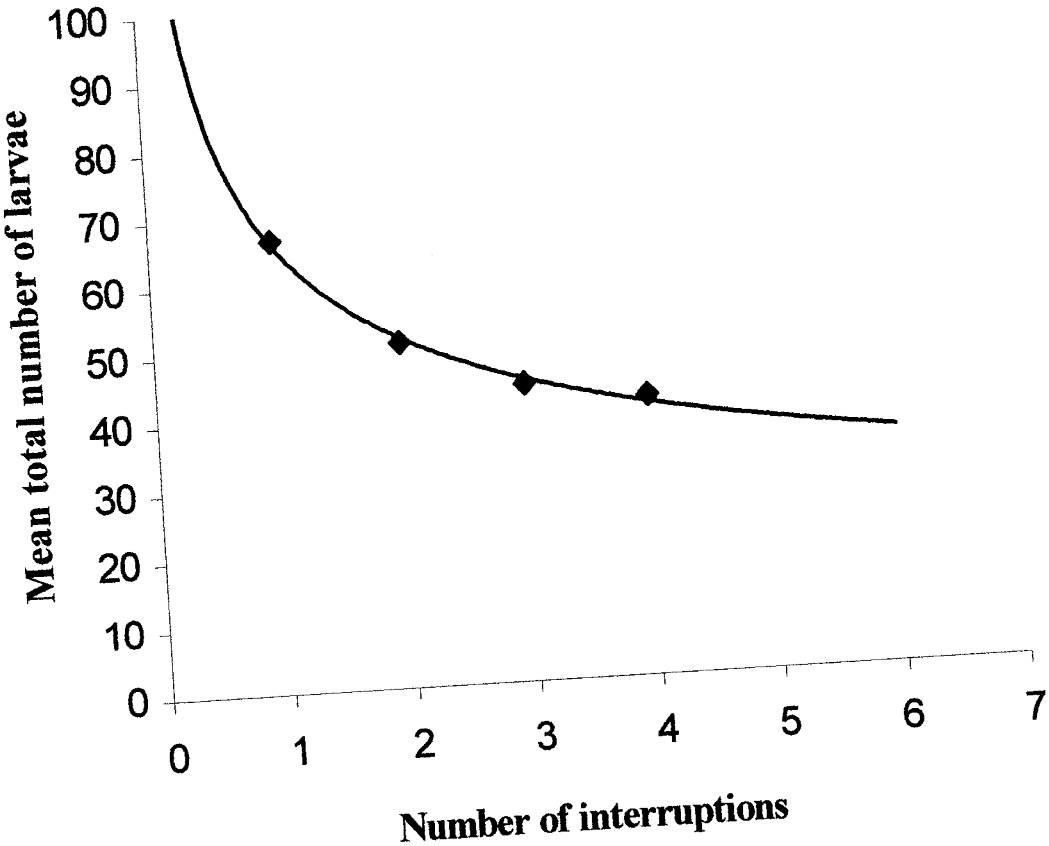

Mean number of larvae per two-week wet period
14
12
10
8
6
4
2
0
0
2
4
6
8
Number of contiguous two-week wet periods

**Figure 2**

**Number of water interruptions in managed and unmanaged basins versus the production of *Anopheles* larvae, over a nine-month period, in the Niono region, Mali 1999.**



Mean total number of larvae
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
Number of interruptions

**Figure 3**

**Rice density versus the production of *Anopheles* larvae, over a nine-month period, in the Niono region, Mali, 1999.**

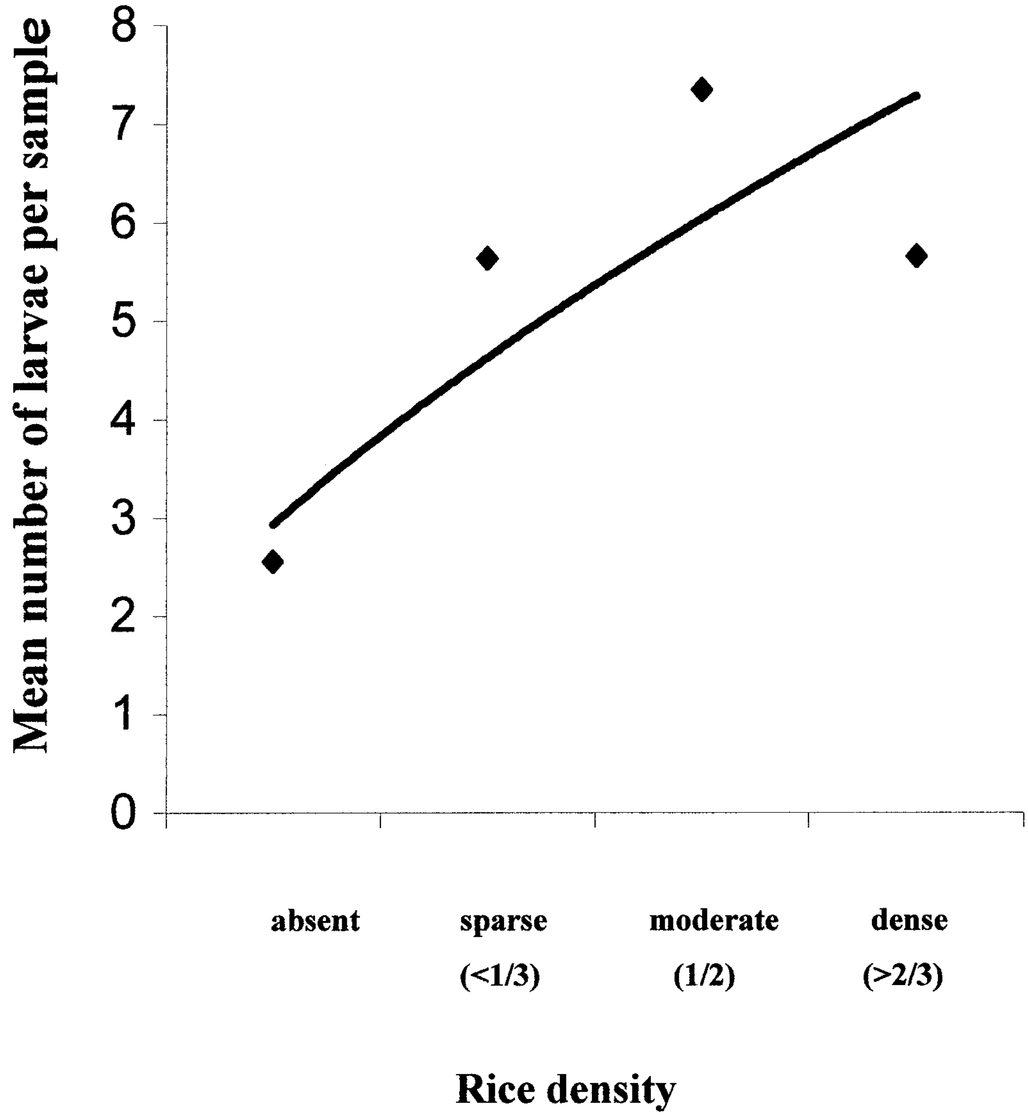
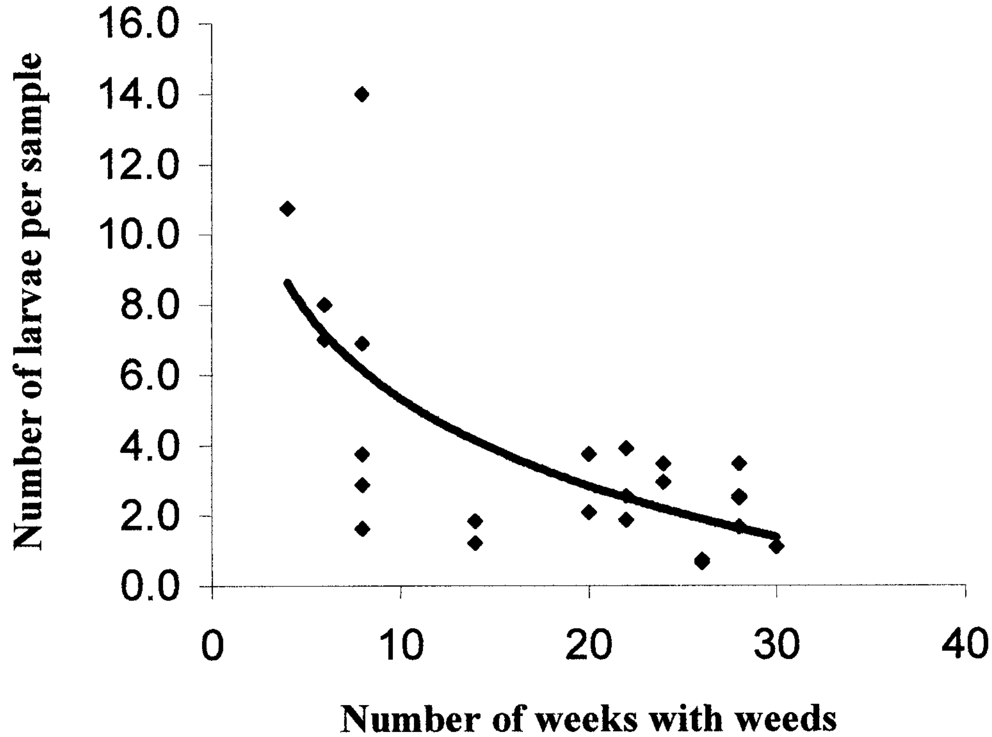

Mean number of larvae per sample
8
7
6
5
4
3
2
1
0
absent
sparse
(<1/3)
moderate
(1/2)
dense
(>2/3)
Rice density

**Figure 4.**

**Influence of floating weed coverage on the production of *Anopheles* larvae, over a nine-month period, in Niono region, Mali, 1999.**


16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Number of larvae per sample
0
10
20
30
40
Number of weeks with weeds

# 5. DISCUSSION

## 5.1 Vector Population Composition

*Anopheles gambiae s.s.* was, by far, the most abundant among the *Anopheles* species as identified by the PCR technique. *Anopheles arabiensis* was relatively uncommon, regardless of water management. To the extent that the larval populations reflect the adult population, this suggests an important subsequent availability of potential malaria vectors. The composition obtained is comparable to that of Coulibali (1996) from the same region.

The low rate of identification may be due DNA degradation during transport from the field to the laboratory. The probability that this was due to errors in the PCR process is unlikely, since a test on larvae taken directly from the insectarium yielded a rate of 100% identification. The presence of other species morphologically identical to *Anopheles gambiae* s.l. but lacking the DNA marker cannot be excluded, and may also explain the low identification rate. Similarly, low levels of identification of mosquitoes were found from Tissana village in 1991 (Bouaré, personal communication).

## 5.2 Water Cycle

The study confirms that excessively deep flooding reduces mosquito production. *Anopheles* larvae prefer shallow habitats, less than 50 cm deep. Since they harbour no rice plants, deep permanent waters are thought to reduce the availability of shelter for mosquito larvae and favour the survival and efficiency of mosquito predators such as fish (Konradsen, 1998a). Indeed, fish were found in these basins. Mukiama & Mwangi (1989a) also reported an inverse relationship between Nile water levels and *An. arabiensis* production. Water depths in excess of 50 cm are rarely encountered in well managed rice paddies since they severely reduce rice production.

Within the normal range of water depths suitable for rice production, the duration of standing water and the number of interruptions of water were the most important factors in determining the number of *Anopheles* larvae in the rice paddies. Since *Anopheles* larvae need at least 5 days to develop to adults, frequent draining of the fields may kill larvae (Mukiama & Mwangi, 1989a). Furthermore, intermittent drying of fields may periodically reduce habitat available for oviposition. Rapid drying of the habitat may also result in severe intraspecific competition for space between larvae and may limit larval population size (Happold, 1965).

Water management may also influence the abundance of predators in the systems studied. For example, if fish are responsible for maintaining mosquito populations at low levels in these systems, the slow drying of their habitat in unmanaged fields may severely reduce their numbers as oxygen levels decrease. This oxygen reduction would not affect mosquito larvae since they use atmospheric oxygen (Happold, 1965).

In managed fields, fish periodically invade fields by way of irrigation canals (Mogi, 1993). In unmanaged fields however, there is no direct connection between the fields and the irrigation system. Flooding is either from runoff or through hand dug channels too shallow for fish to traverse. Fish were only seen in freshly flooded managed basins and in unmanaged basins with deep water (more than 25 cm) where few larvae were observed. The presence of fish in these unmanaged basins is probably the result of deliberate introduction or the occasional overflow of the irrigation system. Differences in predator populations from managed to unmanaged fields may therefore explain the very high *Anopheles* larval production in unmanaged fields.

Some studies have shown that intermittent irrigation may be beneficial for *An. gambiae* production since it is often associated with clear shallow water and a reduction in the number of predators (Mutero et al., 2000). This may occur when the irrigation source is not a reservoir for mosquito predators. This aspect may vary regionally, and should be taken into consideration when attempting to predict the effects of specific water management techniques on mosquito populations.

### 5.3 Rice Density, Rice Development Stages and Aquatic Weeds.

In the current study, beneficial aspects of rice plants to *Anopheles* mosquito larvae seem to override disadvantages. Thus, the number of larvae increased with the density of rice plants to moderate levels. Rice plants may protect the eggs, larvae and pupae from predators (Bradley, 1932; Heck & Thoman, 1981). Predator species vary also as a function of shelter (Happold, 1965). Happold (1965) found that spatial distribution of larvae is frequently attributable to the behaviour of ovipositing female mosquitoes in response to vegetation, the amount of shade and the location of the breeding habitat. Rice plants offer shade and diversify the habitat. As well, biochemical processes of plants or their metabolites may promote oviposition by attracting females (Furlow & Hays, 1972). However, excessively high rice densities may reduce the number of larvae due to physical obstruction of ovipositing females (Teuscher, 1997). Rice plants may also offer both protection to larvae and an environment for their predators.

Rice developmental stage did not show a significant impact on mosquito populations, Perhaps the sampling method did not permit a clear relationship to be established between the two. Since it is difficult to sample close to the rice plants, the sample may not reflect the true abundance of larvae.

The presence of *Azola* and other floating weeds was inversely related to *Anopheles* larval population size. This may be due to impaired oviposition or larval breathing. Mosquito breeding is usually favoured by debris and filamentous aquatic plants as long as these do not cover the entire water surface. Furlow and Hays (1972) found that a complete surface mat of floating plants impairs mosquito breeding. These authors also concluded that mosquito breeding may be completely inhibited at high densities of weeds, due either to a lack of oviposition or reduced access to atmospheric oxygen by larvae and pupae.

# 6. CONCLUSIONS

The objectives of this study were to determine the species composition of *Anopheles* mosquitoes in the Niono region of Mali in West Africa, to compare larval production in the fields under irrigation practices ranging from virtually unmanaged to highly managed and also to assess the concomitant impact of rice density, rice development stage and floating aquatic weeds on larval production. The objectives were achieved and the hypotheses accepted. More is now understood concerning the extent to which irrigation practices in this vast territory may influence the size of larval populations of malaria vectors, and suggestions can be made to reduce such populations by water management. This study showed that water management plays a major role in production of *Anopheles* larvae in Mali rice fields. Unmanaged basins, at one end of the continuum, produced more larvae than managed ones. The presence of *Azola* and rice densities were also shown to determine the size of the larval population in rice lands, whereas no significant impact of rice developmental stages on the *Anopheles* larval population were found.

*Anopheles* mosquitoes are undoubtedly adaptable to a wide variety of breeding places. Intermittently drying fields, if detrimental to predators, may favour larval development in re-irrigated fields reducing predation pressure (Mogi, 1993), but when predators are reintroduced with irrigation water, irrigation and drainage management may become a way to control mosquitoes (Chandler & Highton, 1975). In this study, it was found that intermittent irrigation and drainage significantly reduce *An. gambiae s.s* and *An. arabiensis* larval populations; persistence of shallow water in rice paddies favours *Anopheles* production. Changing water management to avoid pooling may cause a reduction in the number of *Anopheles* mosquito larvae (Konradsen et al., 1998b), but the techniques must be adapted to each situation and take into account the source of intake water. This study focussed on total *Anopheles* mosquito production, regardless of season. Since water temperature is known to have effect on larval development it would be useful to break down the data into seasons to examine the influence of these factors in relation to the temperature of the aquatic environment.

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