Development of environmental tools for anopheline larval control

Susan S Imbahale¹²†, Collins K Mweresa¹²†, Willem Takken¹ and Wolfgang R Mukabana²³

Abstract

Background: Malaria mosquitoes spend a considerable part of their life in the aquatic stage, rendering them vulnerable to interventions directed to aquatic habitats. Recent successes of mosquito larval control have been reported using environmental and biological tools. Here, we report the effects of shading by plants and biological control agents on the development and survival of anopheline and culicine mosquito larvae in man-made natural habitats in western Kenya. Trials consisted of environmental manipulation using locally available plants, the introduction of predatory fish and/or the use of Bacillus thuringiensis var. israelensis (Bti) in various combinations.

Results: Man-made habitats provided with shade from different crop species produced significantly fewer larvae than those without shade especially for the malaria vector Anopheles gambiae. Larval control of the African malaria mosquito An. gambiae and other mosquito species was effective in habitats where both predatory fish and Bti were applied, than where the two biological control agents were administered independently.

Conclusion: We conclude that integration of environmental management techniques using shade-providing plants and predatory fish and/or Bti are effective and sustainable tools for the control of malaria and other mosquito-borne disease vectors.

Introduction

Development activities that entail clearing of forests and/or drainage of swamps for timber, agriculture, human settlement and road construction often create suitable breeding sites for malaria mosquitoes [1-4]. Irrigated fields and areas with vegetable crops are ecologically good breeding sites for anopheline larvae [5-8] and they indirectly lead to sustained levels of malaria transmission [9]. The gradual increase in human population in western Kenya has put pressure on land available for farming and as a consequence, areas that were previously natural swamps and forests have been transformed into agricultural fields that provide suitable breeding habitats for mosquitoes.

One way of adapting to changes in land use and preventing the transmission of mosquito-borne disease may be achieved through the control of immature mosquitoes. The control of immature mosquito populations is advantageous because the larvae are usually concentrated, relatively immobile, and occupy a minimal habitat area compared with adults [10,11]. Several larval control programs in China, India and Sri Lanka have shown great success in controlling mosquitoes through good water management practices [12]. In Africa, malaria prevention through the control of immature mosquitoes has not received as much attention as adult mosquito control [11]. This is despite the fact that successful larval control of mosquitoes in Africa by environmental management and application of larval insecticides was reported more than half a century ago [13-15], and that there is renewed interest by the scientific community to assess the feasibility of these methods of disease control [16-20].

Mosquito larval control can be achieved through environmental (water) management, use of insect growth regulators, biological and chemical control [10,21]. Environmental management entails modification and manipulation of the environment, and modification or manipulation of human habitation or behaviour to prevent propagation of mosquito vectors and subsequently reduce human vector pathogen contact [22,13].
However, specifications for environmental management vary with local ecosystem structure, and hence there is no uniform environmental management recipe that is appropriate in all settings [13, 23]. Biological control methods directed against mosquitoes mostly refer to the use of natural enemies such as predatory fish, invertebrate predators, and toxins produced by microbial agents [10, 21, 24–26].

We recently reported that, while members of communities affected by malaria are willing to take part in mosquito control activities [27], there is lack of evidence-based research on locally-applicable strategies. A longitudinal study carried out in these same communities showed that larval populations of *Anopheles gambiae* Giles are continuously present [5]. In the present study we investigated the potential of environmental manipulation (shade from crop and non-crop plants) and biological agents (larvivorous fish and the microbial insecticide *Bacillus thuringiensis* var. *israelensis*) for the control of anopheline mosquito larvae.

**Materials and methods**

**Study site**

The field study was conducted in Nyalenda (0°06'S and 34°46'E, 1100 m above sea level), a peri-urban, low-income area in Kisumu County, western Kenya. The site represented a swamp transformed to sustain irrigated agriculture. The main economic activities were subsistence agriculture with rice, maize, sweet potatoes and vegetables under cultivation. Commercial nurseries of ornamental plants and trees were also present. The area received a total annual rainfall of 1004 mm and experienced a mean annual relative humidity of 64% and air temperature of 23°C in 2007. The area receives short seasonal rains in the months of October through December, while long rains occur between March and June with year-to-year variation in intensity. Water present at the Nyalenda study site was in parts turbid and polluted with debris and human waste from the adjacent housing estates. Additional experiments were conducted in screen-house at the Centre of Global Health Research (CGHR), KEMRI, Kisian, located 13 km north-west of Kisumu city.

**Mosquito colony**

*Anopheles gambiae* *sensu stricto* larvae (Igulu strain) used in experiments were maintained at the KEMRI insectaries in Kisian. Each larval tray was provided with 100 mg of brewer’s yeast daily (Pharmadass Ltd., Harrow, UK).

**Fish colony**

A colony of *Gambusia affinis* (Cyprinodontiformes: Poeciliidae) was established from a wild-caught population kindly provided to us by staff of the Kenya Marine and Fisheries Research Institute (KEMFRI), Kisumu County. The mosquito-fish colony was maintained in a screen-house at KEMRI, Kisian. The fish were fed on a locally made fish food supplement obtained from KEMFRI. Adult fish were used for screen-house trials and field experiments.

**Mosquito larval sampling**

Larval sampling was done using the standard dipping method with a 350 ml mosquito dipper (Bioquip, Garden, CA, USA) as described by Service [28]. A maximum of 10 dips were sampled from each habitat. Sampled larvae were identified based on morphological characteristics, counted and classified as anophelines and culicines. The larvae were recorded either as early instars (L1 and L2) or late instars (L3 and L4) and mosquito density was expressed as number of larvae per dip. Late instar anopheline larvae were microscopically identified to species level using existing identification keys [29]. Larval sampling was followed on weekly basis unless stated otherwise.

**Identification and characterization of plant cover types**

This was a preliminary study done to determine whether plant cover type had any impact on the abundance of mosquito larvae in habitats within the Nyalenda study site. Plants growing within suitable mosquito breeding habitats were identified and categorized into two broad groups: those which grew along banks of water channels and those which grew inside the water channels. Plants which grew along the banks of the water channels were grouped as food and non-food crops. Plants which grew inside water channels with roots anchored in the soil were classified as rooted emergent plants while those suspended on the water surface were grouped as floating types.

**Identification and mapping of traditional water management practices**

Four habitat types associated with different traditional water management practices were identified and incorporated in the design of this study. These were pools (on average 0.7 m deep × 2.1 m in diameter), small water canals (15 m × 1 m × 0.3 m deep), paddies (15 m × 15 m × 0.5 m deep) and swamps used for control (5 m × 15 m × 0.3 m deep). Except for the swamps (= control), all habitats were man-made. Sampling of mosquito larvae was done on Tuesday and Friday mornings for thirteen weeks (February to May 2008) based on the procedure used under plant cover habitats. The presence or absence of plant cover of each habitat was also recorded and mosquito larvae sampled according to the procedure above.
Establishing the effect of plant cover type on mosquito breeding
Vegetation cover types inside or along the banks of man-made water canals in Nyalenda were identified. These consisted of arrow root (Maranta arundinacea) growing along banks of water canals and inside water canals, sweet potatoes (Ipomea batatas) growing along banks of water channels, African couch grass (Cynodon dactylon) growing inside water channels, water ferns (Azolla filiculoides) growing on the water surface and open sites with no plant cover (control). Each type of habitat (2 m × 0.75 m × 0.3 m) was replicated five times. All habitats were irrigated by running water from large canals (20 m away). Silt was removed, edges of the habitats were slashed while weeds growing between the plants were uprooted on weekly basis. Each habitat type was separated from the other by 10 cm thick wall made up of soil/mud with a narrow inlet on the upper part to allow flow of water. Mosquito larval sampling was followed as described above.

Manipulation of mosquito breeding habitats through shading
This study was conducted for a period of 17 weeks from March to June 2007. Thirty-six mosquito breeding habitats (1 m × 1 m × 0.5 m) were created by building a shallow dyke (0.2 m) around each habitat. Each of the four locally grown plant species Napier grass (Pennisetum purpureum), arrow root (Maranta arundinacea), papyrus reeds (Cyperus spp.) and rice (Oryza sativa) were planted in each habitat and replicated six times. One additional habitat of rice was introduced and left intact to determine if weeding had any effect on mosquito breeding and larval survival. Another series of habitats was left unplanted (control). The habitats filled naturally with water by seepage from groundwater or from rainfall. Weeding was done once per month in all habitats, except in the weeded rice habitats, to remove un-wanted plant species that would cause unforeseen effects on the experiment. Larval sampling was conducted using the standard dipping method as described above.

Biological control of mosquito larvae under field conditions
This study was done for a period of 13 weeks from February to May 2008. Thirty man-made habitats (1 m × 1 m × 1 m) were created as mosquito larval habitats by building a 30 cm shallow dyke around each habitat. Six treatments were randomly administered as follows, Bti, Bti + fish in full required amount, Bti + fish at half the required amount of each, fish introduced once, and fish introduced fortnightly, while one habitat series was left untreated to act as a control. Each treatment was replicated 25 times. Small plastic washbasins (27.5 cm × 17.3 cm × 10 cm) filled with two litres of water collected from the Nyalenda field site to a depth of three cm were used. Sixty larvae consisting of 30 early (L1 and L2) and 30 late (L3 and L4) instars were randomly dispensed into each basin using a rubber pipette. Each basin containing water and larvae, were then randomly assigned the six treatments as shown above. In total, 9000 laboratory-reared larvae of An. gambiae s.s. were used. The optimum Bti dosage and concentration of 5 mg/l of water was determined based on the existing literature [30]. Preliminary trials were done with different numbers of adult fish, which were offered 60 larvae (mixed larval stages of development) and we found that four adult fish were able to consume 60 larvae in 24 h. Different sizes of mosquito fish were used to cater for differences in predation resulting from effect of size. The number of live larvae present after introduction of the treatments in different wash basins was recorded after 24 and 48 h of exposure.

Biological control of mosquito larvae
Investigations of Bti and Gambusia affinis for larval control
This study was done for a period of eight weeks from November to December 2007. The main goal was to estimate the optimum number of fish and the quantity of Bti required for effective control of mosquito larvae. Six different treatments were randomly administered. These included Bti 1 day, Bti 3 days and Bti 5 days (Bti was put in water, left to stay for 1, 3 and 5 days before larvae were introduced), Bti and fish, Bti only and fish only while one series was left untreated to act as a control. Each treatment was replicated 25 times. Small plastic washbasins (27.5 cm × 17.3 cm × 10 cm) filled with two litres of water collected from the Nyalenda field site to a depth of three cm were used. Sixty larvae consisting of 30 early (L1 and L2) and 30 late (L3 and L4) instars were randomly dispensed into each basin using a rubber pipette. Each basin containing water and larvae, were then randomly assigned the six treatments as shown above. In total, 9000 laboratory-reared larvae of An. gambiae s.s. were used. The optimum Bti dosage and concentration of 5 mg/l of water was determined based on the existing literature [30]. Preliminary trials were done with different numbers of adult fish, which were offered 60 larvae (mixed larval stages of development) and we found that four adult fish were able to consume 60 larvae in 24 h. Different sizes of mosquito fish were used to cater for differences in predation resulting from effect of size. The number of live larvae present after introduction of the treatments in different wash basins was recorded after 24 and 48 h of exposure.

A similar procedure was used for biological control of mosquito larvae within man-made water canals with different vegetation cover types. Six treatments were randomly administered in canals habitat with open water...
(control), *Azolla* growing on the water surface, sweet potatoes and arrow roots growing along the banks of water channels, African couch grass and arrow roots growing inside the water canals. Larval sampling of mosquitoes was done 24 h after treatment and thereafter regular sampling of mosquito larvae was conducted twice weekly using the standard dipping method as described above.

**Data analysis**

Data analysis was done using SPSS 15.00 for windows (SPSS Inc, Chicago, IL, USA). The General Linear Model (GLM), multivariate analysis was used to calculate the estimated marginal means for larval densities. Generalized Linear Model (GLM), with probability for normal distribution and log linked function was used for calculation of Odds ratio and comparison of larval densities within different habitats with the control. Only anopheline larval data was included in the analysis.

**Results**

**Water management practices and larval abundance**

Anopheline larval abundance sampled from the pools, paddies and water canals was compared with the control (swamp). The abundance of early instars was significantly different in water canals (P < 0.05) and pools (P < 0.05). Early instars were twice more likely to be sampled in pools (OR 2.328, 95% CI 1.057-5.124) and water canals (OR 2.512, 95% CI 1.151 - 5.482) than in the swamps. Water management practices had no significant (P > 0.05) influence on the abundance of late instar anophelines. However, late instars were twice more likely to be found in pools (OR 2.519, 95% CI 0.281 - 22.610) and four times in water canals (OR 4.240, 95% CI 1.057-5.124) and sweet potatoes growing along the banks of water channels, African couch grass growing inside water bank and arrow roots growing along the banks in Nyalenda were mostly sampled in the open habitats, while water surfaces covered with *Azolla* recorded the lowest number (4.55%, n = 1). Compared with other habitats, *As. funestus* were mainly recorded in habitats with African couch grass (53.85%, n = 7). *Anopheles coustani* Laveran, which has been reported recently as a possible vector species of malaria in East Africa [17] formed 28.81% (n = 208), while other anophelines that are non-vector species of malaria constituted 66.34% (n = 479).

**Vegetation cover and larval abundance**

A total of 722 late instar larvae of anopheline mosquitoes were identified. Table 1 provides a list of plants common in the study area. A comparison of different habitats showed that open sites recorded the highest percentage of anopheline larvae (31.16%; n = 224) while those with arrow roots growing in water had 22.58% (n = 163), arrow roots growing along water banks 14.82% (n = 107), African couch grass growing inside water 14.54% (n = 105) and sweet potatoes growing along the water banks 13.85% (n = 100). The lowest percentage of anopheline larvae (3.05%, n = 23) was recorded where the water surface was covered by *Azolla*. The abundance of both early (OR 0.290, P = 0.001) and late instar larvae (OR 0.264, P = 0.025) of anophelines was reduced by 71 and 73%, respectively, in habitats covered with *Azolla* (Table 2). Although there were significant differences among habitats with other plant cover types (Figure 1), habitats with arrow roots recorded lower densities of both early and late instar larvae.

*Anopheles gambiae* s.l. and *An. funestus* constituted 3.05% (n = 22) and 1.8% (n = 13), respectively, of all late instar larvae of anopheline mosquitoes identified from all habitats. *Anopheles gambiae* s.l. (36.36%, n = 8) were mostly sampled in the open habitats, while water surfaces covered with *Azolla* recorded the lowest number (4.55%, n = 1). Compared with other habitats, *An. funestus* were mainly recorded in habitats with African couch grass (53.85%, n = 7). *Anopheles coustani* Laveran, which has been reported recently as a possible vector species of malaria in East Africa [17] formed 28.81% (n = 208), while other anophelines that are non-vector species of malaria constituted 66.34% (n = 479).

**Manipulation of mosquito breeding habitats through shading**

Young anophelines (L1 and L2) were abundant in all habitat types but the numbers of late stage larvae (L3 and L4) were fewer in most habitats except in the controls, weeded rice and habitats covered by Napier grass (Table 3). The densities of young anophelines were significantly reduced by 58% (OR = 0.414, P = 0.002), 51% (OR = 0.488, P = 0.038) and 42% (OR = 0.577, P = 0.051) in habitats with Napier grass, unweeded rice and arrow roots, respectively, when compared with control habitats. Late stage larvae were significantly reduced by 95% in habitats where arrow roots were grown (OR = 0.045, P = 0.004), and by 91% in habitats containing unweeded rice (OR = 0.091, P = 0.026), when compared with the control habitats (Table 3).

Overall, anophelines comprised 29% of the total larval population sampled (N = 2445); while culicines (71%) were most abundant. Almost 85% of all *Anopheles gambiae* s.l. collected were from the control habitats while

---

**Table 1 Plant species grown in water and along water banks in Nyalenda**

| Category of plants | Plant species |
|--------------------|--------------|
| a) Plants grown along the water banks | (i) Food crops | Zea mays, Phaseolus vulgaris, Phaseolus aureus, Elucine coranaca, Sorghum sativum, Musa paradisica, Brasica spp (eg Kale), Colocasia esculenta, Manihot esculenta, Ipomea batatas, Lycoipersicon sp, Saccharum officinarum, Cucurbita spp. |
| (i) Non food crops | Pennisetum purpurea, Digitaria scalarum, Cydonon nlemfuensis, Cyperus rotundus, Commelina spp, Rincus communns. |
| b) Plants growing in water | (i) Emergent | Colocasia esculenta, Digitaria scalarum, Cydonon nlemfuensis, Cyperus rotundus, Onyza sativa, |
| (i) Floating | Azolla filiculoides, Sipryphya sp, Rhodophyte sp, Phaeophyte sp |
An. coustani was present in all habitats except the unweeded rice habitats (Figure 2).

**Efficacies of Bacillus thuringiensis var israelensis and Gambusia affinis for mosquito larval control**

The percentage of larvae that was alive after 24 and 48 h of exposure to different treatments was quite low. Treatment with Bti recorded a few pupating larvae, but the resulting pupae were unable to develop into adults. Analysis of variance found significant differences among the treatments ($F = 16.457; df = 4; P < 0.001$). Pairwise comparison of different treatments showed that the number of larvae exposed to Bti and fish, Bti 1 day, Bti 3 days and Bti 5 days were not statistically different ($P = 1.0$) from each other. However, apart from the control, treatment with fish recorded significantly more surviving larvae after 24 h when compared to those treated with Bti 1 day, Bti 3 days and Bti 5 days old ($P < 0.001$).

**Efficacy of Bti and fish in man-made habitats**

Anopheles larvae were sampled more from the control and in habitats with fish only (Table 4), whereas more culicine larvae (data not shown) were recorded in habitats treated with full amounts of Bti and fish. The effect of treatment type on young instars were significantly observed in habitats provided with Bti and fish, in half (OR = 0.650, $P = 0.004$) and full (OR = 0.325, $P < 0.001$) quantities of the required amount when compared to the control habitats. However, for the late

### Table 2 Abundance of early and late instar larvae of anopheline mosquitoes in man-made habitats covered with different plant covers

| Parameter                        | Early instars | Late instars |
|----------------------------------|---------------|--------------|
|                                  | EMM | 95% CI of EMM | Odds ratio | 95% CI for Exp | P   | EMM | 95% CI of EMM | Odds ratio | 95% CI for Exp | P   |
| Azolla                           | 0.450 | 0.064-0.964 | 0.290 | 0.144 - 0.584 | 0.001* | 0.236 | 0.030-0.502 | 0.264 | 0.082 - 0.547 | 0.025* |
| Arrow roots inside water         | 1.343 | 0.829-1.857 | 0.866 | 0.491 - 1.526 | 0.620 | 0.757 | 0.491-1.023 | 0.848 | 0.535 - 1.343 | 0.482 |
| Arrow roots on the water banks   | 1.557 | 1.043-2.071 | 1.005 | 0.619 - 1.631 | 0.985 | 0.657 | 0.391-0.923 | 0.736 | 0.446 - 1.216 | 0.231 |
| Couch grass                      | 1.486 | 0.972-2.000 | 0.959 | 0.595 - 1.545 | 0.862 | 0.829 | 0.563-1.095 | 0.928 | 0.599 - 1.437 | 0.738 |
| Sweet potato outside             | 1.757 | 1.243-2.271 | 1.134 | 0.711 - 1.808 | 0.599 | 0.786 | 0.520-1.052 | 0.880 | 0.561 - 1.381 | 0.576 |
| Control                          | 1.550 | 1.036-2.064 | 1    | 0.893 | 0.627-1.159 | 1    |

* shows larval densities that are significantly different from the control. EMM = estimated marginal mean CI = confidence interval; P = significance level at 95%.

---

Figure 1 Abundance of late instar larvae of anopheline species in habitats with: a) arrow roots growing in water, (b) arrow roots growing along water banks, (c) sweet potatoes along water banks, (d) couch grass in the water, (e) Azolla on water surface, and (f) control without plant cover, in Nyalenda.

---

Imbahale et al. Parasites & Vectors 2011, 4:130
http://www.parasitesandvectors.com/content/4/1/130
instars, habitats with *Bti* and fish, half were marginally significant in comparison to the control whereas habitats provided with full quantities of *Bti* and fish were significantly different (OR = 0.344, P < 0.001) (Table 4).

In man-made canals, all treatment types were significantly different from the control (all P < 0.05). There was an overall reduction of 73.03% in the population of all larval stages of anopheline mosquitoes. Late instar larvae of anopheline mosquitoes were reduced by 87% (n = 173), 59% (n = 117) and 92% (n = 183) due to application of *Bti* only, fish only and *Bti* and fish, respectively. When compared with the control, late instars were reduced by 89% (OR 0.106, P < 0.001) and 86% (OR 0.137, P < 0.001) in habitats with *Bti* and fish, and those with *Bti* only, respectively. Generally, more larvae were recorded in both ponds and canals provided with fish as the only control option (Table 4).

*Anopheles gambiae s.l.* was recorded in all habitats except those provided with *Bti* and fish in full quantities, whereas more *An. coustani* were recorded from habitats containing *Bti* alone (Figure 3A). The population of *An. gambiae* reduced by 83.33% due to *Bti* only, 50% by mosquito fish, while both mosquito fish and *Bti* caused a reduction of 100%. *Anopheles funestus* was only recorded in control habitats (Figure 3B).

**Discussion**

Simple strategies such as locally cultivated cover crops and plants to provide shade over mosquito breeding habitats as well as the use of predatory fish in combination with *Bti* are feasible options for the control of immature mosquitoes, including malaria vector species. All habitats provided with shade from *Azolla*, sweet potatoes, arrow root, Napier grass, rice and papyrus

---

**Table 3 Abundance of early and late instar larvae of anopheline mosquitoes in man-made habitats with different plant covers**

| Parameter      | Early instars | Late instars |
|----------------|---------------|--------------|
|                | EMM 95% CI for EMM | Odds ratio 95% CI for Exp (B) | Odds ratio 95% CI for Exp (B) | P   |
| Arrow roots    | 1.100 0.683-1.517 0.577 0.332-1.003 0.051* 0.006 0.036-0.048 0.045 0.005-0.380 0.004* |
| Unweeded rice  | 0.929 0.339-1.520 0.488 0.247-0.962 0.038* 0.012 0.047-0.071 0.091 0.011-0.755 0.026* |
| Weeded rice    | 1.353 0.763-1.943 0.710 0.387-1.303 0.269 0.094 0.035-0.153 0.727 0.227-2.334 0.593 |
| Papyrus        | 1.976 1.386-2.567 1.037 0.621-1.731 0.889 0.035 0.024-0.094 0.273 0.052-1.442 0.126 |
| Napier         | 0.788 0.371-1.206 0.414 0.238-0.718 0.002* 0.065 0.070-0.189 0.500 0.177-1.410 0.190 |
| Control        | 1.906 1.316-2.496 1.000 0.252-2.016 0.129 0.023-0.107 1.000 0.022-1.000 1.000 |

* shows larval densities that are significantly different from the control. EMM = estimated marginal mean CI = confidence interval; P = significance level at 95%.
reeds supported significantly fewer anopheline larvae than the controls. Therefore, this mosquito species thrives best in open, sunlit pools of water, however such habitats become unsuitable for ovipositing females when shade increases [6]. This is probably caused by the action of shade, which lowers the water temperature and reduces algal growth. Gravid female mosquitoes select to oviposit in sun-exposed sites [37].

The numbers of An. gambiae s.l. mosquito larvae recorded 24 h and 48 h after exposure to treatment was significantly influenced by treatment type. However, man-made habitats provided with both Bti and fish resulted in greater reductions of anopheline larval population densities when compared to habitats where only G. affinis was introduced. These results are comparable to the outcome of experiments conducted by Blaustein [38] where G. affinis alone failed to control mosquitoes in experimental rice habitats. These results indicate that the predatory effectiveness of mosquito fish on anopheline mosquito larvae diminished when introduced into the man-made larval habitats. The contrast in the findings could be attributed to other factors that we did not investigate/foresee, such as fish preying on other aquatic organisms, external food or invertebrate sources and physical factors such as turbidity. Homski et al. [39] found that higher turbidity in man-made habitats may have favored a higher abundance of invertebrates and reduced visibility of anopheline larvae for mosquito fish than in sites covered with emergent vegetation. In addition, under natural circumstances other fish species may be better predators on anopheline larvae [40].

| Variable | Parameter | EMM | 95% CI for EMM | Odds Ratio | 95% CI for Exp | P | EMM | 95% CI for EMM | Odds Ratio | 95% CI for Exp | P |
|----------|-----------|-----|----------------|------------|----------------|---|-----|----------------|------------|----------------|---|
| A) Ponds | Bti only  | 2.376 | 1.926-2.826 | 0.891 | 0.679 - 1.169 | 0.404 | 0.570 | 0.364-0.776 | 0.752 | 0.475-1.192 | 0.225 |
|          | Fish only | 3.067 | 2.604-3.505 | 1.145 | 0.899 - 1.460 | 0.272 | 0.903 | 0.697-1.109 | 1.192 | 0.793-1.791 | 0.398 |
|          | Bti-Fish  | 1.733 | 1.283-2.183 | 0.650 | 0.486 - 0.869 | 0.004* | 0.485 | 0.279-0.691 | 0.640 | 0.405-1.012 | 0.056* |
|          | Fish Once | 2.667 | 2.217-3.117 | 1.000 | 0.786 - 1.272 | 1.000 | 0.964 | 0.757-1.170 | 1.272 | 0.844 - 1.916 | 0.250 |
|          | Bti-Fish  | 0.867 | 0.417-1.317 | 0.325 | 0.211 - 0.499 | 0.000* | 0.261 | 0.054-0.467 | 0.344 | 0.190 - 0.623 | 0.000* |
|          | Control   | 2.667 | 2.217-3.117 | 1    | 0.758 | 0.551-0.964 | 1    | 0.068-0.278 | 0.004* |
| B) Water canals | Bti only  | 0.635 | 0.115-1.156 | 0.186 | 0.120-0.289 | 0.000* | 0.161 | 0.042-0.365 | 0.137 | 0.068-0.278 | 0.004* |
|          | Fish only | 1.906 | 1.386-2.427 | 0.558 | 0.374-0.832 | 0.004* | 0.547 | 0.344-0.750 | 0.465 | 0.288-0.750 | 0.002* |
|          | Bti-Fish  | 0.740 | 0.219-1.260 | 0.216 | 0.143-0.327 | 0.000* | 0.125 | 0.078-0.328 | 0.106 | 0.056-0.200 | 0.000* |
|          | Control   | 3.417 | 2.896-3.937 | 1    | 1.177 | 0.974-1.380 | 1    | 1.177 | 0.974-1.380 | 1    |

* shows habitats that are significantly different from the control. EMM = estimated marginal mean; CI = confidence interval; N = number of times sampled; P = significance level at 95%.
carried out in Eritrea [19]. With a two-weeks interval, our results show a low impact of Bti only on larval abundance. However, studies by Fillinger and Lindsay [16] and Majambere et al. [18], report microbial larvicides such as Bti to have greater efficacy (95%) when applied to anopheline larval habitats in optimum quantities on a weekly basis. If weekly application of Bti would have been followed, then habitats provided with Bti only may have been as effective as those provided with Bti and fish on larval abundance. In addition, the persistence of Bti endotoxins in our study may have reduced rapidly under field conditions, hence showing no apparent effect on anopheline larval abundance. As previous studies clearly showed that Bti is non-toxic to non-target organisms [16,30], we used this property of Bti to serve as a basis for integrating this product with G. affinis for increased efficacy of larval control.

The trials in this study were done under field conditions in man-made habitats that were naturally colonized by mosquito larvae. Under these conditions, external factors were not controlled and could have played an important role in the colonization and growth of mosquito larvae in the respective habitats. The variations in water level and occasional flooding of habitats could not be avoided, as the sites were exposed to ambient conditions. Factors such as water turbidity, nutrient content in water, cannibalism, predation of immature stages, parasitism, pathogens, competition, water temperature and plant odours that could have either repelled or attracted female mosquitoes during oviposition [41-47] were not controlled and hence could have played a role in the results obtained. All larval stages of culicine mosquitoes increased as vegetation cover increased progressively from man-made ponds, small

Figure 3 Anopheles gambiae, An. funestus and An. coustani larval distribution in the (A) ponds and (B) canal man-made habitats under different treatments expressed as a percentage of the total number of larvae recorded.
water canals, rice paddies to swamps. Habitats with few anopheline larvae recorded more culicine larvae, while those that recorded more anophelines had fewer culicine larvae. This suggests selective oviposition behaviour among these mosquito families [37,48,49]. In Nyalenda, the water present in breeding habitats was often polluted with debris and human waste, which might have favored proliferation of culicine mosquitoes (data not shown) and at the same time water quality may have had a negative impact on the efficacy of the treatments provided. Competition and differences in the physical-chemical characteristics of the water may have played a role in structuring larval populations, although these factors were not investigated in this study. The standard dipping method was used to estimate mosquito larval densities, which may have underestimated larval abundance [28,50,51] and consequently, may have influenced the amounts of Bti and numbers of G. affinis used, leading to contrasting results.

In western Kenya, areas that were previously natural swamps and forests have been transformed into agricultural fields mainly due to human population pressure [2]. These agricultural developments have an impact on the ecological characteristics of the local mosquito vector in terms of density, local microclimate and malaria incidence [2,33,52,53]. Communities in western Kenya are willing to take part in malaria control [27] and to effect this, simple control strategies suitable for the local of mosquito vectors need to be available as a way of adapting to the changes in land use. Results from this study indicate that locally available leafy plants could be used for mosquito control especially in areas under traditional agriculture. Use of edible fish [40] and mosquito fish are other options that can easily be put into practice, especially in areas where water is always present.

The effectiveness of biological larvicides for the control of African anophelines has already been demonstrated by several studies [16,17,54] and in areas where locally available solutions are not feasible and where water cannot be drained, then application of microbial larvicides could be the best option. Although our results are spatially and temporally limited, the option of using shade from locally available crops and predatory fish seems an easily applicable alternative for the control of mosquito larvae. More importantly, as the level of morbidity resulting from the specific problem of malaria is a net result of a balance between livelihood and ecosystem factors [55] an ecohealth approach to malaria control is bound to produce discernable and long lasting effects.

This study was part of an ongoing project in different agro-ecological settings in two highland villages (Lunyerere and Fort Ternan) and one peri-urban area (Nyalenda), where most larval habitats were man-made [5]. The field studies reported in this paper were done in the peri-urban area of Kisumu town to assess the best options of controlling immature mosquitoes. For Nyalenda, larviciding and use of predatory fish seem promising and can be supplemented with the existing adulticiding options.

Acknowledgements

We are grateful to Tedd Omordi, Paul Mabuka, Amos Wawire and Annet Obukos for their tireless support during field sampling and mosquito identification in the laboratory. We are grateful to Mr. David Madahana for his role in driving us to and from the field. Dr. Andrew Githeko is acknowledged for institutional support provided during the field study. Drs. Ron van Lammeren and Krijn Paaajiemans are thanked for helpful comments and suggestions on the draft version of this paper. We wish to acknowledge the Inland waters manager and the staff at KEMRI Kismu for the tremendous support provided in the establishment of fish colony. Financial support was provided by the Dioraphte Foundation, The Netherlands.

Author details

1Laboratory of Entomology, Wageningen University, P.O. Box 8031, 6700 EH Wageningen, The Netherlands. 2International Centre of Insect Physiology and Ecology, P.O. Box 30772 - 00100 GPO, Nairobi, Kenya. 3School of Biological Sciences, University of Nairobi, P.O. Box 30197-00100 GPO, Nairobi, Kenya.

Conflict of interest statement

We declare that we have no conflict of interest. This work was part of the project funded by the Dioraphte Foundation, The Netherlands. The funding organization had no role in the analysis or interpretation of the results or in the drafting of the manuscript.

Received: 22 April 2011 Accepted: 6 July 2011 Published: 6 July 2011

References

1. Lindbladte KA, Walker ED, Onapa AW, Katungu J, Wilson ML: Land use change alters malaria transmission parameters by modifying temperature in a highland area of Uganda. Trop Med Int Health 2000, 5:263-274.

2. Munga S, Minakawa N, Zhou GF, Mushinzimana E, Barrack ODI, Githeko AK, Yan GY: Association between land cover and habitat productivity of malaria vectors in western Kenyan highlands. Am J trop Med Hyg 2006, 74:69-75.

3. Norris DE: Mosquito-Borne Diseases as a consequence of land use change, EcoHealth 2004, 1:19-24.

4. Walsh JF, Molyneux DH, Birley MH: Deforestation: effects on vector-borne disease, Parasit 1993, 106:55-75.

5. Imbahale SS, Paijmans KP, Mukabana WR, Lammeren R, Githeko AK, Takken W: A longitudinal study on Anopheles mosquito larval abundance in distinct geographical and environmental settings in western Kenya. Malar J 2011, 10:81.

6. Klinkenberg E, Takken W, Huibers F, Toure YT: The phenology of malaria mosquitoes in irrigated rice fields in Mali. Act Trop 2003, 85:71-82.

7. Mathys B, N’Goran EK, Kone M, Koudou BG, Vounatsou P, Cisse G, Tischennab AB, Tanner M, Utzinger J: Urban agricultural land use and characterization of mosquito larval habitats in a medium-sized town of Cote d’Ivoire. J Vec Ecol 2006, 31:319-333.

8. Mwangi JM, Muturi EJ, Shilu JI, Jacob B, Kaburu EW, Mboogo CM, Githure JJ, Novak RJ: Distribution of mosquito larvae within the paddy and its implication in larvicidal application in Mwea Rice Irrigation Scheme, Central Kenya. J Am Mosq Control Assoc 2008, 24:36-41.

9. Imbahale SS: Integrated Malaria vector control in different agro-ecosystems in western Kenya, PhD Thesis Wageningen University, The Netherlands, 2009.
10. Flore TG: Mosquito larval control practices: past and present. J Am Mosq Control Assoc 2006, 22:527-533.
11. Killeen GF, Fillinger U, Knols BG: Advantages of larval control for African malaria vectors: low mobility and behavioural responsivity of immature mosquito stages allow high effective coverage. Malar J 2002, 1:8.
12. Liu WH, Xin K, Chao CZ, Feng SZ, Yan L, He RZ, Zhang ZH, Gibson G, Kang WM: New irrigation methods sustain malaria control in Sichuan Province, China. Act Trop 2004, 89:241-247.
13. Kesper J, Singer BH, Utzinger J: Reducing the burden of malaria in different eco-epidemiological settings with environmental management: a systematic review. Lancet Infect Dis 2005, 5:685-708.
14. Kiron U, Spielman A: Suppression of transmission of malaria through source reduction: antinomopheline measures applied in Israel, the United States, and Italy. Rev Infect Dis 1989, 11:391-406.
15. Utzinger J, Tozan Y, Doumani F, Singer BH: The economic payoffs of integrated malaria control in the Zambian coppertbelt between 1930 and 1950. Trop Med Int Health 2002, 7:657-677.
16. Fillinger U, Lindsay SW: Suppression of exposure to malaria vectors by an order of magnitude using microbial larvicides in rural Kenya. Trop Med Int Health 2006, 11:1629-1642.
17. Geissbuehler Y, Kannady K, Chaki PP, Emidi B, Govella NJ, Mayagaya V, Kiama M, Matiwa D, Mhinda H, Lindsay SW, Tanner M, Fillinger U, Castro MC, Killeen GF: Microbial Larvicide Application by a Large-Scale, Community-Based Program Reduces Malaria Infection Prevalence in Urban Dar Es Salaam, Tanzania. PLoS ONE 2009, 4(3):e5107.
18. Majambere S, Fillinger U, Sayer DR, Green C, Lindsay SW: Spatial distribution of mosquito larvae and the potential for targeted larval control in The Gambia. Am J Trop Med Hyg 2008, 79:19-27.
19. Shiklo A, Trevorde GM, Brantly E, Githure JI, Mbogo CM, Beier JC, Fusco R, Novak RJ: Efficacy of Bacillus thuringiensis var israelensis, Bacillus sphaericus and temephos for managing Anopheles larvae in Entirea. J Am Mosq Control Assoc 2003, 19:251-258.
20. Vanek MJ, Shoo B, Matiwa D, Kiama M, Lindsay SW, Fillinger U, Kannady K, Tanner M, Killeen GF: Community-based surveillance of malaria vector larval habitats: a baseline study in urban Dar Es Salaam, Tanzania. BMC Public Health 2006, 6:154.
21. Rose P: Pesticides and Public Health: Integrated Methods of Mosquito Management. Emerg Infect Dis 2001, 20:1.
22. Beales PF, Gilleps HM: Rationale and technique of malaria control. Essential Malanology London: Arnold, a member of the Hodder Headline Group, 2002.
23. Shift C: Integrated approach to malaria control. Clin Microbiol Rev 2002, 15:278-293.
24. Becker N: Ice granules containing endotoxins of microbial agents for the control of mosquito larvae—a new application technique. J Am Mosq Control Assoc 2003, 19:63-66.
25. Mathis RJ, Adnas AQ: The use of annual killifish in the biocontrol of the aquatic stages of mosquitoes in temporary bodies of fresh water: an apotential new tool in vector control. Parasit Vectors 2010, 3:66.
26. Reichard M, Watters BR, Wildekamp HR, Sonnenberg R, Nagy B, Pola, Goma LKH: Integrated Malaria Control (East Africa), IDRC report number 100482.
27. Homski D, Goren M, Gasith A: Comparative evaluation of the larvivorous fish Gambusia affinis and Aphanius dispar as mosquito control agents. Hydrobiologia 1994, 284:137-146.
28. Service MW: Larvicides for malaria control in The Gambia. Urban Dar Es Salaam, Tanzania.
29. Kaande M, Mtasiwa D, Mshinda H, Lindsay SW, Tanner M, Fillinger U, Castro MC, Killeen GF: Microbial Larvicide Application by a Large-Scale, Community-Based Program Reduces Malaria Infection Prevalence in Urban Dar Es Salaam, Tanzania. PLoS ONE 2009, 4(3):e5107.
30. Fillinger U, Knols BG, Becker N: Efficacy and efficiency of new Bacillus thuringiensis var israelensis and Bacillus sphaericus formulations against Afrotropical anophelines in Western Kenya. Trop Med Int Health 2003, 8:37-47.
31. Petracca V, Sabatelli G, Toure YT, di Deco MA: Morphometric multivariate analysis of field samples of adult Anopheles arabiensis and An. gambiae s.s. (Diptera: Culicidae). J Med Entomol 1998, 35:16-25.
32. Foley DH, Torres EP, Mueller I: Stream-bank shade and larval distribution of the Philippine malaria vector Anopheles flavirostris. Med Vet Entomol 2002, 16:347-355.
33. Munga S, Minakawa N, Zhou GF, Barron OOI, Githeko AK, Yan GY: Oviposition site preference and egg hatchability of Anopheles gambiae: Effects of land cover types. J Med Entomol 2005, 42:993-997.
34. Mwangangi JM, Mutunji EJ, Shillu L, Muriu S, Jacob B, Kaburu EW, Mbogo CM, Githure JI, Noval RJ: Environmental covariables of Anophelines arabiensis in a rice agro ecosystem in Mwea, Central Kenya. J Am Mosq Control Assoc 2007, 23:371-377.
35. Wannae PM, Githeko AK, Menya MD, Takken W: Shading by Napier grass reduces Malaria vector larvae in Natural habitats in Western Kenya highlands. EcolHealth 2010, Online July 2010.
36. Goma LKH: Experimental Breeding of Anopheles gambiae Giles in Papyrus Swamps. Nature 1960, 187:1137-1138.
37. Tuno N, Okeka W, Minakawa N, Takagi M, Yan G: Surviviorship of Anopheles gambiae sensu stricto (Diptera: Culicidae) larvae in western Kenya highland forest. J Med Entomol 2005, 42:270-277.
38. Blaustein L: Larvivorous fishes fail to control mosquitoes in experimental plots. Hydrobiologia 1992, 232:219-232.
39. Homski D, Goren M, Gasith A: Comparative evaluation of the larvivorous fish Gambusia affinis and Aphanius dispar as mosquito control agents. Hydrobiologia 1994, 284:137-146.
40. Howard AF, Zhou G, Omlin FX: Malaria mosquito control using edible fish in western Kenya: preliminary findings of a controlled study. BMC Public Health 2007, 7:199.
41. Koenraadt CJM, Takken W: Cannibalism and predation among larva of the Anophelinae gambiae complex. Med Vet Entomol 2003, 17:61-66.
42. Liu WH, Xin K, Chao CZ, Feng SZ, Yan L, He RZ, Zhang ZH, Gibson G, Kang WM: New irrigation methods sustain malaria control in Sichuan Province, China. Act Trop 2004, 89:241-247.