

Is Mosquito Larval Source Management Appropriate for Reducing Malaria in Areas of Extensive Flooding in The Gambia? A Cross-over Intervention Trial

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Abstract. Larviciding to control malaria was assessed in rural areas with extensive seasonal flooding. Larval and adult mosquitoes and malaria incidence were surveyed routinely in four 100-km² areas either side of the Gambia River. Baseline data were collected in 2005. Microbial larvicide was applied to all water bodies by hand application with water-dispersible granular formulations and corn granules weekly from May to November in two areas in 2006 and in the other two areas in 2007 in a cross-over design. The intervention was associated with a reduction in habitats with late stage anopheline larvae and an 88% reduction in larval densities ($P < 0.001$). The effect of the intervention on mosquito densities was not pronounced and was confounded by the distance of villages to the major breeding sites and year ($P = 0.002$). There was no reduction in clinical malaria or anemia. Ground applications of non-residual larvicides with simple equipment are not effective in riverine areas with extensive flooding, where many habitats are poorly demarcated, highly mobile, and inaccessible on foot.

INTRODUCTION

The realization that successful malaria control cannot rely on a single tool and the need for evidence-based vector control methods has prompted the World Health Organization to promote a global framework for integrated vector management.^{1,2} This framework is designed to rationalize vector control by using evidence collected in the field to make decisions about what combination of control measures to use to achieve maximum control of one or more vector-borne diseases. Control measures such as the use of long-lasting insecticide-treated nets (ITNs) and indoor residual spraying are effective tools for malaria control and are currently the mainstay of vector control in sub-Saharan Africa. However, the increasing resistance of malaria vectors to pyrethroids in Africa,^{3,4} the capacity of adult mosquitoes to avoid interventions,⁵ and the recent call for malaria eradication⁶ has lead to a renewed interest in the use of larval source management (LSM)⁷ for inclusion in integrated vector management programs for malaria control in Africa. Over the past five years, a series of small-scale studies have been undertaken to investigate the efficacy of LSM in different biomes in sub-Saharan Africa. It was shown that larval control works well where breeding sites are aggregated in urban^{8,9} and rural areas.^{10–14}

In contrast, we describe the results of an LSM trial with microbial larvicides in a markedly different habitat in The Gambia, an area of extensive wetland. The floodplain of lower reaches of the Gambia River floods each rainy season to produce extensive areas of pooled sediment, which are ideal breeding sites for mosquito larvae.^{15–17} Habitats most often colonized are found in the first 1 km of the landward edges of the floodplains in shallow water bodies. Because these sites appeared to be readily accessible, we hypothesized that larviciding could decrease larval numbers sufficiently to reduce malaria transmission. Moreover, because malaria transmission

is confined largely to the rainy season,¹⁸ which lasts from June to October, we assumed this intervention would be effective when targeted solely during this period. Before the large-scale trial the commercial larvicide VectoBac® (*Bacillus thuringiensis* var. *israelensis* [Bti]; Valent BioSciences Corporation, Libertyville, IL) was found to be highly effective at killing mosquito larvae in rural Gambia.¹⁹ The application of larvicide was designed to be locally appropriate, and the microbial larvicides were delivered by hand by field teams using simple equipment.

This pilot trial was part of a series of studies to assess the feasibility of LSM and its impact on malaria morbidity in different eco-epidemiological settings (rural towns,²⁰ highland valleys,¹³ desert fringe,¹⁴ urban areas^{16,21}). The aim of this study was to evaluate the impact of LSM using microbial larvicides on vector populations and malaria incidence in the extensive seasonally flooded areas of the lower reaches of the river in rural Gambia.

METHODS

Study design. The study was carried out in four separate areas (referred to as zones 1 to 4) two on the north banks and two on the south banks of the Gambia River east of Farafenni (universal transverse mercator zone 28 1500200mN, 435500mE; Figure 1). The area was flat open Sudan savannah broadly consisting of farmlands, sparse woodland and the extensive alluvial floodplains of the river. Villages were discrete entities populated by mainly subsistence farmers predominantly of the Wolof, Mandinka, and Fula ethnic groups. The four study zones were approximately 12 × 8 km in area and divided into three parallel 4 km-wide bands (subzones) perpendicular to the river. Study villages were recruited from the central band of each zone. We assumed that when larvicide was applied to an entire study zone, the two 4-km bands, either side of the central band, would be sufficiently wide to minimize mosquito movement from untreated sites outside the study zone into the central band, where the study villages were located.^{22,23}

Baseline entomological data, but not clinical data, were collected during July–November 2005. In 2006 and 2007, entomological and clinical data collection started in May and ended in

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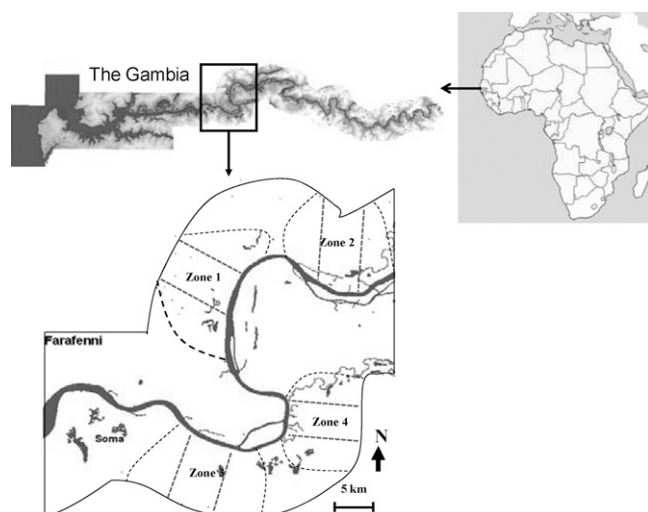


FIGURE 1. Study area in The Gambia. Larval control was applied in each zone over the area enclosed by the two broken lines furthest from the center of each zone and the study villages are enclosed by the two broken lines nearest the center of each zone.

November. A cross-over design was used for the application of larvicide. From June to November 2006, larvicide was applied to all accessible aquatic habitats in zones 1 and 3 at weekly intervals and zones 2 and 4 served as controls. From May to November 2007, larvicide intervention was applied to zones 2 and 4 and zones 1 and 3 served as controls.

Eligibility criteria for study participants. A census of residents, including children 6 months to 10 years of age, was carried out in 50 study villages during the dry season in 2006. In addition to demographic data, information was also collected on malaria risk factors from all consenting inhabitants, including use of bed nets and ITNs, the presence of open or closed eaves in sleeping rooms, and ethnicity. A total of 14,112 inhabitants were enumerated and children were selected from random lists with the total in each village proportional to village size. Informed consent was obtained from parents or caregivers, and children were enrolled to enable a study of approximately 500 children in each zone (Figure 2). In 2007, participants who had reached 10 years of age were replaced

by infants 6–18 months of age and those who left the study were replaced by additional children from the same village. All children were provided with a study photo identity card to verify their study number and village at clinical consultations and during cross-sectional surveys.

Intervention. Detailed descriptions of the application of microbial larvicides are provided elsewhere.^{16,20,24} Briefly, water-dispersible granular formulations (WDG) and corn granules (CG) of the commercial strain of *Bti* (VectoBac® strain AM65-52; Valent BioSciences Corporation) were applied weekly from the end of the dry season in May until the end of the rainy season in November to all water bodies within an intervention zone that could be reached by applicators on foot. The WDG formulations were applied as liquid with knapsack compression sprayers (15-liter capacity diaphragm knapsack sprayers, Solo 475; Solo Kleinmotoren GmbH, Sindelfingen, Germany) at 0.2 kg/hectare in areas with low vegetation coverage. The CG was applied by hand from buckets held with a strap around the waist or neck or motorized knapsack granule-blowers (13-liter capacity motorized sprayers; MD 150DX-13; Maruyama, Tokyo, Japan) at 5.0 kg/hectare when aquatic habitats were covered by vegetation and difficult to access.

Field applicators were recruited from communities within each zone to make use of their local knowledge of the environment. They were supervised by one field supervisor in each zone and trained for one month before larviciding. Applicators worked five days a week from 7:00 AM to 1:00 PM to avoid the hottest time of the day. Teams of 3–4 applicators walked abreast 8 meters apart. Each applicator covered a 180° swath in front of him as he walked and applied larvicide from the edge of a water body to the end of it or until progress was impossible because of deep water.

Objective and outcome measures. To assess the impact of LSM we used clinical and entomological outcome measures. The primary clinical outcome was the incidence of clinical malaria in study children defined as a history of fever within the last 48 hours or axillary temperature $\geq 37.5^{\circ}\text{C}$ plus the presence of *Plasmodium falciparum* identified microscopically. The primary entomological outcomes were adult densities in houses, a proxy measure for indoor human biting rates, and mosquito larval abundance, which served to evaluate the effectiveness of the larvicide.

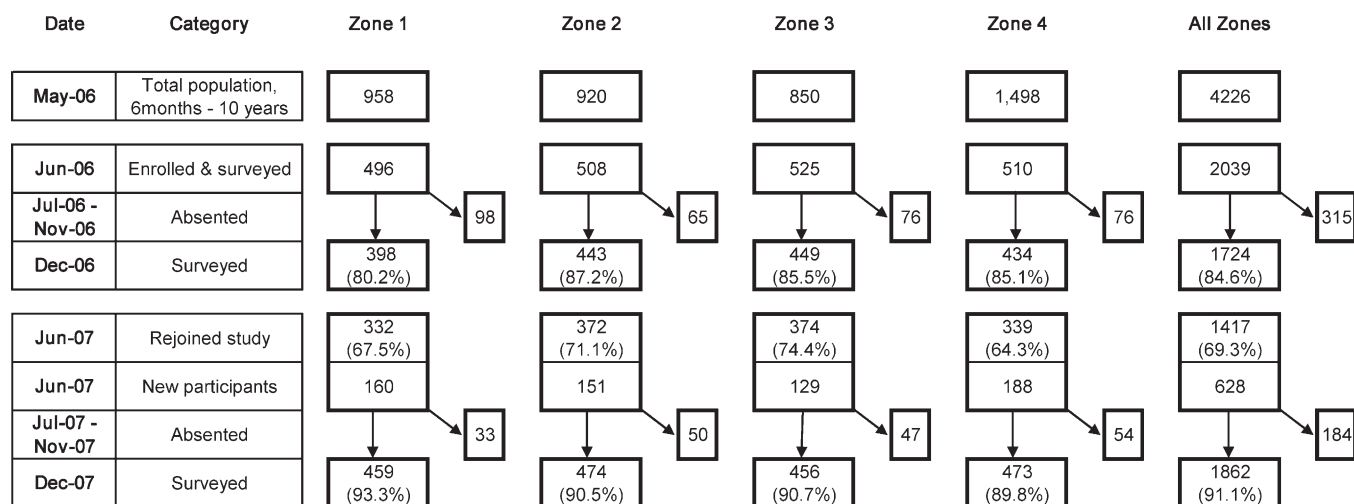


FIGURE 2. Trial profile, The Gambia.

Larval vector abundance. Larval surveys were carried out continuously by the zone supervisor. In 2005, during the baseline period, all aquatic habitats in each zone were visited and the presence or absence of anopheline and culicine larvae recorded as described elsewhere.²⁴ Each habitat was visited monthly.

During the intervention years (2006 and 2007) random larval spot checks were implemented throughout the season to estimate the proportion of habitats containing early and late instar larvae to determine the effectiveness of larvicide application. Of the total number of habitats identified in each zone during baseline ($n = 1,076$), 40 habitats were randomly (computer-generated) selected every day for each zone respectively by the program manager (S.M.) and the habitat identification number, including global positioning system coordinates, forwarded to the field supervisor for habitat inspection as described above. Selection of sites was stratified according to subzone and the timetable of larvicide application to ensure that inspection of sites took place 1–2 days after the habitat was treated with larvicide and that an equal number of sites were visited weekly in all three subzones in each zone. In addition, 10 sentinel habitats per zone were randomly selected after the first round of complete habitat surveys in 2005 and larval densities measured weekly in these.

At each site visit, purposive dipping was used to sample larvae (10 dips per site), which were categorized as early (first and second instars) stages and late (third and fourth) stages. Late instar anopheline larvae and all pupae were stored in 98% ethanol and taken to the laboratory for species identification by polymerase chain reaction (PCR).²⁵

Adult vector abundance. Adult vector surveys were implemented in 39 villages (10 in zone 1, 11 in zone 2, 9 in zone 3, and 9 in zone 4) at two-week intervals from July through November in 2005 and for the duration of larviciding in the intervention years. Each zone had 15 traps divided between the villages with 1–3 sentinel houses per village proportional to village size. Within randomly selected compounds, all houses with open eaves, a thatched roof, no ceiling, and where a single man slept were numbered and one was selected randomly. Mosquitoes were sampled using miniature CDC light traps (Model 512; John W. Hock Company, Gainesville, FL) positioned one meter above the floor at the foot end of the bed where a man slept under an untreated bed net. Traps were set at 7:00 PM and collected at 7:00 AM the following morning. If the occupant moved house, the trap was moved to the nearest similar house in the same village. If the occupant did not spend the night in the selected room or the trap was faulty, the data were excluded from the analysis.

Mosquitoes were identified to the level of species by microscopy and the numbers of *Anopheles gambiae* s.l. females recorded. The presence of sporozoites was identified using an enzyme-linked immunosorbent assay.²⁶ In 2005 and 2006, a 1% random sample of the *An. gambiae* s.l. females, stratified by zone and sampling period was typed to the species by PCR.²⁷

Malaria in children. Cross-sectional surveys were implemented before (May–June) and after (November–December) the main transmission season in 2006 and 2007. At each survey, children were questioned and examined for malaria signs and symptoms, including axillary temperature, recent clinical history, drug ingestion, anemia, and splenomegaly. In children with an axillary temperature $\geq 37.5^{\circ}\text{C}$ or recent history of fever, a rapid diagnostic test (RDT; ICT Malaria

Pf Cassette Test; ICT Diagnostics, Cape Town, South Africa) was conducted in the field and treatment was given if it was positive. Anemia was measured in all children at each survey (Hemocue AB, Ängelholm, Sweden) and thick blood smears collected for subsequent determination of parasitemia. At surveys and consultations, clinical conditions were treated according to standard Gambian treatment guidelines. A short history of each child's health and mosquito prevention and control measures at their home were recorded at each survey. Travel history was collected at the end of season survey.

From June to December each year, passive case detection was used to monitor clinical cases of malaria. Parents or caregivers were encouraged to consult study nurses if a study child became ill. One study nurse was stationed in each of two centrally located study villages in each zone (total = 8), and they collaborated closely with government village health workers (VHWs) to cover their zones. At any consultation, children were identified by their study cards, signs and symptoms were recorded, a blood sample was tested for parasites by the RDT, and a thick blood smear made if fever was present. If parasites were detected by RDT, children were referred to the VHW for treatment immediately. The thick blood film was stained and read immediately. If the result was positive, but the result of the RDT was negative, children were treated the next day. All conditions were treated according to current standard Gambian treatment guidelines. Moderate anemia ($\text{Hb} < 8 \text{ g/dL}$) was treated with iron sulfate, and severe cases ($\text{Hb} < 5 \text{ g/dL}$) were transported to a health facility. Uncomplicated malaria was treated with chloroquine and pyrimethamine/sulfadoxine. Formal and on-the-job training was provided to all VHWs by the study doctor and nurses according to the Gambian VHW training guidelines.

Blinding. Reading of blood films and ELISA results was blinded. Entomological data collection was not blinded to the assignment of mosquito larval control interventions in the study areas. However, field applicators were blinded to the sites selected for larval surveys. Residents were aware of ongoing interventions. Light-trap collections of adult mosquitoes were identified and counted by technicians blinded to the identity of the village.

Protection of human subjects. Institutional and ethical clearance was granted by the National Institutes for Health, the Gambian Government/Medical Research Council Laboratories Joint Ethics Committee, and the Ethics Advisory Committee of Durham University. Verbal consent for the study was obtained from local leaders and the community at large before collecting entomological baseline data. Before implementation of larval control operations, the community was again briefed on the nature of the intervention. Informed consent was obtained from house occupiers for setting and collecting the adult mosquito traps. Interviews and malaria parasite screening were only started after the purpose of the study had been clearly explained to the participants and parents or guardians of children and an informed consent form was read in an appropriate language and signed by the parents/guardian and a witness. Assent was also sought and obtained from older children. Approval to use microbial larvicides was granted by the National Environment Agency of The Gambia.

Statistical analysis. All data were collected on forms, checked for completeness, double entered into Access databases, verified and checked for consistency. The incidence of clinical

malaria was calculated from the number of study children who consulted with malaria/100 child-years of exposure. The time of exposure for each child was the duration of passive surveillance corrected for absences of over one week and by subtracting 28 days if a child received anti-malaria drugs. Time of exposure was censored at the first attack in children with clinical malaria. The potential effect of mosquito larval control was examined by calculating the incidence of clinical malaria, prevalence of parasitemia and splenomegaly, and mean $\pm$ SD Hb levels (g/dL) for each survey, zone, and year. Hemoglobin levels in intervention and control groups each year were compared at the end of each season by using a *t*-test. The incidence of malaria allowing for time of exposure was analyzed as a cross-over study, with a multilevel generalized linear model (GLLAMM, Stata version 9.1; Stata Corporation, College Station, TX). The clustering effects of village and subject were included as random effects (the intra cluster correlation for zone was minimal once subject and village were included in the model).

The impact of larviciding on the presence of late stage larvae in water bodies was analyzed as a cross-over study by using GLLAMM with clustering by water body and zone included as random effects. Odd ratios were adjusted for the year of intervention. The density of female anopheline in traps was highly over-dispersed, and we used a generalized estimating equation with a negative binomial distribution to examine the effect of larviciding on this. Comparisons were adjusted for month, baseline densities in 2005, distance of villages to the edge of alluvial floodplains, and clustering by trap and village. Binary logistic regression was used to examine the presence and absence of *An. gambiae* with sporozoites by zone over the two intervention years. Seasonal entomological inoculation rates were calculated by multiplying the mean density of mosquitoes collected in light traps from July to November in each zone by the proportion positive for *P. falciparum* sporozoites and by 153, the number of days in the season.

RESULTS

Participants, demographics, and follow-up. Approximately 500 children were surveyed in each zone at the start of the transmission season in 2006 and 2007 (Figure 2). Most children were surveyed again at the end of each season; 84.9% in 2006 and 90.8% in 2007. Of those not surveyed most had traveled for a religious holiday for a few weeks. Most participants were enrolled in the study both years (Figure 2).

The age and sex of children were similar in each zone and between intervention arms, although there were slightly more boys in zone 4 (Table 1). There were 16% more clinical consultations in 2007 than in 2006 (787 compared with 678), which indicated that the lower incidence of malaria in 2007 (Table 2) was not caused by fewer consultations that year. At consultation, 99% (1,458 of 1,466) of the patients had a recorded temperature of $\geq 37.5^\circ\text{C}$ or reported history of fever, 34% (488 of 1,458) of these patients were slide positive, and 72% (352 of 488) had a fever $\geq 37.5^\circ\text{C}$. Slides results were available for > 98% of clinical and survey visits; 2% (85 of 4,443) missing in 2006 and 1% (44 of 4,828) in 2007.

Malaria risk factors. Data on risk factors were available for > 94% of children for both years of the study. Most risk factors varied in a similar manner between the zones each year; these included the distance of homes from the floodplain, the

TABLE 1
Characteristics of children enrolled by study zones, The Gambia*

Characteristic	Zone 1				Zone 2				Zone 3				Zone 4			
	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007
Year																
Larviciding																
No. children	496	492	508	523	525	523	525	503	510	509	422	527	510	527	416	416
Females (%)	50.6	52.6	49.2	49.3	51.1	49.3	51.1	50.9	42.2	48.8	52.2	41.6	42.2	41.6	41.6	41.6
Age, years	5.2	5.1	4.9	4.9	4.7	4.9	4.7	4.8	5.2	4.8	5.2	4.9	5.2	4.9	5.2	4.9
% Houses with open eaves (95% CI)	57.4 (53.0–61.8)	46.9 (42.3–51.5)	74.9 (71.1–78.7)	68.4 (64.1–72.6)	46.3 (42.0–50.6)	43.4 (38.8–48.0)	46.3 (42.0–50.6)	43.4 (38.8–48.0)	39.2 (34.9–43.4)	43.4 (38.8–48.0)	39.2 (34.9–43.4)	28.5 (24.4–32.6)	39.2 (34.9–43.4)	28.5 (24.4–32.6)	39.2 (34.9–43.4)	28.5 (24.4–32.6)
% Children using bed nets (95% CI)	32.7 (28.5–36.8)	43.4 (38.8–48.0)	69.7 (65.6–73.8)	97.7 (96.3–99.0)	75.8 (72.1–79.5)	91.9 (89.3–94.4)	75.8 (72.1–79.5)	91.9 (89.3–94.4)	78.9 (75.3–82.5)	91.9 (89.3–94.4)	78.9 (75.3–82.5)	88.9 (86.1–91.8)	78.9 (75.3–82.5)	88.9 (86.1–91.8)	78.9 (75.3–82.5)	88.9 (86.1–91.8)
% Children using treated bed nets (95% CI)	27.6 (23.6–31.5)	37.2 (32.8–41.7)	6.1 (4.0–8.2)	81.4 (77.9–84.9)	38.3 (34.1–42.5)	71.2 (67.0–75.4)	38.3 (34.1–42.5)	71.2 (67.0–75.4)	34.3 (30.2–38.5)	71.2 (67.0–75.4)	34.3 (30.2–38.5)	70.4 (66.3–74.6)	34.3 (30.2–38.5)	70.4 (66.3–74.6)	34.3 (30.2–38.5)	70.4 (66.3–74.6)
% Villages < 1 km from floodplain (95% CI)	17.9 (14.6–21.3)	21.3 (17.7–25.0)	12.2 (9.3–15.1)	11.7 (8.9–14.4)	82.3 (79.0–85.6)	83.9 (80.7–87.1)	82.3 (79.0–85.6)	83.9 (80.7–87.1)	90.0 (87.4–92.6)	83.9 (80.7–87.1)	90.0 (87.4–92.6)	88.8 (86.1–91.5)	90.0 (87.4–92.6)	88.8 (86.1–91.5)	90.0 (87.4–92.6)	88.8 (86.1–91.5)
Ethnicity																
% Wolof	82.5	83.1	27.0	27.1	0	0	0	0	0.8	0	0.8	0.8	0.8	0.8	0.8	0.8
% Mandinka	0.4	0.4	0	0	12.8	13.0	12.8	13.0	73.9	73.9	73.9	75.1	73.9	75.1	73.9	75.1
% Fula	16.1	16.3	68.1	68.5	37.0	37.7	37.0	37.7	19.4	19.4	19.4	19.7	19.4	19.7	19.4	19.7
% Serrehule	0	0	0.4	0.4	40.6	41.4	40.6	41.4	0	0	0	0	0	0	0	0

* CI = confidence interval.

TABLE 2
Impact of the intervention on malarial indices in children 6 months to 10 years of age, The Gambia*

Variable	Zone				All zones
	1	2	3	4	
Incidence of malaria cases/100 child-years (95% CI)					
2006	70.9 (58.8–85.6)	30.3 (23.1–39.7)	44.1 (35.2–55.2)	29.1 (22.1–38.4)	42.9 (38.2–48.1)
2007	7.2 (4.3–11.9)	17.0 (12.4–23.5)	27.2 (20.9–35.4)	24.7 (18.8–32.3)	19.0 (16.3–22.2)
Prevalence of <i>Plasmodium falciparum</i> infection (no. parasitemic/total)					
Start 2006	38.4% (158/411)	16.8% (85/505)	16.0% (84/524)	9.5% (48/508)	19.3% (375/1,948)
End 2006	41.0% (163/398)	12.2% (54/443)	12.8% (57/447)	10.5% (45/430)	18.6% (319/1,718)
Start 2007	17.0% (82/482)	3.3% (17/514)	1.0% (5/502)	2.3% (12/513)	5.7% (116/2,011)
End 2007	20.7% (95/458)	8.2% (39/474)	10.4% (47/452)	22.3% (105/472)	15.4% (286/1,856)
Mean hemoglobin level, g/dL (SD)					
Start 2006	10.4 (1.7)	10.4 (1.9)	10.8 (1.7)	10.7 (1.6)	10.6 (1.8)
End 2006	10.2 (1.8)	10.5 (1.9)	10.7 (1.7)	10.7 (1.7)	10.5 (1.8)
Start 2007	10.4 (1.6)	10.2 (1.7)	10.5 (1.5)	10.4 (1.7)	10.3 (1.7)
End 2007	10.4 (1.6)	10.6 (1.9)	10.4 (1.6)	10.0 (1.6)	10.3 (1.7)
Prevalence of splenomegaly (Hackett's score > 0)					
Start 2006	11.6% (57/493)	3.9% (20/507)	4.2% (22/524)	3.9% (20/510)	5.9% (119/2,034)
End 2006	12.0% (47/393)	5.9% (26/442)	6.5% (29/447)	5.8% (25/434)	7.4% (127/1,716)
Start 2007	4.5% (22/491)	1.9% (10/524)	2.2% (11/503)	1.1% (6/527)	2.4% (49/2,045)
End 2007	7.7% (35/456)	2.6% (12/471)	2.6% (12/455)	3.8% (18/471)	4.2% (77/1,853)
Prevalence of gametocytemia					
Start 2006	4.2% (17/411)	1.4% (7/505)	1.9% (10/524)	0.4% (2/508)	1.9% (36/1948)
End 2006	5.5% (22/398)	2.5% (11/443)	3.1% (14/447)	4.7% (20/430)	3.9% (67/1718)
Start 2007	3.1% (15/482)	0.8% (4/514)	0.2% (1/502)	0.8% (4/513)	1.2% (24/2011)
End 2007	6.3% (29/458)	4.2% (20/474)	2.7% (12/452)	6.4% (30/472)	4.9% (91/1856)

* CI = confidence interval. Incidence of malaria was estimated by passive case detection during the main malaria transmission seasons. Prevalence of *P. falciparum* infection and anemia was estimated at the start and end of each transmission season. Start or end denote surveys done in June or November/December, i.e., at the start and end of the malaria transmission season each year.

percentage of subjects living in houses with closed eaves, bed net use, and ethnicity (Table 1). Comparison of risk factors by intervention for each year shows that only bed net and ITN use varied with the intervention in both years; ITN use increased from a range of 6.1–38.3% in 2006 to 37.2–81.4% in 2007.

Larvicide application. Sixty-four men applied 4,933 kg of *Bti* WDG and 2,712 kg of *Bti* CG to zones 1 and 3 in 2006, and 6,705 kg of *Bti* WDG and 7,553 kg of *Bti* CG to zones 2 and 4 in 2007.

Mosquito abundance. Each year there were five months of rain from June to October, with peaks in rainfall pattern between July and September (Figure 3). Total annual rainfall decreased slightly each year from 858.3 mm in 2005 to 807.9 mm in 2006 and 751.4 mm in 2007. The proportion of sampled habitats colonized with late instar *Anopheles* larvae at baseline was 31% (439 of 1,408). A similar proportion was found in 2006 in the untreated zones (40%, 515 of 1,288), whereas only 1% (12 of 1,380) of sites were colonized with *Anopheles* in zones where larviciding took place. However, in 2007 only

12% of sites (165 of 1,389) were colonized in untreated zones compared with 4% (55 of 1,439) in zones where larviciding took place. The overall crude relative risk of sites being colonized in the presence of larviciding was thus 0.12. Taking into account clustering by zone and site, we observed that the intervention significantly reduced the likelihood of water bodies being colonized in both years, but was more effective in 2006 (odds ratio [OR] = 0.01, 95% confidence interval [CI] = 0.01–0.02, $P < 0.001$) than 2007 (OR = 0.27, 95% CI = 0.18–0.41; $P < 0.001$). Similar results were found in the sentinel sites where the proportion of sites with late anopheline larvae in the absence of larviciding was 51% (415 of 817) in 2005, 48% (198 of 410) in 2006, and 22% (107 of 487) in 2007. In the presence of larviciding, the proportion was 2% (9 of 489) in 2006 and 9% (42 of 488) in 2007. In these sentinel sites, when we took clustering by zone and site into account, similar reductions in colonization by larvae were found in 2006 (OR < 0.01, 95% CI = 0.003–0.020, $P < 0.001$) and 2007 (OR = 0.44, 95% CI = 0.22–0.83, $P < 0.01$). The mean density of anopheline larvae per dip per sentinel habitat was significantly reduced in all zones during larviciding ($P < 0.001$; Figure 4).

On each sampling round, 60 CDC light traps were set in the selected 39 villages and traps were sampled on 97.5% (2,053 of 2,100) occasions during the study. A subsample of 626 *An. gambiae* s.l. females caught in houses in 2005 and 2006 was identified to species level by PCR; 13% ($n = 82$) of these samples did not amplify and the rest consisted of 54% *An. gambiae* s.s., 27% *An. melas*, and 19% *An. arabiensis*. There was a clear seasonal trend in adult vector densities with peaks in August or September (Figure 4). The density of adult vectors collected varied between zones with the lowest levels in zone 1 and highest in zone 3, except in the year of the intervention (Figure 4 and Table 3). Adult vector densities were higher in the baseline year than subsequent years, even in the absence of the

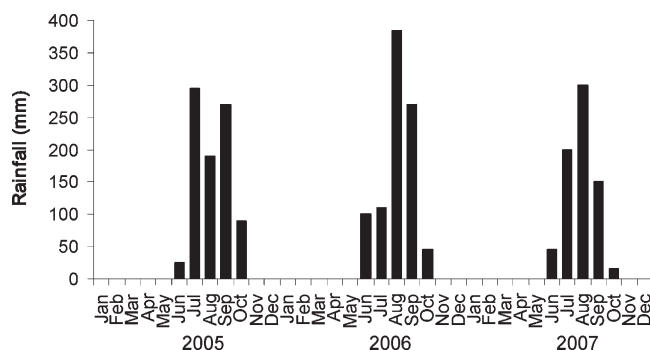


FIGURE 3. Rainfall during the study period, The Gambia.

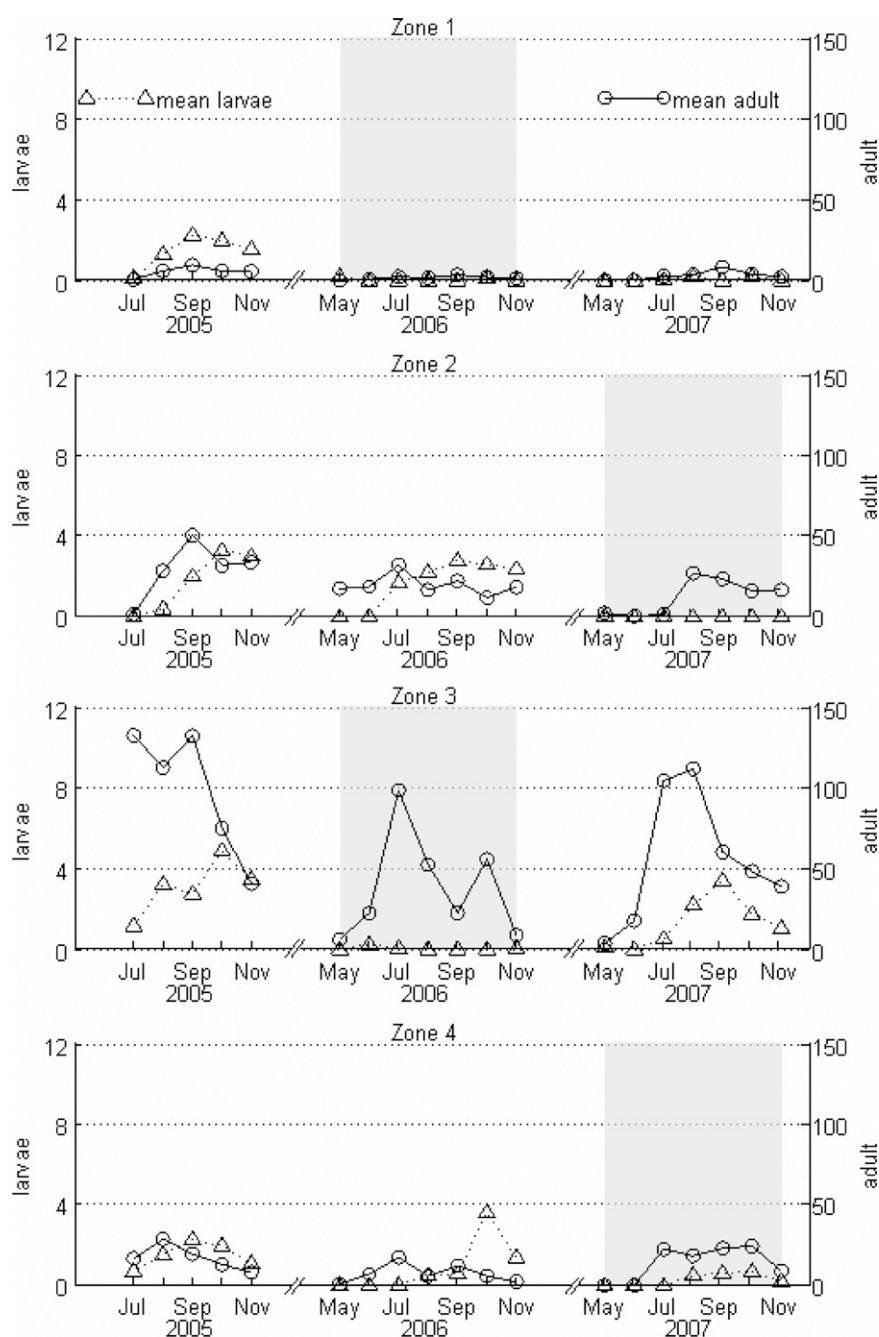


FIGURE 4. Seasonal abundance of larval and adult *Anopheles gambiae* during the study, The Gambia. Gray bars represent periods of larvicide application.

intervention (median = 10, interquartile range [IQR] = 2–32 compared with median = 7, IQR = 1–25, $P = 0.011$). Adult vector density was lower in zones 1 and 3 during larviciding but there was little change in zones 2 and 4 (Figure 4 and Table 3). Vector densities varied not only with larviciding and year, but also with distance of villages from the floodplains (Figure 5). In villages further from the floodplain, larviciding was associated with a reduction in vector densities in 2006, but not in 2007. Conversely, in villages closest to the river, larviciding was associated with reduced vector density in 2007, but not 2006. This three-way interaction between the intervention, time, and location relative to the major breeding sites was highly significant when modeled ($P = 0.002$), making an estimation of the

effect of larviciding alone on vector density an unreasonable simplification.

Sporozoite rates (Table 3) were generally lower during larviciding. However, binary logistic regression, when adjusted for year and zone, showed no significant association between larviciding and the presence of infective mosquitoes (OR = 0.65, 95% CI = 0.36–1.20, $P = 0.17$). Seasonal entomological inoculation rate varied from below the level of detection to 19.5 (Table 3).

***Plasmodium* spp. infection.** There was considerably more malaria in 2006 than 2007 (Table 2). Incidence of clinical episodes of malaria in 2006 was twice that in 2007. This finding may have resulted from higher rainfall in 2006, and

TABLE 3
Mean female *Anopheles gambiae* s.l. density and the sporozoite rate per zone, June–November, The Gambia*

Parameter	Zone			
	1	2	3	4
Median female <i>An. gambiae</i> s.l./trap/night (IQR)				
2005	3 (0–7)	19 (4–44)	24 (6–78)	11 (3–26)
2006	1 (0–3)	13 (6–26)	12 (4–31)	3 (1–11)
2007	2 (0–5)	13 (4–26)	34 (10–69)	9 (2–26)
Sporozoite rate, no. with <i>Plasmodium falciparum</i> sporozoites/total				
2005	1.09% (13/1,191)	0.19% (16/8,332)	0.11% (16/15,136)	0.23% (7/3,008)
2006	0% (0/469)	0% (0/4,105)	0.08% (7/9,315)	0.24% (4/1,633)
2007	0.37% (2/546)	0.08% (3/3,493)	0.16% (25/15,796)	0.14% (6/4,154)
Overall	0.68% (15/2,206)	0.12% (19/15,930)	0.12% (48/40,247)	0.19% (17/8,795)
Seasonal EIR				
2005	8.80	8.29	16.55	6.13
2006	0	0	5.82	3.13
2007	2.24	2.32	17.00	3.91

* IQR = interquartile range; EIR = entomological inoculation rate.

particularly the heavy rains early in the season, compared with 2007 or changes in ITN coverage (Figure 3 and Table 1). There was also a large variation between zones, with zone 1 having approximately twice the malaria incidence found in the other zones in 2006. This finding may have been a consistent pattern because the prevalence of parasitemia in zone 1, at the start of 2006, which reflected the intensity of malaria transmission in 2005, was also double that of its neighbors. These differences in incidence were also reflected by the differences in the rates of enlarged spleens, with rates in zone 1 approximately twice those in the other three zones and lower rates in 2007 than 2006 (Table 2). Gametocyte rates were higher in zone 1 in the dry season surveys, and as expected these rates increased in the wet season survey but only slight differences were apparent between the zones in the wet season surveys, and there was no apparent association with the intervention (Table 2). Mean Hb levels remained fairly constant throughout the study and between zones (Table 2). Overall the malaria indices seen in each zone appear largely unaffected by the intervention.

Analysis of the effect of larviciding on malaria incidence, taking into account the cross-over trial design and adjusting for individual time of exposure and clustering by subject and

village, indicated an increase in malaria incidence associated with larviciding in 2006, but not 2007 (2006: OR = 2.89, 95% CI = 1.79–4.68, $P < 0.001$; 2007: OR = 1.25, 95% CI = 0.74–2.09, $P = 0.404$). Sex, age, bed net use, ITN use, sleeping in a room with open eaves, distance of villages from the floodplains, and ethnicity did not significantly impact on the OR for a model including year. Year of study was the only variable with a significant effect ($P = 0.031$). There was also no significant impact on anemia ($P > 0.05$; Table 3).

DISCUSSION

Larval control with microbial larvicides is effective in areas with relatively few well-defined habitats^{13,14,20,21} but there has been no detailed evaluation of this method in areas with extensive habitats. This is the first large-scale assessment of the impact of larviciding in a rural ecosystem that is dominated by large areas of flooding.

Although our data indicate that larviciding with microbials reduced the proportion of water bodies containing *Anopheles* larvae and the mean densities of late stage larvae per habitat by an order of magnitude, the reduction in exposure to transmission was unsatisfactory and did not lead to any reduction in clinical malaria, parasite prevalence, or anemia. The impact of larviciding on adult vectors in this study was limited. This finding is in marked contrast to studies in urban Tanzania and lowland and highland Kenya where LSM reduced exposure to transmission by 65–93%^{13,20,21} and was associated with a 68–72% reduction in new parasite infections.^{13,21}

The study does not show any consistent change in malaria associated with larval control. This result is heavily influenced by the exceptionally variable level of malaria found in zone 1; being extremely high during the intervention and extremely low in the non-intervention year. The reasons for this variability are not understood because there is relatively little change in net use in this zone during the study. This decrease may reflect a trend in this area because similarly decreasing levels were found in the community to the east of zone 1²⁸ and also in selected hospitals in The Gambia.²⁹ This finding illustrates the general study design issue of finding large clusters that have similar malaria ecologies.

Nonetheless we have demonstrated that larviciding reduced the aquatic stages of anophelines, but only limited success in reducing adult numbers in three of the four study zones. The

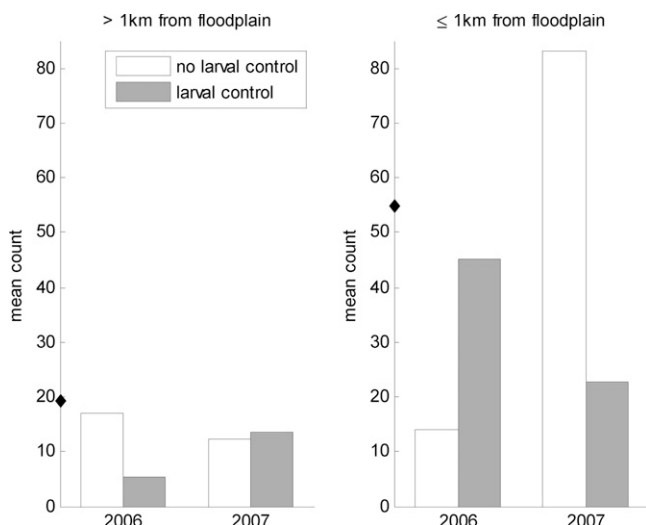


FIGURE 5. Impact of larval control on female *Anopheles gambiae* s.l. densities stratified by intervention year and distance of the villages to the nearest riverine floodplain, The Gambia.

question arises: why were we not able to achieve a significant reduction in transmission? There are a number of possible explanations. First, it is possible that mosquitoes might have invaded the intervention zones from surrounding areas outside the untreated areas. Earlier studies in The Gambia indicate that although most *An. gambiae* s.l. fly no more than 2 km, a small proportion may fly much further.²³ This long-distance flight may be a consequence of the local ecology of the study area, which is flat, where persons live in small and discrete communities and where breeding sites are often far from the villages. Thus, vectors flying from the abundant breeding sites in the floodplain find it difficult to locate a human blood meal, particularly because those persons living closer to the floodplains are more likely to sleep under bed nets.³⁰ Second, not all water bodies in the alluvial floodplains were treated with larvicide because deep water, especially during times of high tides, made it impossible to reach some parts of the wetlands close to the river. Based on earlier published work,³¹ we assumed that this might not affect the intervention because these sites were more than 4 km from the study villages and we expected most adult mosquitoes to emerge from the landward edge of the floodplain. Nevertheless, more recent work has shown that low densities of larvae can be found over the entire floodplain area, even close to the river.^{24,32} Away from the landward edge, raised areas within the large flooded areas can create edges suitable for colonization by mosquitoes compromising the success of targeted interventions such as the current one. Third, in our study area the flooded areas close to the Gambia River are subject to daily tidal movements³³ that might disperse mosquito larvae away from sites regularly treated with larvicide and dilute the larvicides in areas treated at low tide. Furthermore, mosquito eggs can survive on damp soil for several days³⁴ and once these sites are flooded with water, the eggs hatch and larvae develop successfully to adults. A similar situation might occur in The Gambia when eggs laid at low tide on damp soil remain viable and hatch at high tide when these sites are flooded. In such cases, a successful intervention would either require larviciding at shorter intervals or the application of a more residual larvicide, which remains viable even if it falls dry periodically, such as the insect growth regulator pyriproxyfen.³⁵ Fourth, even though considerable effort was made to supervise the application of larvicides, we cannot exclude the possibility that field applicators may have missed aquatic habitats.

In this study, a low technology approach was used to apply the larvicide because it was considered most appropriate for community involvement in resource-poor countries in Africa.^{20,24} However, in areas with extensive flooding, such as river floodplains and major areas of irrigated rice, significant impact might only be achieved with aerial application because large areas can be treated rapidly at full coverage. Clearly, it is imperative that appropriate methods are developed for the large-scale application of larvicides in areas of extensive flooding in Africa.

Notably, LSM can improve as the field teams gain more experience, as has been demonstrated for the Urban Malaria Control Program in Dar es Salaam, Tanzania.¹⁶ In a large and difficult study area such as ours with locally recruited field teams that have had relatively little experience with LSM, a strategy implementing the intervention in the same zones over consecutive years might have been more successful. The same applies if the study would have been implemented 200 km

upriver where water bodies are less influenced directly by the river, are no longer tidal and often much smaller.

The recent successes that have been achieved with LSM in rural and urban settings in east Africa^{13,21} are clearly associated with differences in the transmission setting and habitat characteristics compared with the extensive floodplains of the Gambia River. Significant control of vectors can be achieved by hand application of larvicide where malaria transmission is focal and when water bodies are defined and accessible, where the water is stagnant and the flight range of the adult vector is not more than several kilometers. In such settings LSM has a significant added benefit to personal protection measures such as ITNs.³⁶

This trial was part of a series of studies to assess the impact of LSM on malaria morbidity in different eco-epidemiological settings. Despite a major effort, we were not able to reduce malaria in this ecosystem dominated by riverine floodplains. Because LSM using simple, low-cost technology is not an intervention that works everywhere, careful consideration needs to be given to the habitat characteristics responsible for the proliferation of malaria vectors. Ground-based manual application of larvicides is not an appropriate tool for areas with extensive flooding (e.g., large floodplains or large-scale rice cultivation where habitats are not defined and largely inaccessible on foot and/or where the water is tidal). It is therefore crucial to develop a decision support system for national malaria control programs to guide where and when it is appropriate to consider LSM in an integrated vector management approach.

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