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Spatial Distribution of Mosquito Larvae and the Potential for Targeted Larval Control in The Gambia

Silas Majambere, Ulrike Fillinger, David R. Sayer, Clare Green, and Steven W. Lindsay*
School of Biological and Biomedical Sciences, Durham University, Durham, United Kingdom; Medical Research Council Laboratories, Fajara, The Gambia

Abstract. We examined the distribution of aquatic stages of malaria vectors in a 400-km^2 area in rural Gambia to assess the practicality of targeting larval control. During the rainy season, the peak period of malaria transmission, breeding sites were 70% more likely to have anopheline larvae in the floodplain of the Gambia River than upland sites ($P < 0.001$). However, mosquitoes were found in some examples of all habitats, apart from moving water. Habitats most often colonized by anopheline larvae were the largest water bodies, situated near the landward edge of the floodplain, where culicine larvae were present. In the wet season, 49% of sites had anophelines versus 19% in the dry season ($P < 0.001$). Larval control targeted at specific habitats is unlikely to be successful in this setting. Nonetheless, larval control initiated at the end of the dry season and run throughout the rainy season could help reduce transmission.

INTRODUCTION

There is a growing interest in using larval control as a tool for integrated vector management programs for malaria control in sub-Saharan Africa (SSA).[1–10] The first operational larviciding programs in modern Africa recently started in the city of Dar es Salaam, Tanzania.[11] Data needed to inform these programs are starting to grow. Recently, pilot studies in lowland and highland Kenya showed that microbial larvicides could reduce *Anopheles* larval densities by 95% with a concomitant reduction in exposure to mosquito bites of > 90%.[9] Most importantly, in highland sites, larviciding was associated with a 50% reduction in malaria parasite infection (U Fillinger and others, unpublished data). Similar reductions in vector productivity have been achieved with larval source management using microbial larvicides in Eritrea.[5]

The eradication of *Anopheles gambiae*, the principal vector of malaria in SSA, from large flooded areas in Brazil in the 1930s suggested a similar approach could be effective in comparable habitats in SSA, including those in rural Gambia.[12] Before embarking on a larval control campaign, it is crucial to understand where and when the aquatic stages of the vectors are found to direct control activities at these sites. Because larviciding in large river ecosystems would be logistically complicated and expensive, identifying the sites where *Anopheles* larvae occur most frequently and/or in highest density for targeting larval control[1,13] would increase the cost-effectiveness of the operation.

In SSA, *Anopheles* larval habitats are frequently associated with human activity.[14] These are typically open sunlit pools created when depressions made by people and their animals fill with rain or ground water. Such sites are common close to human habitation and in fields. In addition, regions with large river systems including The Gambia often face seasonal flooding, which creates large areas of standing water for extended periods of time and provides potential breeding sites for mosquitoes.[15,16]

Few larval surveys have been conducted in The Gambia,[16–19] and most of these studies were small scale or along transects confined largely to the floodplains in the rainy season, making it difficult to generalize the findings from these studies. Originally it was recognized that higher numbers of adult mosquitoes were captured in villages close to the Gambia River compared with those further away.[20] Because the river is too fast flowing to provide mosquito breeding sites, a study of the riverine habitats was carried out. This study showed that the number of adult mosquitoes found in a village was positively related to the proximity and extent of pooled sediments bordering the river, suggesting that this was the most productive area for anophelines.[19] This finding was confirmed several years later when the highest densities of mosquito larvae collected along transects were close to the landward edge of the alluvial plains, although high numbers could also be found > 1 km into the alluvial floodplains.[16] *An. gambiae* s.s. and *An. melas* were found mainly within the flooded areas, whereas *An. arabiensis* occurred mainly in rain-fed rice fields close to this area.

This study was carried out to prepare for a large trial of microbial larvicides where we needed to identify all potential breeding habitats within the study area. We used the larval data collected during this study to determine and characterize those water bodies commonly frequented by anopheline larvae, both in the floodplains and the upland sites, during the dry and wet seasons. This information is essential for determining whether targeting interventions at a limited number of specific habitats would be a viable option for malaria control in rural Gambia and in other areas with major river systems. This study represents a comprehensive longitudinal survey of potential larval breeding sites; we surveyed every accessible water body in a 400-km^2 area from the river to the borders of the country repeatedly over a 2-year period.

MATERIALS AND METHODS

Study area. The study was carried out east of Farafenni town in The Gambia from June 2004 to May 2006. The study area was selected to comprise the most common habitats found in large river ecosystems, where many water bodies contained brackish and fresh water. Four zones, each ~100 km^2 in area, were selected (Figure 1): two on the north bank of River Gambia around Balanghar Ker Nderry (Zone 1 13°40′0″N, 15°24′0″W and Bantanto Jawara (Zone 2 13°41′60″, 15°15′0″W and two on the south bank, near Jalangbereh (Zone 3 13°22′60″N, 15°24′0″W and Sutukung (Zone

* Address correspondence to Steven W. Lindsay, School of Biological and Biomedical Sciences, Durham University, Durham DH1 3LE, United Kingdom. E-mail: S.W.Lindsay@durham.ac.uk

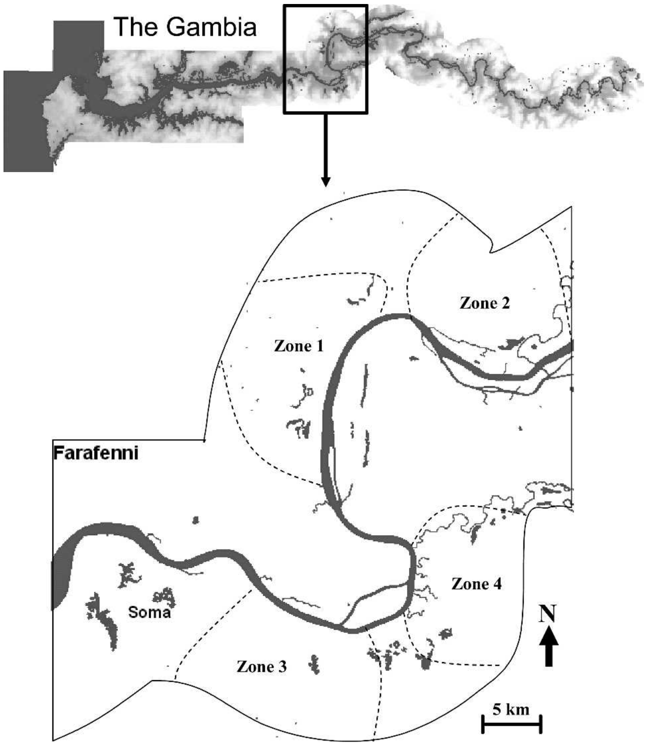

FIGURE 1. Map of study area. Discontinuous lines show zone limits.

4 13°28′0″, 15°16′0″). The study reported here describes data collected during the pre-intervention period of a larval control trial. This trial was designed as a cross-over study based in the four study zones. Each zone can be divided broadly into 1) the upland area that is predominantly woodland savannah and farmland, where the main crops are millet and groundnuts, and 2) the river floodplains, where large areas of alluvial soils are flooded during the rainy season and rice is grown. The rainy season is from June to October, and the average annual rainfall during the study period was 837 mm. All study villages within the study zone were located between 1 and 8 km from the Gambia River.

Water body measurements. The depth, size, type of water body, and surrounding land cover was recorded for every accessible aquatic habitat found during the study. Surveys were carried out continuously, with each zone being surveyed six to eight times during the study. Each water body was given a unique identification number, and its position was recorded using a handheld Global Positioning System (GPS, 15-m accuracy, Garmin GPS 12 XL, Southampton, UK). Water depth was classified as shallow when the water level was below knee-high and deep when it was above the knee. The perimeter of each breeding site was categorized by eye as 1) < 10, 2) 10–100, or 3) > 100 m. Each aquatic habitat was classified into one of the following categories that are usually found in succession from the village toward the main river: 1) brick or sand pits, borrow pits (> 2 m diameter) resulting from brick-making or other construction activities; 2) cattle troughs attached to village pumps; 3) pools, discrete (< 200 m diameter) and shallow (< 50 cm) standing water bodies, usually drying out toward the end of the dry season; 4) edges of floodwater, the shallow landward edges of the extensive floodwater in the floodplains of the river or its tributaries, partly barren and partly associated with grass (*Paspalum* and *Sporobolus* spp.) and sedge (*Eleocharis* spp.); 5) ponds, discrete and permanent water bodies, > 100 m in circumference fed by groundwater and deeper than pools; 6) water channels, used for irrigation or drainage; 7) stream fringes, the shallow edges of permanent streams associated with grass or sedge, and tall reeds in deeper parts; 8) puddles or tire tracks, small natural or vehicle-made depressions; 9) footprints, made by people, cattle, or other animals where water collects, often associated with edges of large water bodies (floodwater, streams, pools and ponds); 10) floodwater, inundated areas in the floodplain further away from the landward edge, toward the river; 11) rice fields, seasonally flooded areas used to grow rice; and 12) mangrove, water body characterized by densely growing mangrove trees (*Rhizophora* and *Avicennia* spp.) near the main river. Additionally, the dominant land cover around each aquatic habitat was recorded as follows: 1) upland grassland (Poaceae), vegetation dominated by *Paspalum* and/or *Sporobolus* species and not affected by the river; 2) upland agriculture, such as fields of groundnuts, maize, pumpkins, sorghum, and millet; 3) shrubs of the West Sudanian savannah ecoregion; 4) forest, densely growing, tall trees; 5) barren floodplain: under tidal influence without any vegetation; 6) sea-purslane (*Sesuvium* spp.), a succulent salt indicator plant forming a low carpet of thick leaves; 7) grass (Poaceae) on the floodplain, vegetation dominated by *Paspalum* and/or *Sporobolus* species; 8) sedge (Cyperaceae), vegetation dominated by the spike-rush (*Eleocharis* spp.); 9) rice (*Oryza sativa*) plantations; 10) reeds, *Phragmites karka* and *Cyperus papyrus* form the reed beds, usually found in deep water; and 11) mangrove forest of *Rhizophora* or *Avicennia* spp. usually next to the main river and large tributaries.

Larval sampling. Purposeful sampling was done to maximize collection of the aquatic stages of mosquitoes using a 350-mL dipper (Clarke Mosquito Control Products, Roselle, IL). At each site, 10 dips were made in places likely to harbor mosquito larvae, such as around tufts of submerged vegetation or substrate, edges of water bodies, and around floating debris. In extensive water bodies, dipping was carried out over a 100-m walk. Larvae were classified either as anophelines or culicines. Anopheline larvae were stored in 100% ethanol, which was refreshed on reaching the laboratory. Randomly selected subsamples of anopheline larvae were selected during the routine mapping of the area and sibling species of the *An. gambiae* complex identified by amplification of ribosomal DNA using polymerase chain reaction (PCR).[21]

Statistical analysis. Coordinates of each water body were entered into a Geographical Information System (ArcGIS-ArcInfo Version 9.1 software; ESRI, Redlands, CA) and plotted on a map of The Gambia using the Geographic Coordinate System: GCS WGS 1984, Datum: D WGS 1984. The map templates were obtained from the Department of Lands and Surveys (The Government of the Republic of The Gambia, 2004). These maps were used to localize and visualize all surveyed aquatic habitats. The distance between a specific breeding site and the nearest human settlement was obtained by measuring the distance between points (breeding sites) and polygons defining the human settlements using the ArcGIS software. A layer was defined along the edges of the alluvial floodplains and the nearest distance between all breeding sites and this edge determined. Habitats close to the alluvial edge, created by floodwater, were referred to as "edge of floodwater." For the purpose of estimating the area of this habitat type, its width was assumed to be 50 m based on maps from the Department of Lands and Surveys. Further into the floodplain, the habitats are usually deeper and semi-permanent and are described as "floodwater," which is another category.

The impact of different water body characteristics on the presence or absence of mosquito larvae was explored individually. Comparisons between proportions were made using χ^2 analysis. All variables were incorporated in a mathematical model, and their overall impact on the presence of anopheline larvae tested using generalized estimating equations (GEEs). This model was used because it takes account of repeated measures in the analysis, because the same water bodies were repeatedly sampled during the study. The habitat identification was used as subject unit for repeated measures assuming an exchangeable correlation matrix. Larval data (presence or absence) were fitted to a binomial distribution with a logit link function. After testing for collinearity of predictors in the model, those that were not highly correlated ($R > 0.9$) were used together in the model. The "edge of floodwater" was selected as the reference group in the model for comparison of different habitat types, because this habitat was identified in an earlier pilot study as most likely to be colonized by mosquito larvae.[16] Various vegetation types as land cover were compared with floodplain areas without any vegetation, which is characteristic of many parts of the edge of floodwater. Regression analysis was used to test for relationships between key variables. Logistic regression was used to elucidate any differences between sites with *An. gambiae* s.l. and sites with other anophelines and between anopheline early- and late-instar larvae. Analyses were performed with SPSS (Chicago, IL) version 15 and EpiInfo, Atlanta, GA version. Missing data were excluded from the analysis.

Ethics. Ethical approval for this study was given by the Joint Gambian Government and Medical Research Council's Laboratories in The Gambia, as well as Durham University's Ethics Advisory Committee. The need for sampling water bodies, including fields and backyards, was explained to local communities. Verbal consent was obtained from village leaders and from home owners before the start of household surveys.

RESULTS

Characteristics of aquatic habitats. A total of 6,038 visits were made to 1,076 different water bodies in the four study zones over 2 consecutive years. Seventy-one percent of the water bodies in the floodplain contained anopheline larvae on at least one occasion (528/739) compared with 50% in the upland (138/337; $P < 0.001$). Sixty percent (3,673/6,038) of visits took place in the dry season, and 40% (2,410/6,038) took place in the rainy season. Most habitats were visited on six to eight occasions over the study period, with 35% of habitats occurring in Zone 3 (373/1076), 25% in Zone 4 (269/1076), 21% in Zone 1 (224/1076), and 19% in Zone 2 (210/1076). Although there were more aquatic habitats in Zone 3, the risk of habitats being colonized by anopheline larvae in Zone 3 was less compared with other zones ($P < 0.001$). On occasions when sites were visited, 84% (2031/2410) contained water during the rainy season, whereas only 45% (1,666/3,673) had water during the dry season ($P < 0.001$). Sites contained water on 88% of occasions in the floodplains and 67% of occasions in the uplands during the rainy season ($P < 0.001$). In the dry season, sites were wet on 58% of occasions in the river's floodplains and only 15% of occasions in the upland ($P < 0.001$).

Characteristics of larval habitats. Because the presence of late-instar anopheline larvae was strongly correlated with early instars ($R^2 = 0.59$, $P < 0.001$), the results for early and late instars were pooled for all further analyses. Forty-two of 3,695 (1%) records for anophelines were missing in the dataset and were not included in the analysis.

GEE modeling for the entire data set adjusting for study zone, the location of the habitat in the upland or in the floodplains, the season of sampling, the habitat type, the habitat size, distance to the landward edge of the alluvial plains, and dominant land cover type showed that anopheline larvae were four times more likely to be found during the rainy season than during the dry season (odds ratio [OR] = 4.06; 95% confidence interval [CI] = 3.31–4.99; $P < 0.001$) and were less common in the upland than in the floodplains, although this was of borderline significance (OR = 0.64; 95% CI = 0.41–1.01; $P = 0.055$). However, when only the rainy season was considered, the likelihood of finding anophelines was significantly less in the upland sites compared with the floodplain (OR = 0.30; 95% CI = 0.22–0.39; $P < 0.001$).

Given these differences between occurrence of larvae in floodplain and upland sites and between the dry and rainy season, data were analyzed in subsets to identify potential risk factors for the presence of mosquitoes in the floodplains and upland during rainy (June–October) and dry season (November–May) separately. The distance from a habitat to the nearest village and the water depth of habitats were not significantly associated with the presence of anopheline larvae and were therefore not included in any of the final models.

In the floodplains during the rainy season (Table 1), habitats farther away than 1 km from the landward edge of the alluvial plains were 58% less frequently colonized than those within the first 1 km ($P < 0.001$), and larger habitats, with > 100 m in perimeter were seven times more frequently colonized than smaller ones ($P = 0.006$). Notably, habitats with these characteristics represent those most frequently encountered (> 80% of the site visits).

Rice fields ($N = 413$), open floodwater ($N = 439$), stream fringes ($N = 295$), and pools ($N = 105$) were most frequently flooded in the floodplains during the rainy season, and the majority of the *Anopheles* samples were taken from these sites ($N = 272$, 190, 125, and 105, respectively). Nevertheless, although some habitat types were available more frequently, GEE modeling showed that, when adjusting for the location of the habitat and its size, the risk of finding *Anopheles* larvae was the same for the majority of habitat types. There was a positive association between anopheline abundance and habitats in areas dominated by grass, sedge, and rice compared with floodplain areas without vegetation.

The risk of finding *Anopheles* larvae in the floodplains in the dry season (Table 2) was the same for the entire width of the floodplain area and independent of the size and type of habitats or the dominant land cover type.

In the upland area during the rainy season (Table 3), the most frequently recorded aquatic habitats were pools

Table 1
Factors associated with the presence and absence of anopheline larvae in the floodplain in the rainy season

Factor	Number of visits (N)	Anophelines present: Occasions	Anophelines present: Proportion (%)	Odds ratio	Lower CI	Upper CI	P
Zone							
Zone 4	411	234	56.9	0.55	0.37	0.83	0.004
Zone 3	346	122	35.3	0.24	0.16	0.37	< 0.001
Zone 2	469	143	51.8	0.26	0.17	0.38	< 0.001
Zone 1	304	205	67.4	1.00			
Distance to edge of alluvial plains							
1–3 km	142	45	31.7	0.42	0.27	0.64	< 0.001
< 1 km	1,388	759	54.7	1.00			
Perimeter							
> 100 m	1,285	696	54.2	6.81	1.72	26.92	0.006
10–100 m	222	101	45.5	3.20	0.82	12.55	0.095
< 10 m	23	7	30.4	1.00			
Habitat types							
Brick or sand pits	10	8	80.0	0.56	0.13	2.49	0.450
Cattle troughs	8	4	50.0	0.67	0.14	3.28	0.625
Pool	165	105	63.6	0.93	0.46	1.86	0.831
Pond	14	10	71.4	1.42	0.19	10.62	0.730
Water channel	33	8	24.2	0.56	0.20	1.57	0.266
Stream fringe	295	125	42.4	0.49	0.25	0.95	0.036
Puddles or tire tracks	50	23	46.0	1.17	0.42	3.32	0.762
Footprints	5	1	20.0	0.13	0.02	0.82	0.030
Floodwater	439	190	43.3	0.56	0.31	1.01	0.056
Rice fields	413	272	65.9	0.62	0.27	1.43	0.265
Edge of floodwater	98	58	59.2	1.00			
Land cover							
Mangrove	39	3	7.7	0.59	0.15	2.42	0.467
Reeds	298	107	35.9	1.78	0.71	4.44	0.220
Sea-purslane	20	4	20.0	0.58	0.12	2.76	0.490
Bush	52	29	55.8	1.24	0.41	3.73	0.706
Sedge	260	138	53.1	3.11	1.26	7.63	0.013
Rice	355	238	67.0	3.33	1.09	10.16	0.035
Grass	476	280	58.8	2.92	1.25	6.84	0.013
Barren floodplain	30	5	16.7	1.00			
Culicines							
Present	656	556	84.8	18.35	13.19	25.54	< 0.001
Absent	859	233	27.1	1.00			

CI = 95% confidence interval.

(N = 152), cattle troughs (N = 140), and puddles (N = 115). The majority of *Anopheles* records were taken from pools (N = 80), cattle troughs (N = 42), puddles (N = 21), and rice fields (N = 21). Risk factor analyses showed that habitats > 10 m in perimeter were three times more often associated with anopheline larvae than smaller ones (P = 0.009), but the risk of finding *Anopheles* larvae in the most frequently encountered aquatic habitats in the upland was not significantly associated with any land cover or habitat type. The presence of larvae was significantly less when aquatic habitats were > 3 km away from the edge of the alluvial plains. In the dry season, aquatic habitats were rarely encountered in the upland. Of 214 sampling events, only 14 had *Anopheles* larvae; 64% of these were found in cattle troughs and the rest in puddles and pools.

Rice fields and pools were most frequently found, especially during the rainy season (Tables 1–3), but differed greatly in the area they covered (Table 4). Rice fields stretched in total over ~2,150 ha (21.5 km^2), whereas pools covered < 1 ha within the entire study area. In comparison, the edge of floodwater was ~500 ha.

Independent of location and season, there was a very strong positive association between the presence of anophelines and the presence of culicines in the aquatic habitats (Tables 1–3; Figure 2).

PCR analysis. Of a subsample of 124 anopheline habitats, PCR analysis conducted on 1,401 samples showed that 52% of these habitats were occupied by *An. gambiae* s.l. (35% *An. gambiae* s.s., 11% *An. melas*, 6% *An. arabiensis*). Most *An. arabiensis* (86%) and *An. gambiae* s.s. (58%) were found in rice fields and in pools. *An. melas* was predominantly found in floodwater and edges of floodwater (57%; Figure 3). Binary logistic regression showed no significant difference between characteristics of habitats occupied by *An. gambiae* s.l. and those of other anophelines.

DISCUSSION

This study represents the most comprehensive survey of mosquito larvae in The Gambia and is of relevance to other parts of the Sahel, where large river systems dominate the local malaria ecology. We mapped aquatic habitats in an area of ~400 km^2 over 2 years, including both floodplain and upland areas during the dry and wet seasons. Although we attempted to achieve full coverage of the study area, some sites may have been missed in deeper water close to the river. Our study is unique in that it covers such a large area over an extended time period in contrast to the majority of published ecology studies, which were small scale in space and time.[22–25]

TABLE 2
Factors associated with the presence and absence of anopheline larvae in the floodplain in the dry season

Factor	Number of visits (N)	Anophelines present: Occasions	Anophelines present: Proportion (%)	Odds ratio	Lower CI	Upper CI	P
Zone							
Zone 4	284	62	21.8	2.90	1.57	5.38	0.001
Zone 3	343	28	8.2	0.92	0.44	1.93	0.830
Zone 2	459	144	31.4	2.43	1.55	3.81	< 0.001
Zone 1	329	61	18.5	1.00			
Distance to edge of alluvial plains							
1–3 km	216	47	21.8	1.09	0.67	1.78	0.719
< 1 km	1,199	248	20.7	1.00			
Perimeter							
> 100 m	1,234	278	22.5	1.61	0.30	8.76	0.583
10–100 m	152	14	9.2	0.73	0.12	4.45	0.733
< 10 m	29	3	10.3	1.00			
Habitat types							
Brick or sand pits	5	1	20.0	0.40	0.06	2.83	0.361
Cattle troughs	9	1	11.1	0.53	0.04	7.79	0.644
Pool	59	14	23.7	1.02	0.39	2.67	0.961
Water channel	69	1	1.4	0.15	0.02	0.96	0.045
Stream fringe	387	74	19.1	0.90	0.47	1.71	0.736
Puddles or tire tracks	6	1	16.7	4.31	0.30	62.43	0.284
Floodwater	425	57	13.4	0.57	0.29	1.12	0.101
Rice fields	375	120	32.0	2.10	0.60	7.38	0.248
Edge of floodwater	80	26	32.5	1.00			
Land cover							
Mangrove	76	2	2.6	0.22	0.02	1.99	0.178
Reeds	391	58	14.8	0.53	0.07	3.88	0.529
Sea-purslane	9	1	11.1	1.03	0.11	9.40	0.982
Bush	36	6	16.7	0.50	0.05	5.62	0.578
Sedge	250	58	23.2	1.30	0.18	9.09	0.794
Rice	328	107	32.6	0.73	0.08	6.89	0.785
Grass	308	60	19.5	0.85	0.12	5.82	0.869
Barren floodplain	17	3	17.6	1.00			
Culicines							
Present	346	213	61.6	18.86	13.23	26.89	< 0.001
Absent	1,066	79	7.4	1.00			

CI = 95% confidence interval.

Only large-scale studies allow making generalizations about the larval ecology of malaria vectors relevant for operational larval control programs. Specifically, our research was carried out to determine whether it is possible to identify habitat characteristics associated with the presence of anopheline larvae using practical operational tools. It was hoped that any such characteristics could be used to guide interventions to target larval control at specific sites or time periods.

Most anopheline breeding habitats were confined to the floodplains, in agreement with previous studies.[16,19] These habitats are created by flooding from the river and heavy rainfall in the rainy season and persist because of the high water table and impervious clay ground, unlike the dry and porous sandy upland.[26] The importance of naturally flooded areas for mosquito proliferation is supported by earlier studies in The Gambia, where the salt water malaria vector *An. melas* was associated with *Avicennia* mangrove in flooded areas of the River Gambia.[27] Similarly, *An. gambiae* s.l. was associated with flooded areas in Liberia[28] and Nigeria.[29,30]

The risk of finding anopheline larvae in the floodplains during the rainy season was increased when habitats were located within 1 km of the landward edge, were large in size (100 m or more in perimeter), and located in areas where grassy vegetation (including rice and sedge) dominated the land cover. This includes > 80% of all habitats encountered during the 2 years of rainy season surveys and does not represent selection criteria that could easily be used to guide antilarval interventions. One exception might be the distance from the edge of the alluvial plains. The farther away from the edge and the closer to the river, the more difficult it is to access habitats and to implement antilarval interventions. Specifically, the application of larvicides becomes difficult in these highly tidal environments. For operational reasons, it would be wrong to target larviciding operations only at the landward edge of the floodplains, because mosquitoes found further into floodplains would be missed.

During the dry season, the small water bodies in the uplands dried out, leaving those in the floodplains as the main refugia for anophelines. Hence the probability of finding anopheline larvae during the dry season was reduced by 75% compared with the rainy season. Presumably, this was a consequence of the lower water level and the reduction in habitat availability. In the floodplains, habitats suitable for larval development were found everywhere, irrespective of land cover, habitat type, or size.

There were fewer aquatic sites in the upland areas compared with the floodplains. Specific risk factors for finding sites that could be targeted for antilarval interventions were not identified. Even though fewer in number, these upland habitats are important for malaria transmission in The Gambia because of their closeness to human settlements. Mosquitoes emerging from these sites are more likely to feed on people and become infected with malaria parasites than mosquitoes that have to fly far to reach people. The upland sites,

TABLE 3
Factors associated with the presence and absence of anopheline larvae in the upland area in the rainy season

Factor	Number of visits (*N*)	Anophelines present		Odds ratio	Lower CI	Upper CI	*P*
		Occasions	Proportion (%)				
Zone							
Zone 4	145	61	42.1	0.63	0.37	1.07	0.089
Zone 3	116	45	38.8	1.24	0.65	2.35	0.515
Zone 2	52	13	25.0	0.70	0.30	1.60	0.395
Zone 1	168	68	40.5	1.00			
Distance to edge of alluvial plains							
> 3 km	76	17	22.4	0.33	0.13	0.84	0.020
1–3 km	238	89	37.4	0.84	0.52	1.38	0.494
< 1 km	167	81	48.5	1.00			
Perimeter							
> 100 m	119	58	48.7	3.35	1.36	8.28	0.009
10–100 m	235	97	41.3	2.69	1.29	5.64	0.009
< 10 m	127	32	25.2	1.00			
Habitat types							
Brick or sand pits	9	7	77.8	6.11	0.82	45.78	0.078
Cattle troughs	140	42	30.0	0.76	0.38	1.51	0.430
Water channel	5	4	80.0	5.61	1.85	17.01	0.002
Stream fringe	4	3	75.0	0.73	0.04	12.60	0.831
Puddles or tire tracks	115	21	18.3	0.73	0.34	1.54	0.407
Floodwater	4	2	50.0	0.93	0.27	3.19	0.908
Rice fields	31	21	67.7	0.65	0.12	3.48	0.612
Pond	21	7	33.3	0.45	0.15	1.33	0.148
Pool	152	80	52.6	1.00			
Land cover							
Forest	5	1	20.0	1.07	0.16	7.18	0.941
Upland agriculture	41	5	12.2	0.53	0.19	1.48	0.223
Upland grassland	111	39	35.1	1.70	0.71	4.08	0.234
Shrubs	237	96	40.5	1.09	0.52	2.28	0.827
Sedge	3	2	66.7	1.47	0.22	9.75	0.690
Rice	30	22	73.3	3.27	0.50	21.39	0.215
Grass	54	22	40.7	1.00			
Culicines							
Present	243	146	60.1	7.95	4.65	13.61	< 0.001
Absent	238	41	17.2	1.00			

CI = 95% confidence interval.

unlike the extensive breeding sites in the floodplains, could be reduced by filling unused pits and pools and ensuring that pooling does not occur around water pumps and cattle troughs. However, larval control cannot be successfully targeted only at the upland sites close to human settlements because of the greater propensity of larvae to be found in the floodplains. Adult studies also showed a gradient, indicating that the majority of adults emerged from the landward edge of the floodplains.[19,31] These findings are consistent with the hypothesis that blood-fed mosquitoes have a long flight range in The Gambia, a situation typical of sparsely populated savanna.[14,19,32] Unlike urban or densely populated areas where flight range for anophelines is often ~1 km,[32,33] settlements in rural Gambia are tightly clustered and not widely dispersed as in many other African countries. Thus, for a blood-questing mosquito in the floodplain, it would seem to be more difficult to locate a blood meal than elsewhere, particularly because people living close to the floodplains are more likely to use bednets.[34] This implies that larval control cannot focus on breeding sites close to human settlements alone but must also attack those farther away.

Even though rice fields presented as much a risk factor for anopheles breeding as any other large water body with grassy vegetation, it is important to emphasize that rice fields were by far the most common aquatic habitats, covering a surface area of > 20 km^2 in our study area. Most of these are found in the floodplain, although rice is also grown in valley depressions in the uplands. It is well known that rice cultivation encourages mosquito production,[35–37] although most studies described the importance of irrigated rice rather than the traditional "swamp" rice grown in floodplains of the River Gambia. During the rainy season when rice was cultivated, a high proportion of all rice fields (66–68%) was colonized by

TABLE 4
Sampling frequency and size of major anopheline breeding habitats

Habitat	Frequency	Area (ha*)				
		Zone 1	Zone 2	Zone 3	Zone 4	Total
Rice fields	20% (1,234/6,083)	647	256	116	1,136	2,155
Pools	16% (997/6,083)	0.225	0.040	0.388	0.161	0.814
Edge of floodwater	5% (296/6,083)	155	75	145	125	500

* 1 ha = 10,000 m^2.

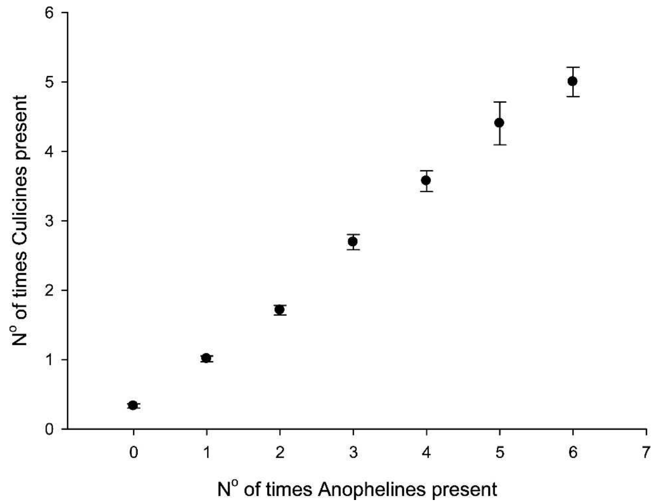

FIGURE 2. Relationship between the frequencies at which culicine and anopheline larvae occurred together. Bars represent SE.

anopheline larvae. Although rice fields cannot be singled out as preferred *Anopheles* larval habitats in The Gambia, their sheer abundance and the ease of recognizing them in the field makes them an important target for antilarval interventions. Nevertheless, given the large variety of suitable water bodies for anopheline development, targeting rice fields exclusively might not be enough to reduce malaria transmission to such an extent to be cost effective.

The large areas covered by rice fields and the fact that one in four habitats colonized by anophelines in the upland close to human settlements is man-made emphasizes the importance of breeding sites created by people in the ecology of malaria in The Gambia.

Anopheline larvae were predominantly found in habitats covered by relatively short vegetation such as (early stage) rice, grass, and sedge in accordance to larval ecology studies elsewhere in Africa.[11,37–41] These types of vegetation allow water bodies to be exposed to sunlight, a situation preferred by ovipositing mosquitoes,[42] unlike tall and thick vegetation such as reeds and mangrove.

The finding that large water bodies are more important than small ones for mosquito breeding in both the floodplains and upland areas in the rainy season contradicts a common view, held since the 1950s, that small water bodies are typical habitats for *An. gambiae*.[42,43] Indeed the lack of enthusiasm for antilarval measures for malaria control in SSA was partly because of the idea that such small sites were too common and difficult to locate.[44]

Anopheline larvae were rare and difficult to find in the field. We found anophelines in only 309 site visits, after ~15,000 dips, during the dry season, and on 992 site visits, after ~18,000 dips, during the rainy season. The small number of anophelines found is likely to be caused by a combination of factors. Larvae are frequently clustered,[45] and these clusters were distributed over a huge area in the floodplains, making sampling challenging. Although dipping is a simple sampling tool, it is inefficient and only likely to capture a small proportion of the mosquito larvae present in any habitat.[46] We recommend that other methods such as area sampling or sweep nets be used to increase chances to capture larvae. However, these methods would be time consuming and could only be considered in experimental settings.[10]

Interestingly, anophelines and culicines were commonly found together. Similar findings have been reported in habitats in East Africa.[47] The over-riding impression in The Gambia is that, although some water bodies support a wide diversity of life, others are truly inimical for invertebrates. This would explain why anophelines and culicines shared the few prolific habitats available in the area. Niche partitioning, occurring at a finer spatial scale,[16,48–52] was only apparent in our study when we examined the habitat preferences of different members of the *An. gambiae* complex. *An. gambiae* s.s. predominated in pools, *An. arabiensis* was more common in rice fields, and *An. melas* was most frequently found in floodwater, which was likely to be saline.

In this study, we describe the characteristics of sites with anopheline mosquitoes and not those specifically relating to *An. gambiae* s.l., which is the major malaria vector in The Gambia. We consider that our approach is relevant to determining where *An. gambiae* s.l. is found, because members of the complex inhabited the same water bodies as other anophelines. Larval control programs mostly concentrate on monitoring the density of late stage larvae or pupae, because they represent sites most likely to produce adult vectors.[11] However, for a large routine program such as ours, it would be too demanding to measure larval density in each habitat, and pupae are rare and difficult to sample with a dipper as shown in earlier surveys.[10] Collecting data on mosquito emergence from each habitat would present the optimal way to estimate habitat productivity but would require a lengthy and thorough study that would be difficult to implement in a routine control program. The most feasible approach under operational conditions is to use local residents with relevant training to collect data on the presence and absence of larval stages.[11] In The Gambia, the probability of finding late instars was positively and strongly correlated with finding early-stage larvae. Thus, we consider that our findings for both early and late instars of anopheline mosquitoes are also generally applicable for determining sites most commonly occupied by

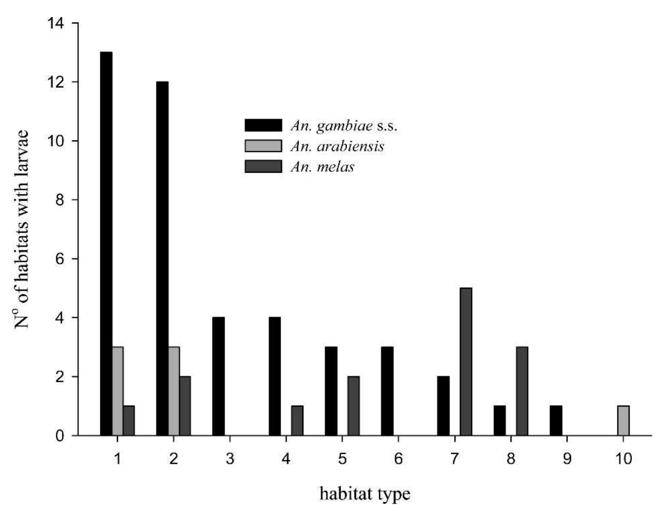

FIGURE 3. Frequency of *An. gambiae* s.l. in different habitat types: 1, pool; 2, rice fields; 3, pond; 4, puddles and tire tracks; 5, stream fringe; 6, construction; 7, floodwater; 8, edge of floodwater; 9, brick or sand pits; 10, water channel.

late stages of *An. gambiae* s.l. Furthermore, in large-scale operational programs, evidence-based decisions on re-treatment intervals need to be made instantly in the field and will therefore be based on the presence of any anopheline larva and not necessarily the presence of *An. gambiae*.[11]

Our findings, based on practical operational monitoring and evaluation tools, showed that anopheline larvae are present in a wide variety of habitats and associated characteristics, implying that successful larval control cannot be targeted at specific habitats in The Gambia. This calls for blanket treatment of all available aquatic habitats at regular intervals and the implementation of sustainable environmental modifications where applicable.

Whereas the comparatively small number of habitats during the dry season (November–May) would in principle suggest that there could be an advantage for dry season larval control, which might lead to a large reduction in overall population size, the wide distribution of few sites over a vast area of floodplains and upland without any risk factors to guide the intervention to specific sites would be logistically demanding, and the overall impact on malaria transmission would be questionable. Nevertheless, to delay the rise of adult mosquito numbers during the rainy season[25] and also to allow field teams to adapt slowly to the changing environment and increasing habitat numbers, we propose that antilarval measures should be started 1–2 months before the rainy season. Furthermore, the quickly increasing risk of vector proliferation and malaria transmission with the start of the rains makes it necessary to implement antilarval intervention throughout the wet season.

Because mosquito habitats are distributed over a large area and involve extensive water bodies situated far away from human settlements, larval control will be logistically demanding. However, these sites are largely accessible, and less effort is needed to control larvae in moving and deeper water bodies covered by tall reeds or mangrove forest. The long flight range of *An. gambiae* in this country means that larval control activities would have to be carried out over large areas to reduce the likelihood of adults flying into control areas from surrounding locations.

Although there would be considerable advantage in targeting larval control to specific breeding sites if they would be identifiable as the most productive habitats for malaria vectors,[13] it is necessary to be cautious about this approach because the heterogeneity in productivity of different breeding sites is not always predictable, and breeding sites are highly dynamic and influenced largely by the rainfall and river.[1]

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Authors' addresses: Silas Majambere, Ulrike Fillinger, David R. Sayer, Clare Green, and Steven W. Lindsay, School of Biological and Biomedical Sciences, Durham University, Durham DH1 3LE, UK, and Medical Research Council Laboratories, Fajara, The Gambia, Tel: 44-191-374-1289, Fax: 44-191-374-1289, E-mail: S.W.Lindsay@durham.ac.uk.

Reprints requests: Steve Lindsay, School of Biological and Biomedical Sciences, Durham University, Durham DH1 3LE, UK.

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