

The practical importance of permanent and semipermanent habitats for controlling aquatic stages of *Anopheles gambiae sensu lato* mosquitoes: operational observations from a rural town in western Kenya

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Summary

Control of aquatic-stage *Anopheles* is one of the oldest and most historically successful interventions to prevent malaria, but it has seen little application in Africa. Consequently, the ecology of immature afro-tropical *Anopheles* has received insufficient attention. We therefore examined the population dynamics of African anopheline and culicine mosquitoes using operationally practicable techniques to examine the relative importance and availability of different larval habitats in an area of perennial malaria transmission in preparation for a pilot-scale larval control programme. The study was conducted in Mbita, a rural town on the shores of Lake Victoria in Western Kenya, over 20 months. Weekly larval surveys were conducted to identify the availability of stagnant water, habitat characteristics and larval densities. Adult mosquitoes were collected indoors at fortnightly intervals. Availability of aquatic habitats and abundance of mosquito larvae were directly correlated with rainfall. Adult mosquito densities followed similar patterns but with a time-lag of approximately 1 month. About 70% of all available habitats were man-made, half of them representing cement-lined pits. On average, 67% of all aquatic habitats on a given sampling date were colonized by *Anopheles* larvae, of which all identified morphologically were *A. gambiae sensu lato*. Natural and artificial habitats were equally productive over the study period and larval densities were positively correlated with presence of tufts of low vegetation and negatively with non-matted algal content. The permanence of a habitat had no significant influence on larval productivity. We conclude that *A. gambiae* is broadly distributed across a variety of habitat types, regardless of permanence. All potential breeding sites need to be considered as sources of malaria risk at any time of the year and exhaustively targeted in any larval control intervention.

keywords *Anopheles gambiae*, larval ecology, mosquito larval habitat, malaria vector, mosquito control, Kenya

Introduction

In Suba District, western Kenya, malaria transmission is meso- to holoendemic, with an average parasite prevalence in the human population of 60% (Gouagna *et al.* 2004). Malaria in this area is associated with ideal climatic conditions for transmission (Bayoh & Lindsay 2003) and the most efficient vector species in the world (Beier *et al.* 1999; Hay *et al.* 2000): *Anopheles gambiae* Giles, *A. arabiensis* Patton and *A. funestus* Giles (Mutero *et al.* 1998; Minakawa *et al.* 1999, 2002). National malaria

control efforts in Kenya, as in most African countries, focus on case management and the use of insecticide-treated bednets (MOH 2003). Domestic vector control interventions against adult mosquitoes in the form of insecticide-treated bednets (Kazura 2003) or indoor residual spraying (Kouznetsov 1977), combined with improved access to effective diagnosis and treatment, have enormous potential to Roll Back Malaria and constitute the primary means to achieve the Abuja targets (WHO/UNICEF 2003). However, even these highly effective interventions are insufficient to eliminate malaria transmission from most

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endemic parts of Africa (Killeen *et al.* 2000b; Gu *et al.* 2003). Additional interventions are clearly required to build truly integrated malaria control programmes that can make further inroads to the enormous burden of malaria in African communities (Beier *et al.* 1999; Killeen *et al.* 2000a,b; Shiff 2002). The potential of integrated vector control for malaria prevention in Africa is now being reappraised (Castro *et al.* 2004; Keiser *et al.* 2004; Killeen *et al.* 2004) including underused interventions such as routine application of larvicides (Fillinger *et al.* 2003) and source reduction through removal of *Anopheles* larval habitats. Such approaches have received little attention in recent decades despite proven successes in diverse settings (Watson 1911; Soper & Wilson 1943; Muirhead-Thomson 1945; Shousha & Pasha 1948; Watson 1953; Killeen *et al.* 2002a). Since the advent of dichlorodiphenyltrichloroethane (DDT), most efforts on vector control have focused on mosquito adults and research on the larval ecology of African malaria vectors has been largely neglected. As a consequence, little is known about the habitats, abundance and distribution of the larvae of the main African malaria vectors, with most information in relation to practical larval control dating back to publications in the first half of the previous century (Hopkins 1940; Muirhead-Thomson 1945, 1951; Watson 1953; Holstein 1954; Clyde 1967).

To control mosquitoes, whether adults or larvae, it is crucial to understand the relevant ecology of the target species. This requires the study of not only the fluctuations of the adult populations, but also the factors affecting larval abundance and distribution. While considerable progress towards understanding the aquatic stages of *A. gambiae sensu lato* has been made in recent years (Chinery 1984; Minakawa *et al.* 1999; Gimnig *et al.* 2001, 2002; Bøgh *et al.* 2003; Keating *et al.* 2003; Klinkenberg *et al.* 2003; Koenraadt *et al.* 2003; Koenraadt & Takken 2003; Ye-Ebiyo *et al.* 2003), systematic research on the larval ecology of the main malaria vectors in Africa is still limited and often represents short data collection periods, frequently considering mosquito-infested habitats only. In contrast, more intensive larval ecology studies evaluating and acknowledging options for larval vector control have been conducted in central and southern America on various malaria vectors in the last decade (Berti *et al.* 1993; Rodriguez *et al.* 1993; Fernandez-Salas *et al.* 1994; Manguin *et al.* 1996a,b; Rejmankova *et al.* 1999; Grillet 2000).

We report our observations on the population dynamics of African anophelines and associated culicine mosquitoes in order to evaluate the importance and availability of different larval habitats in an area of moderate and perennial malaria transmission. This study was a baseline survey to inform an operational larval control programme.

Thus, the methods applied are those that are considered practical and useful under operational conditions in health programmes and generalizable to larger, sustainable programmatic scales (Gubler & Clark 1994; Habicht *et al.* 1999; Durrheim *et al.* 2002; Booman *et al.* 2003). This survey was conducted intensively over a period of 20 months from October 2000 to May 2002. It is the first detailed longitudinal study describing the nature of the aquatic habitats of malaria vectors in Africa.

Methods

Study area

The study was carried out in the area surrounding the International Centre of Insect Physiology and Ecology's (ICIPE) Mbita Point Research and Training Centre in Mbita (0°30' S; 34°15' E), a rural town on the shores of Lake Victoria, in Suba District, western Kenya. About 8000 people live in Mbita and most are resident fishermen with a few traditional farmers. Mbita is a peninsula bordered by the lake from the east and west and consists of several small housing groups. In the north Rusinga Island is connected with the mainland via a causeway. The data presented here were collected in an area of approximately 1.5 km², along the eastern shores of the lake and in an inland area known as 'Hillside' because of its closeness to a small central hill (Figure 1). During the study period, mosquito larval and adult surveys were conducted and the relative availability, changes and colonization of aquatic habitats recorded.

The ICIPE operates a meteorological station where daily climate data are recorded. Mbita's average annual rainfall between 2000 and 2002 was 1423 mm, and temperatures varied between an average minimum of 18 and maximum of 29 °C. Two rainy seasons occur annually from approximately March to June and October to November. The peak rainfall is generally expected between March and May, but the seasons are usually not well defined with some years of more or less regular rains and others with prolonged dry periods.

Total annual rainfall in the year 2000 was 1597 mm, with more than a third of this falling in the last 3 months of the year. The year 2001 was comparatively dry with an annual rainfall of 1139 mm, and only 438 mm during the long rains (March–May). This rainy season was thus much drier than the one in 2000 with 683 mm, and the one in 2002 with 513 mm from March to May.

Mosquito adult survey

Adult mosquitoes were collected indoors (resting catches) from 12 houses in close vicinity to the surveyed larval

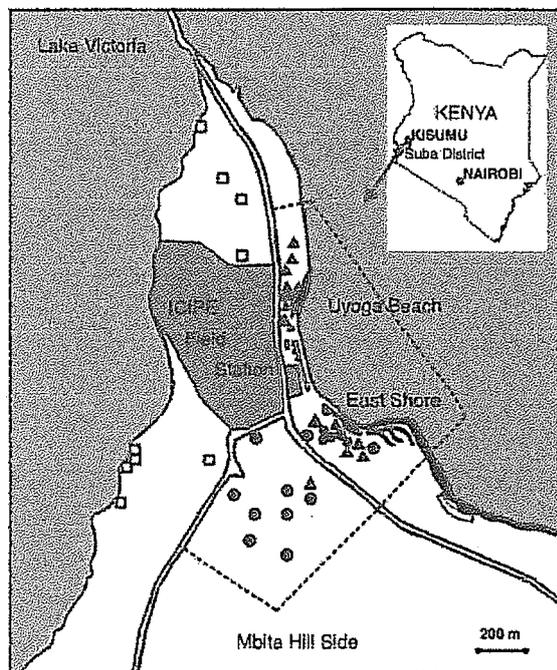
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Figure 1 Map of the study area, Mbita town, Suba district, western Kenya. Black triangles (▲) indicate sentinel houses for mosquito resting catches, black circles (●) indicate surveyed larval habitats with semipermanent character, grey areas indicate larval habitats with temporary character, transparent squares indicate potential habitats outside the investigation area. The investigation area is enclosed between dotted lines and main roads.

habitats. The adult survey was conducted at fortnightly intervals. All selected houses were traditional mud huts with iron roofing representing the most common houses in the area. The distance of the surveyed houses to the nearest, surveyed breeding habitat varied between 15 and 91 m. Every sampling day, the number of people that slept in the house the previous night was recorded.

Indoor-resting mosquito adults were sampled using the pyrethroid spray catch (PSC) technique following WHO guidelines (WHO 1992). Before spraying inside the hut, large furniture was carefully removed and white sheets placed on the floor. Ten minutes after spraying, all dead mosquitoes found on sheets were collected and transported to the laboratory where *A. gambiae s.l.* and *A. funestus* were separated from the other mosquito species using morphological criteria. Because of operational resource allocation priorities, the *A. gambiae* complex was not identified to species level using the polymerase chain reaction (PCR) methodology. According to earlier

investigations in Mbita location (Minakawa *et al.* 1999, 2002), *A. gambiae s.s.* accounts for an average of 80–90% of both the larval and adult population of the species complex, with the rest being *A. arabiensis*. Other, far less abundant *Anopheles* species identified in the area are *A. funestus* and *A. coustani*; especially the latter is only found sporadically (Minakawa *et al.* 2002) and not at all during our survey.

The number of males and females, and the number of bloodfed and unfed females were recorded for all *Anopheles* and culicine mosquitoes. Culicine mosquitoes were not identified to species level, but their general inclusion in the study was considered important as they represent a significant nuisance in the area and need to be considered in larval control operations to gain community support.

Mosquito larval survey

Relative availability of habitats. During a preliminary survey in September 2000, most aquatic habitats were found at the eastern shores and in the hillside inland area (Figure 1). These areas were therefore selected for detailed study. The study area was examined twice a week for stagnant water bodies, with the presence or absence of the aquatic stages of *Anopheles* and culicine mosquitoes being noted in each water body. All 21 mosquito-containing habitats identified in the preliminary September 2000 survey were selected for biweekly determination of larval densities from October 2000 to December 2001, and further weekly surveys from January to May 2002. Thirteen of these sites were located between the lake shore and the main road at Uyoga Beach and East Shore, with the remaining eight sites being located inland (farther than 100 m away from the lake shores), in the hillside area enclosed by the two major roads in Mbita (Figure 1). We estimated the proportion of habitats that actually contained water at a given time as an index of relative availability (Killeen *et al.* 2001). For the purposes of simplicity of terminology, we define habitats as being available when they contain water.

Larval densities and habitat characteristics. Larvae were sampled using a WHO-standard 250 ml capacity mosquito dipper (WHO 1992) in order to (i) score the presence or absence of larvae from (water containing) habitats, (ii) to determine the preferred breeding sites of vector and other mosquito species, in relation to larval densities and continuous colonization of a site and (iii) to measure changes in larval density over time. Sampling was conducted in a deliberately non-random fashion to maximize sensitivity of collections using standard procedures (Bøgh *et al.* 2003).

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To measure the average number of larvae per dip per breeding site per sampling day, constant number of dips (six in sites smaller and 10 in sites larger than 6 m²) were taken. Larvae were classified into three categories: early instars (stages I and II), late instars (stages III and IV) and pupae. Larval instars were classified separately for *Anopheles* and culicines, but pupae were not distinguished in the field and summarized in the data collection since their species-dependent morphological differences cannot easily be distinguished in the field. After quantification, larvae and pupae were returned to the water.

Anopheles larvae were identified according to morphological criteria in the field. Furthermore, to separate *A. gambiae s.l.* and *A. funestus*, samples of five to 10 *Anopheles* larvae per habitat were identified morphologically in the laboratory in approximately 2-monthly intervals.

To investigate habitat characteristics associated with *Anopheles* and culicine development, we classified habitats as being

- in their origin man-made or natural,
- in their appearance natural (man-made and natural habitats can contain soil and natural vegetation) or an artificial pit structure (cemented), and we recorded
- the surface area in m²,
- water depth in cm,
- turbid or clear water,
- non-matted algal content (according to the intensity of the green colour of the entire water surface area we categorized four levels: absent, low, medium and high), and
- vegetation present or absent (recorded vegetation included grass, *Eichhornia crassipes*, *Lemna* sp. and algal mats).

Statistical methods

General linear models (GLM) have been applied to analyse statistical differences between treatment groups in Table 1 and Figure 3. Proportions were arcsine transformed to normalize the data. Spearman's non-parametric rank correlation of Williams mean values and Mann-Whitney *U*-tests were used to identify correlations between abundances of aquatic stage mosquitoes or between habitat characteristics and mosquito abundance. All correlation analyses present summary statistics of one value per habitat over the entire study period. The specific tests applied are mentioned in the text.

The relationship between the abundance of immature or adult mosquitoes and rainfall or temperature, as well as

with each other, was evaluated by autoregressive time-series analysis fitted by the exact maximum likelihood method. Monthly total rainfall, and 1 month lags thereof, were treated as independent variables in autoregression models which were selected manually beginning with a full set of potential determinants and sequentially removing the least significant variables, including the constant, until only significant variables remained. All analyses were carried out using SPSS 11 for Windows.

Results

Mosquito adult densities

During the 20 months of the study, 42 sampling events took place to collect adult mosquitoes in 12 sentinel houses in the investigation area. Altogether 14 226 mosquitoes were collected, 29% (4182) of them *A. gambiae s.l.*, 1% (153) *A. funestus* and 70% (9891) culicines of various species. A considerable proportion of males could be found resting inside houses as well. Males represented 50% of all culicines, 32% of all *A. gambiae s.l.* and 10% of all *A. funestus* adults. About 92% of all *A. gambiae s.l.*, 75% of all *A. funestus* and 77% of all culicine females were bloodfed.

The average number of bloodfed *Anopheles* mosquitoes per house ranged from a high of 17.4 in December 2000 to 0.2 in September 2001. Taking into account the number of persons that slept in the house the night before any adult PSC took place, the number of bloodfed *Anopheles* per person ranged from 6.5 in December 2000 to 0.1 in September 2001. The entomological inoculation rate (EIR) could not be directly calculated since no data were collected on sporozoite incidence and human biting index. Assuming that all bloodfed *Anopheles* females fed on humans [there are very few cattle or other alternative hosts in Mbita and we would expect human blood indices to exceed 90% (Killeen *et al.* 2001)] the night before and taking into consideration a sporozoite incidence of 3.5% as previously identified for all three *Anopheles* species in a location nearby (Mathenge *et al.* 2004), we can approximate a relatively modest EIR of 20 infective bites per person per year which is comparable with previous rapid assessments for the township (Shililu *et al.* 2003) [for standard methods of EIR calculations see Hay *et al.* (2000), Drakeley *et al.* (2003)].

Particularly high numbers of *Anopheles* adults were observed from November 2000 to February 2001 (Figure 2c) following extended rain fall (Figure 2a) after which habitats were available in greatest numbers (Figure 3). After February 2001, bloodfed, female densities

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Table 1 Larval habitat characteristics in Mbita between October 2000 and May 2002 (20 months, dipping twice a week up to December 2001, once a week, i.e. January-May 2002)

Habitat Number	Location	Type*	Total number of larvae/dip (sum of 20 months)	Proportion of <i>Anopheles</i> (%)	Time habitat flooded (%)	Proportion of time flooded habitat was colonized (%)		Proportion of late instars dipped (%)		Proportion of time species colonized habitats together (%)	Average number of larvae/dip per sampling date with water	
						All larvae	<i>Anopheles</i>	<i>Anopheles</i>	Culicines			
1	Lakeside	Concrete	441.5	20	93	67	45	30	22	49	4.20	0.84
2	Lakeside	Natural	315.6	68	67	74	68	21	11	89	4.15	2.83
3	Lakeside	Concrete	58.3	86	43	86	84	28	2	29	1.19	1.02
4	Lakeside	Natural	73.7	88	32	75	75	24	3	22	2.05	1.80
5	Lakeside	Concrete	23.1	83	6	100	100	34	54	86	3.30	2.74
6	Lakeside	Concrete	395.3	65	73	69	66	32	30	49	4.76	3.09
7	Lakeside	Concrete	46.1	8	10	91	64	13	26	60	4.19	0.35
8	Lakeside	Concrete	190.4	41	42	83	75	31	30	65	3.97	1.64
9	Lakeside	Natural	312.8	21	41	80	63	27	22	78	6.80	1.42
10	Hillside	Concrete	401.8	56	92	93	88	33	15	57	3.86	2.18
11	Hillside	Concrete	238.1	31	88	51	36	24	16	49	2.38	0.75
12	Hillside	Concrete	514.7	1	31	97	34	37	37	35	14.71	0.16
13	Hillside	Concrete	422.9	8	65	99	44	28	25	42	5.79	0.47
14	Hillside	Concrete	576.6	19	81	88	50	25	25	46	6.34	1.23
15	Hillside	Concrete	199.1	25	36	78	59	13	11	66	4.86	1.22
16	Hillside	Concrete	1196.9	1	75	93	25	19	33	25	14.08	0.17
17	Hillside	Concrete	287.4	24	62	73	47	33	16	55	4.11	0.99
18	Lakeside	Natural	525.4	41	99	95	86	29	22	67	4.69	1.92
19	Lakeside	Natural	417.5	34	35	90	73	25	27	75	10.44	3.57
20	Lakeside	Natural	372.7	11	28	91	53	32	46	59	11.65	1.25
21	Lakeside	Natural	149.1	48	16	100	100	18	45	94	8.28	0.70

* Habitat sizes varied between 2 m² for habitat no. 9 and 75 m² for habitat no. 18, water depths of all habitats varied between 1 and 106 cm (average 27.3, SD = 20.1).

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Figure 2 The dependence of mosquito abundance upon rainfall from October 2000 to May 2002. (a) Monthly rainfall in mm. (b) Immature abundance: O, proportion of habitats containing water; ◊, *Anopheles* larvae of all stages; ◻, culicine larvae of all stages; ×, combined *Anopheles* and culicine pupae. All points represent the monthly Williams mean of mean counts per dip for all samples from surveyed habitats. (c) Biting adult abundance: ◊, *A. gambiae sensu lato*; ⋆, *A. funestus*; ◻, culicine species. All points represent the monthly Williams mean of total catches of bloodfed mosquitoes per person.

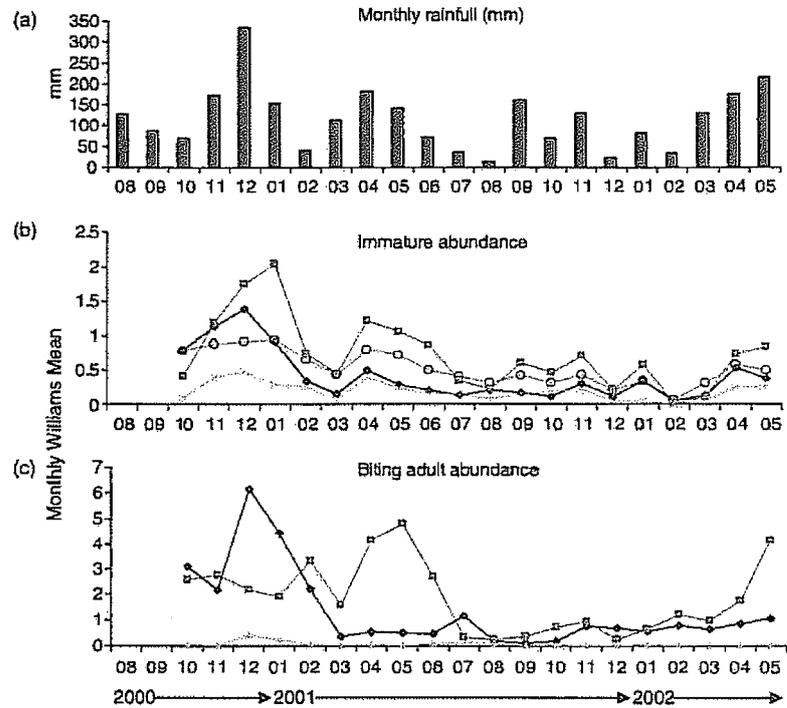
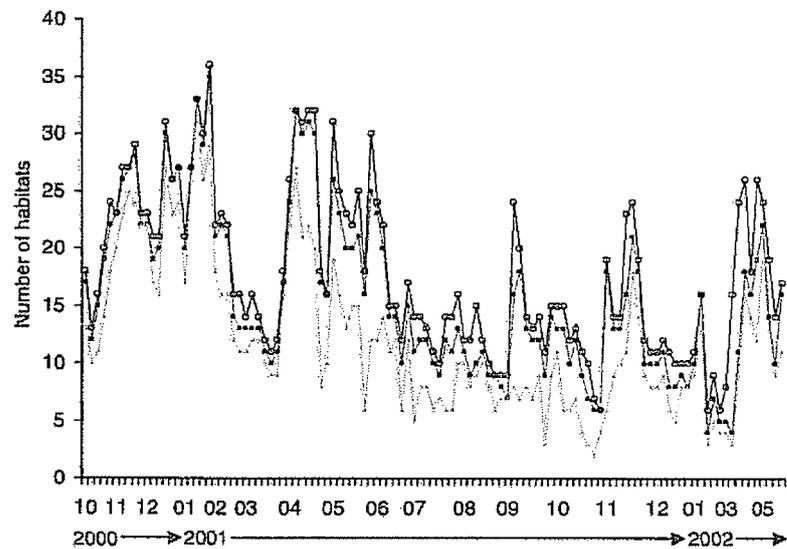


Figure 3 Aquatic habitat availability and colonization by mosquito larvae during study period from October 2000 to May 2002. O, number of available aquatic habitats in study area per sampling date; ◻, number of habitats colonized by any mosquito larvae; ⋆, number of habitats colonized by *Anopheles* larvae.



per person varied between 1.2 and 0.1 with a peak abundance in July 2001, decrease of densities up to October 2001 and a slow and steady increase since November 2001 up to the end of the study (Figure 2c).

Dynamics of relative availability of larval habitats

Over the study period, 53 stagnant water bodies representing potential mosquito larval habitats were identified,

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including the 21 habitats where weekly density measures took place. Most of the habitats were man-made (70%). The man-made habitats had either a natural character containing soil, grass or other vegetation (57%) or were cement-lined pits (43%). The latter (Figure 4) are the most common habitats in the entire location of Mbita town, constituting 60–80% of all larval habitats found in the rainy and dry seasons (U. Fillinger, unpublished data). Most of the possible natural habitats identified were extremely ephemeral, not keeping water long enough to allow a complete life cycle of mosquito development. Only during periods of long and heavy rain, as from October 2000 to January 2001 (Figure 2a), did the water level of the lake increase, resulting in a high water table, water saturated soil and presence of natural puddles and pools for several weeks (Figures 3 and 5).

On average, 89% of all available aquatic habitats on any given sampling date contained mosquito larvae, with an average of 67% being colonized by *Anopheles* per sampling date (Figure 3). There is evidence of a difference in the occupation of possible habitats between the periods from

October 2000 to March 2001 (where the *Anopheles* colonization is closely associated with the occurrence of potential habitats), and the time after March 2001, when *Anopheles* colonization followed the occurrence of habitats to a much lesser extent. For example, between October 2000 and March 2001 an average of 83% of all available habitats were colonized by *Anopheles* larvae compared with an average of 60% after March 2001. The difference between these two time periods was significant ($F_{1,109} = 57.4, P < 0.01$).

Larval identification and distribution

The characteristics of the 21 surveyed mosquito larval habitats are summarized in Table 1. *Anopheles funestus* was not found in those habitats at any time. Species within the *A. gambiae* complex (later referred to as *Anopheles*) were not further identified and we assumed that *A. gambiae* s.s. represented the majority of the



Figure 4 A cement-lined pit – the most common larval habitat in Mbita. This artificial habitat is created any time a modern brick house is built in the area. The cement-lined pit will be filled with water that is then used during construction work, for example, to mix cement.

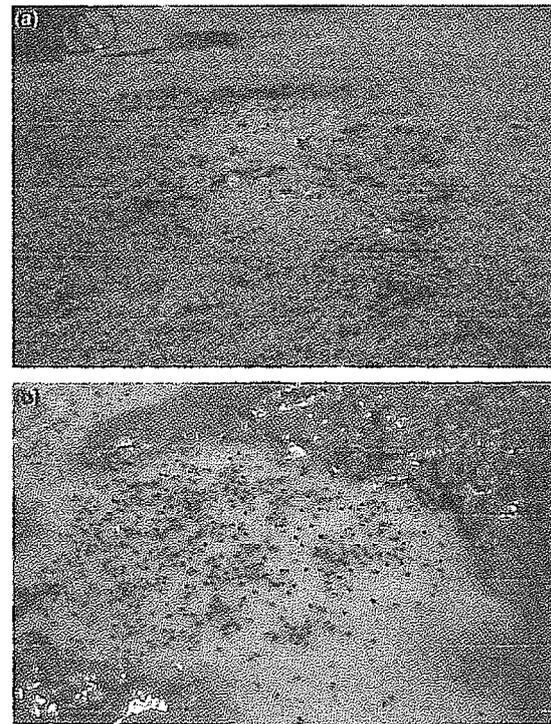


Figure 5 Puddles are natural larval habitats that occur in Mbita only temporary in larger numbers after extended rainfall. (a) Puddle in overview. (b) Puddle close up: high density of *Anopheles* larvae and pupae, freshly emerged adults at the edges.

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specimens (Minakawa *et al.* 1999, 2002). Previous work has shown no differences in *A. gambiae* s.s. and *A. arabiensis* habitat preferences in western Kenya, other than proximity to humans (Minakawa *et al.* 1999, 2002; Gimnig *et al.* 2001, 2002).

Variation in larval densities over space and time was substantial. Considering only sampling dates when habitats contained water, the average number of *Anopheles* larvae per dip varied between 0.16 and 3.57 (average 1.44, SD = 0.98). Culicine mosquitoes occurred in higher densities, with their average density being three times that of *Anopheles* (average 4.55, SD = 4.12). Maximum numbers of *Anopheles* and culicine larvae dipped in a single habitat at a single sampling date were 38 and 52 larvae per dip, respectively.

The mosquito larval productivity varied substantially between the different sites with a total of 23.1 larvae/dip in habitat no. 5 and 1196.9 larvae/dip in habitat no. 16 for the entire study period, respectively. The proportion of *Anopheles* larvae in all available habitats varied between 1 and 88%. The proportion of time that single habitats contained water and were therefore available for oviposition varied from 6 to 99% of the entire study period. *Anopheles* larvae were detected in all habitats, at one time or another, being present at 25–100% of all occasions when habitats contained water. Late larval instars accounted for only 25% of all mosquito larvae dipped, indicating high larval mortality. There was no significant difference between the proportions of late instars of *Anopheles* and culicine larvae ($F_{1,40} = 0.30$, $P = 0.58$) in the studied habitats. This proportion remained constant for *Anopheles* and culicine development even when different micro- and macrohabitat types were compared (proportion of late instars in natural *vs.* cement-lined pit structures: $F_{1,19} = 0.01$, $P = 0.91$, in habitats situated at the lake *vs.* those at the hillside: $F_{1,19} < 0.01$, $P = 0.99$).

All surveyed habitats were shared by *Anopheles* and culicine larvae for at least a certain percentage of the time the habitat was available (co-existence in wet habitats 57% of the time). A few of the cement-lined pit structures were characterized by very high culicine densities and few *Anopheles* larvae (Table 1). In these sites, these species co-occurred less frequently (51% of time) than in natural sites (69% of time, $F_{1,19} = 4.35$, $P = 0.05$).

The area in which habitats were located (e.g. hillside *vs.* lakeside) appeared to be a more important determinant of vector potential than microhabitat variation. For example, comparing lake and hillside habitats, the proportion of *Anopheles* larvae differed significantly (47% and 20%, respectively, $F_{1,19} = 5.53$, $P = 0.03$). Also the average number of *Anopheles* larvae per dip was significantly higher in lakeside habitats (1.8 larvae per dip) than in

hillside habitats (0.9 larvae per dip) ($F_{1,19} = 4.86$, $P = 0.04$). Furthermore, *Anopheles* was found in lakeside habitats more often (present 73% of the time in lakeside habitats *vs.* 48% in hillside habitats, $F_{1,19} = 10.4$, $P < 0.01$). As a consequence of the apparently lower preference of *Anopheles* mosquitoes for hillside habitats, *Anopheles* and culicine larvae co-existed only 47% of the time in contrast to 63% of the time in lakeside habitats ($F_{1,19} = 3.6$, $P = 0.07$).

As all hillside habitats are cemented (Table 1) pits, one could argue that these observations are not spatial effects but dependent on the habitat type. However, a comparison between natural and cemented habitats within the lakeside area showed that the proportion of *Anopheles* larvae recorded is on average 47% and does not significantly differ between the two habitat types ($F_{1,11} = 0.14$, $P = 0.72$). Furthermore, the average number of *Anopheles* larvae per dip for all lakeside habitats was 1.8 and no significant difference could be detected between natural sites and cement-lined pits ($F_{1,11} = 0.3$, $P = 0.6$).

Seasonality of mosquito population dynamics

The bulk of rainfall occurring in Mbita during the study period was concentrated outside of what is traditionally considered 'the long rains' from March to June (Figure 2a). Months with the heaviest total rainfall typically included a handful of days with torrential downpours of >50 mm. The presence of water in the 21 surveyed habitats, as well as the abundance of *Anopheles* larvae, culicine larvae and combined pupae (see Methods) were directly correlated with rainfall in that particular month (Figure 2b and Table 2) and in the case of culicine larvae and adults of *A. gambiae* and culicines, to rainfall the previous month (Table 2). The fact that no constant term was significant or included in any of the models indicates that these habitats are more or less directly fed by rainfall through either direct precipitation, runoff or basin recharge, suggesting more stable water sources such as groundwater do not support many of these habitats through dry periods. The constant in these models represents the mosquito densities expected if no rain fell. Thus, the set of larval habitats surveyed seem largely dependent on direct rainfall. The adult mosquito populations are similarly dependent upon rain suggesting that more stable habitats maintained by the groundwater reservoir through the dry season do not contribute substantially to those populations. Interestingly, the total number of aquatic stage mosquitoes were more closely related to rainfall than the proportion of habitats containing water, suggesting that fresh water, and possibly fresh nutrients picked up by runoff, promote proliferation of both *A. gambiae* s.l. and culicine species. Adult densities

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Dependent variable	d.f.	LL	Independent variables	Parameter estimate	P-value
Aquatic stages					
<i>Anopheles</i> larvae	18	-16.8	Autoregressive	0.781 ± 0.144	<0.0001
			Rainfall that month (mm)	0.0042 ± 0.0015	0.0097
Culicine larvae	17	-20.2	Autoregressive	-0.472 ± 0.212	0.0399
			Rainfall that month (mm)	0.0099 ± 0.0017	<0.0001
			Rainfall previous month (mm)	0.0092 ± 0.0018	0.0001
Pupae	18	2.65	Autoregressive	-0.237 ± 0.232	0.3199
			Rainfall that month (mm)	0.0034 ± 0.0003	<0.0001
Adults					
<i>Anopheles gambiae</i>	17	-40.1	Autoregressive	0.856 ± 0.116	<0.0001
			Rainfall that month (mm)	0.0112 ± 0.0048	0.0316
			Rainfall previous month (mm)	0.0160 ± 0.0048	0.0042
<i>Anopheles funestus</i>	18	-6.4	Autoregressive	0.380 ± 0.230	0.1154
Culicine	17	-61.1	Rainfall that month (mm)	0.0022 ± 0.0007	0.0084
			Autoregressive	0.351 ± 0.243	0.1676
			Rainfall previous month (mm)	0.0337 ± 0.0136	0.0236
			Rainfall previous month (mm)	0.0308 ± 0.0142	0.0440

Table 2 Time series autoregression analysis of the dependence of mosquito abundance on rainfall

Degree of freedom (d.f.), log likelihood (LL) and statistical significance (P-value) are presented.

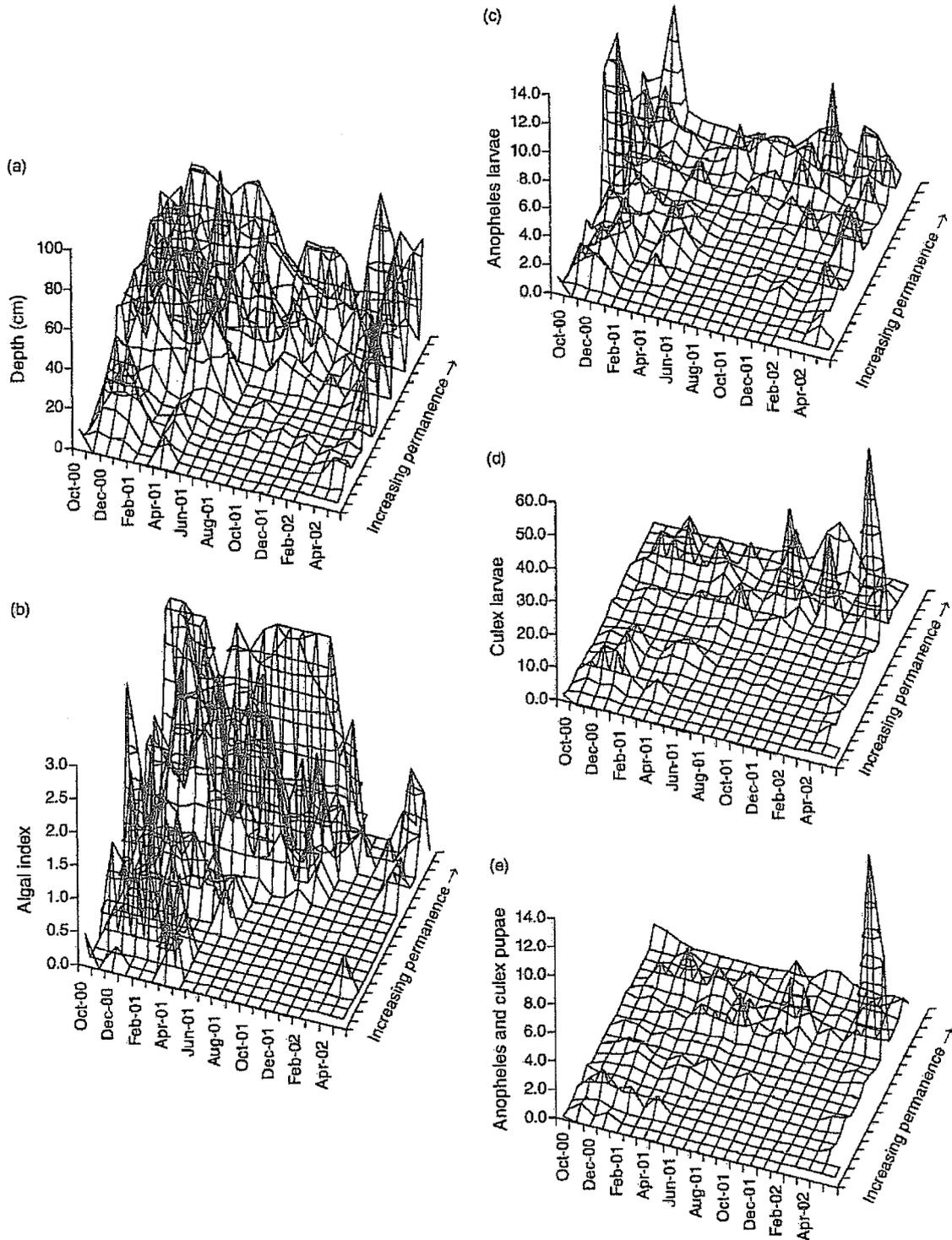
of *A. gambiae s.l.* and culicine species both generally followed a similar pattern but with a clear lag of approximately 1 month (Figure 2c and Table 2). Although not detected in the larval habitats surveyed, *A. funestus* adults appeared in small numbers during and after the heavy rains of late-2000 and mid-2001. Notably, the heavy rains of mid-2001 stimulated proliferation of both *Anopheles* and culicine aquatic stages, resulting in a surge of culicine adults but relatively few *Anopheles* adults (Figure 2).

Figures 2 and 3 reveal that these peaks of heavy rainfall in late-2000 and early 2001 resulted in the majority of the habitats containing both water and aquatic stage mosquitoes. Non-matted algal content was positively associated with habitat stability in the 21 surveyed habitats and highest after long periods of consistently holding water. *Anopheles* larvae were more

evenly distributed across habitats and time than culicines (Figure 6). While *Anopheles* were found at varying densities in habitats of all stability levels, culicines were most abundant and commonplace in semipermanent and temporary habitats. Notably, the three most stable habitats contained *A. gambiae s.l.* larvae throughout the study period, whereas culicine larvae were conspicuously and almost completely absent from these essentially permanent water bodies (Figure 6). When we examined the relationship between the characteristics and productivities of the individual habitats over the duration of the study we found that the overall abundance of pupae was more closely correlated with that of culicine larvae than with *Anopheles* larvae, in terms of both overall productivity throughout the study period and productivity when water was present (Table 3). This suggests that the bulk of pupae collected were in fact culicines,

Figure 6 Permanence, depth, non-matted algal index and productivity of individual habitats over time. The permanence of individual habitats was ranked and plotted according to the proportion of the study period during which they contained water. All productivity values for aquatic stage mosquitoes reflect Williams mean values of monthly mean counts per dip.

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U. Fillinger *et al.* Importance of habitats for controlling *Anopheles* larvae**Table 3** Correlations between abundances of aquatic-stage mosquitoes

	Culicine larvae		Pupae	
	<i>r</i>	<i>P</i> -value	<i>r</i>	<i>P</i> -value
Throughout study period*				
<i>Anopheles</i> larvae	0.005	0.982	0.076	0.736
Culicine larvae	NA	NA	0.869	<0.001
Only when water present				
<i>Anopheles</i> larvae	-0.369	0.091	-0.400	0.065
Culicine larvae	NA	NA	0.808	<0.001

* Samples from periods when the habitat were dry considered to be zero. Correlation coefficients (*r*) and statistical significance (*P*-value) are presented for Spearman's non-parametric rank correlation of Williams mean values of mean counts per dip. Bold values represent *P* < 0.05.

although these were not distinguished from *Anopheles* in the field. A negative association between the abundance of *Anopheles* larvae and those of both culicine larvae and pupae approached significance when the habitats contained water (Table 3), possibly as a result of differential habitat preference as discussed above. However, it might also reflect competition between these species in certain habitats.

Correlation of habitat characteristics and mosquito abundance

Table 4 gives associations between the abundances of aquatic-stage mosquitoes and characteristics of individual habitats. Mean depth over the study period was positively correlated with the abundance of *Anopheles* larvae, pupae and, to a lesser extent, culicine larvae (the latter only approached significance). Similarly, the overall productivity of habitats for culicine larvae was positively associated with mean algal content over the entire period. However, depth and algal content had no association with the productivity of all types of immature mosquitoes when only sampling occasions on which the habitat contained water were considered. This indicates that these correlations are artefacts arising from the covariance between depth or algal content and the presence of water.

In contrast, the correlation of *Anopheles* larval density with the presence of grass and low algal content appeared to be genuine because it was only apparent when counts from periods, when the habitats were wet only, were considered (Table 4). The clear negative association between *Anopheles* larval density and non-matted algal content (Table 4) may help to explain the low densities of *Anopheles* larvae and adults in Mbita during the wet period of mid-2001 (Figures 2 and 6c) because algal

Table 4 Correlations between habitat characteristics and aquatic-stage mosquito abundance

	All samples from habitat		When habitat held water	
	<i>U</i> † or <i>r</i> *	<i>P</i> -value	<i>U</i> † or <i>r</i> *	<i>P</i> -value
<i>Anopheles</i> larvae				
Habitat type†	44	0.581	21	0.026
Depth*	0.452	0.035	-0.209	0.349
Grass*	0.029	0.900	0.427	0.047
Non-matted algae*	-0.043	0.850	-0.549	0.008
Proportion of time containing water*	0.579	0.005	NA	NA
Permanence rank*	0.584	0.004	-0.208	0.352
Culicine larvae				
Habitat type†	51	0.945	42	0.490
Depth*	0.379	0.082	-0.278	0.210
Grass*	-0.046	0.838	0.124	0.582
Non-matted algae*	0.575	0.012	0.117	0.603
Proportion of time containing water*	0.572	0.005	NA	NA
Permanence rank*	0.574	0.005	-0.267	0.230
Pupae				
Habitat type†	47	0.731	42	0.490
Depth*	0.497	0.018	-0.016	0.942
Grass*	-0.030	0.894	-0.037	0.869
Non-matted algae*	0.628	0.002	0.239	0.283
Proportion of time containing water*	0.563	0.006	NA	NA
Permanence rank*	0.565	0.006	-0.090	0.691

Correlation coefficients (*r*) and statistical significance (*P*-value) are presented for Spearman's non-parametric rank correlation* of Williams mean values of mean counts per dip *vs.* the mean values for each habitat characteristic for either the entire study period or only when the habitat contained water. The *U* statistics are presented for Mann-Whitney *U*-test† of Williams mean values of mean counts per dip *vs.* habitat type (number of habitats, *n* = 21). Bold values represent *P* < 0.05.

content was very high in those habitats containing water during this period (Figure 6a,b).

Total productivity for larvae of both *Anopheles* and culicine and for pupae over the full course of the study period was clearly and positively correlated with the proportion of that time during which the habitats contained water. We ranked habitats according to an index of permanence so that we could assess the importance of stability in terms of simply containing water. The right hand side of Table 4 reveals that the permanence of a habitat has no significant influence on its productivity so long as water is present. This confirms that the limiting factor of the abundance of mosquitoes is the availability of water and thus rainfall.

Although both natural and artificial habitats appeared equally productive over the course of the entire study

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period, natural habitats had higher densities of *Anopheles* larvae than artificial habitats when they contained water (1.41 *vs.* 0.58 larvae per dip, respectively; Table 4). As *Anopheles* larval density was also positively correlated with the presence of grass or other low vegetation and negatively correlated with non-matted algal content, and parametric Pearson's correlation analysis yielded essentially identical results to those presented in Table 4, we carried out a parametric partial correlation analysis to determine whether such factors could account for the higher productivity of natural habitats for *Anopheles* larvae. Partial correlation analysis, controlling for the presence of grass and algal index, confirmed that when these factors were taken into account, natural habitats were no more productive than artificial ones when they contained water (correlation coefficient = -0.168, $P = 0.48$). Thus, the seven surveyed natural habitats were more productive when containing water than were the 14 artificial habitats because they more commonly contain grass (90% *vs.* 27% of all sampling occasions, respectively; Mann-Whitney $U = 7.5$, $P < 0.01$) and have lower mean algal indices (0.29 *vs.* 1.10, respectively; Mann-Whitney $U = 15$, $P < 0.01$). Although *E. crassipes* and *Lemna* sp. were also observed in one and three habitats, respectively, and when present seemed to result in lower counts of aquatic stage mosquitoes, they were not common enough to enable meaningful statistical analysis.

Discussion

The generalized and frequently cited view most likely dating back to Muirhead-Thomson's (1951) observations in West Africa that *A. gambiae s.l.* larvae develop in freshwater habitats that are small, temporary, clean and sun-exposed is correct, but our results suggest it might mislead those interested in working on larval control of African malaria vectors. Muirhead-Thomson (1951) himself emphasized, 'unfortunately *gambiae* does not lend itself to generalization'. This is supported by Holstein's (1954) observation that '*A. gambiae* does not follow the textbook in its habitat selection and it can be found in a wide variety of water bodies independent of size'. He furthermore highlighted 'that it is difficult to attribute a definite type of breeding place to *gambiae* and that this anopheline is likely to breed in almost any water that happens to be available; admittedly, it can be said to show a slight preference for small sunlit pools, but it does not follow that these represent the characteristic breeding-place of this mosquito'. Furthermore, work in larger urban settings has indicated that *A. gambiae* can adapt to new habitat types over a relatively short number of years (Chinery 1984). So the flexibility of this species complex

should never be underestimated in operational larval control programmes where high effective coverage are necessitated by high levels of endemicity (MacDonald 1957; Beier *et al.* 1999; Smith *et al.* 2001b; Killeen *et al.* 2002b; Gu *et al.* 2003).

Our study further quantifies the ubiquitous nature of *A. gambiae* breeding habitats showing that semipermanent and permanent habitats can be as suitable for the proliferation of both *Anopheles* and culicines as temporary habitats. For example, of all available habitats, the majority were colonized by mosquito larvae at any sampling date, regardless of permanence rank.

However, small-scale habitat variation in the characteristics of water-containing habitats also influenced *Anopheles* abundance, with their larval density being negatively associated with non-matted algal content and positively with the occurrence of tufts of low vegetation-like grass. The latter observation was described by Muirhead-Thomson (1945) from breeding sites in Sierra Leone. However, the negative association we report between *Anopheles* density and non-matted algal content contradicts the conclusion of Gimnig *et al.* (2002) that algal food plays a key role in *Anopheles* habitats. Since in our study, high non-matted algal content was associated with older habitats, this combination was probably responsible for abiotic (e.g. chemical water quality) and biotic (predatory fauna) conditions that reduced oviposition and/or larval survival. The study of Gimnig *et al.* (2002) specifically investigated artificial habitats of very small-size (35 cm in diameter, 2-3 l of water) and habitat conditions are therefore not comparable with the habitats surveyed in this study. Thus, we conclude that there does not as yet appear to be any straightforward association between larval density and algal productivity and further investigations are needed.

Man-made larval habitats in close vicinity to human habitation are known to play an important role in *Anopheles* proliferation (Holstein 1954; Minakawa *et al.* 2002; Girardin *et al.* 2004), especially in the dry season when supported by human activity. The very common cement-lined pits in Mbita provide excellent conditions for both *Anopheles* and culicine larvae as they keep water for considerably longer time than natural habitats. Furthermore, they are often re-filled with fresh lake water so that the number of available habitats is artificially kept higher than rain alone would support.

While anopheline and culicine larvae co-existed in all investigated habitats, there appears to be some evidence of differential habitat use. *Anopheles gambiae s.l.* was found in habitats more frequently and in higher densities than in hillside habitats, indicating that macrohabitat variations because of complex microhabitat features play an

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important but not yet well understood role in *Anopheles* larval distribution.

Despite some evidence of habitat separation, even habitats with extremely high culicine larval proliferation held consistent numbers of *Anopheles* throughout the year, indicating that larval control of selected *Anopheles* habitats will not be sufficient for malaria control in endemic areas of Africa because very large reductions of transmission are required to achieve useful alleviations of clinical disease burden (Beier *et al.* 1999; Smith *et al.* 2001a; Gu *et al.* 2003).

The long-term nature of our study has revealed that many of the direct associations concluded for *Anopheles* larval distribution (e.g. algal content and mosquitoes, habitat preference) may be the result of selective, cross-sectional sampling and may be more complicated than previously thought. Short-term studies and spot-check observations on mosquito behaviour, larval ecology and larval habitats bear the risk of overlooking important aspects and to generalize conclusions. *Anopheles gambiae*'s ability to use a great variety of larval habitats and to colonize even those considered less favourable must be taken into account in the course of antimalarial campaigns against larval instars. When Watson (1953) describes the larval habitats of *A. gambiae* in the copperbelt of Zambia he concluded for some sites 'larvae were never found there but it was a potential breeding place for this species and was most probably occupied by them at certain times'. As presented here, all monitored habitats did contain *Anopheles* larvae at least once during the study period of 20 months. We conclude that previous, short-term investigations probably greatly underestimated the range and importance of different potential *Anopheles* habitats. We therefore suggest that there is a great need for longitudinal larval ecology studies over extended time periods in preparation of antilarval measures in various African settings.

From our involvement in recent larval control projects in a variety of settings across East Africa, we found that an excessively generalized and dogmatic view of the nature of *A. gambiae* larval habitats has found its way in practical applications. Our work challenges this perception, and highlights the risk that important breeding habitats or those representing dry season refugia may be overlooked in control operations. Recent reports on urban malaria (Chinery 1984; MacIntyre *et al.* 2002; Keating *et al.* 2003) suggest that *A. gambiae* adapted to urban environments by changing their behaviour and colonizing dirty and polluted habitats. It is possible, however, that these habitats have always been capable of supporting larvae, if not to the same frequency as 'cleaner' sites. Indeed, the definition of 'clean', 'dirty' and 'polluted' water itself is unclear when

examining the original literature (Muirhead-Thomson 1945, 1951; Holstein 1954). For example, Muirhead-Thomson (1951) concluded that 'pools and puddles in which *gambiae* breeds become unsuitable very quickly when polluted with cut and rotting vegetation, but animal pollution does not seem to have such an effect'. The latter was often observed during our study, and suggests distinctions between clean and dirty water are not obvious. *Anopheles* larval development has been described previously for essentially all conceivable water bodies with the potential to harbour them, and exclusions have hardly been made (Holstein 1954).

The term 'preferred site' was historically associated with larval densities per site, but several recent studies from southern America (Berti *et al.* 1993; Fernandez-Salas *et al.* 1994; Grillet 2000) support the view that sites with highest larval densities per surface area are not necessarily the most important habitats for vector proliferation over space and time. Larval ecology studies of malaria vectors in Venezuela have shown that a large number of low density, but continuously productive anopheline habitats contribute more than single high-density larval habitats to the density of adults and the risk of malaria (Grillet 2000). Furthermore, Berti *et al.* (1993) concluded for the same study area that one cannot assume that wet season habitats are more favourable than dry season habitats for malaria vector development, but that there is an important interplay among habitat types over space and time. The authors hypothesize that although highest larval densities have been measured in temporary larval habitats, permanent habitats seem to be of more importance for malaria transmission in the area.

The importance of dry season habitats for the perennial transmission of malaria needs to be considered in vector control operations. Dry season habitats are usually well defined and limited in numbers although responsible for continuous production of adult mosquitoes. Antilarval measures are very well suited to targeting these sites, thus reducing the overall mosquito population before the increase of habitat availability during the rainy season (Soper & Wilson 1943; Shousha & Pasha 1948; Fernandez-Salas *et al.* 1994; Grillet 2000). However, endemic malaria transmission in Africa is often viewed as being associated with a very high number of temporary habitats. Consequently, antilarval measures for malaria control are often considered impossible. While this is surely true for some regions in tropical Africa where intensive and extended rainfall leads to vast areas of breeding habitats inaccessible by foot, in areas like Suba District, western Kenya, the high number of temporary habitats occurring only over a very short period of time do not represent a major challenge for larval control interventions as they are

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easily accessible and only partly responsible for the *Anopheles* production in the area.

Conclusion

We conclude that *A. gambiae* s.l. in a setting such as Mbita, a small town in western Kenya, is broadly distributed across a variety of habitat types, regardless of permanence and all potential breeding sites should be considered as sources of malaria risk at any time of the year and exhaustively targeted in any larval control intervention. Man-made habitats that occur in larger numbers in all environments contribute significantly to the transmission of malaria throughout the year. We also conclude that there are various areas in rural Africa with perennial malaria transmission and seasonally larger number of habitats where larval control provides an option to be integrated into malaria control programmes.

Acknowledgements

The authors thank the residents of Mbita for their continuous support and for allowing access to private compounds and houses at any time. We are particularly grateful to Dr Heather Ferguson for her help with the statistical analyses and the valuable comments on the manuscript. The administrative support of ICIPE and the facilitation of the project through Dr John Githure are highly appreciated. Funding for this study was provided by Valent BioSciences Corp., Illinois, USA and GFS, Waldsee, Germany. GK was supported by the Swiss Tropical Institute.

Conflict of interest

This research was funded by Valent BioSciences Corp., USA, a commercial manufacturer of microbial larvicides.

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