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Characteristics of Larval Anopheline (Diptera: Culicidae) Habitats in Western Kenya

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ABSTRACT A longitudinal survey of mosquito larval habitats was carried out in Asembo Bay, western Kenya, during the rainy season of 1998. All pools of standing water along a 700-m transect were sampled twice per week. For each habitat, eight environmental variables were recorded and a sample of anopheline larvae was collected for identification. In total, 1,751 *Anopheles gambiae* s.l. and 2,784 *Anopheles funestus* Giles were identified. Identification of *An. gambiae* s.l. by polymerase chain reaction (PCR) indicated that 240 (14.7%) were *An. gambiae* Giles and 858 (52.4%) were *An. arabiensis* Patton; PCR failed to identify 539 (32.9%) specimens. Repeated measures logistic regression analysis indicated that *An. gambiae* and *An. arabiensis* larvae were associated with small, temporary habitats with algae and little or no aquatic vegetation. *Anopheles funestus* larvae were associated with larger, semipermanent bodies of water containing aquatic vegetation and algae. Direct comparison of habitat characteristics associated with either *An. gambiae* or *An. arabiensis* revealed that algae were associated more commonly with habitats containing *An. gambiae*; no other differences were detected. Chi-square analysis indicated that these species were collected from the same habitat more frequently than would be expected by chance alone. Together, these results indicate that *An. gambiae* and *An. arabiensis* have similar requirements for the larval environment and that, at least in western Kenya, they do not segregate into separate habitats.

KEY WORDS *Anopheles gambiae* complex, *Anopheles funestus*, larval habitats

Anopheles (*Cellia*) *gambiae* Giles, *Anopheles* (*Cellia*) *arabiensis* Patton, and *Anopheles* (*Cellia*) *funestus* Giles are the primary vectors of malaria in sub-Saharan Africa. Despite their importance as vectors, relatively little is known about their larval biology. The few existing reports consist of brief habitat descriptions based on collections made during limited periods. There are two likely reasons for the dearth of larval studies on these vectors. First, malaria control in Africa traditionally has been directed at the adult stages; studies of larval ecology have been thought to be irrelevant by some workers. This narrow view clearly is obsolete: an understanding of population dynamics—which includes an understanding of fluctuations in adult populations—requires a thorough appreciation of factors affecting larval abundance. The second reason for the lack of studies of larval *An. gambiae* is methodological. Until the development of a polymerase chain reaction (PCR)-based diagnostic tool by Scott et al. (1993), no method existed for identifying early instars of this species complex. As a consequence of these philosophical and methodological obstacles, the only systematic studies of the larval stages of *An. gambiae* s.l. are those of Service (1971, 1973, 1977), describing sampling methods and the mortality rates of the larval stages of *An. arabiensis*. However, these studies were conducted in an area of intensive rice cultivation where *An. gambiae* is rare or absent and were not representative of much of sub-Saharan Africa. Systematic studies of the larval stage of *An. funestus* are entirely lacking.

Most observations of the larval habitats of *An. gambiae* s.l. have noted a preference for temporary, sunlit pools (Gillies and DeMeillon 1968, Gillies and Coetzee 1987), whereas *An. arabiensis* appears to exploit permanent, artificial habitats such as rice fields (White et al. 1972, Githeko et al. 1996) and garden wells (Robert et al. 1998). However, consistent differences in habitat use by *An. gambiae* or *An. arabiensis* have not been observed and both species often have been found occupying the same habitat (Service 1970, White and Rosen 1973, Service et al. 1978, Charlwood and Edoh 1996, Minakawa et al. 1999). Observations of adult populations of *An. gambiae* and *An. arabiensis* indicate a spatial or temporal separation of these species. In Mali, Touré et al. (1998) found that *An. gambiae* (Savannah taxon) predominated in humid areas with larval production occurring almost exclusively during the rainy periods, whereas *An. arabiensis* prevailed in arid areas and likely reproduced throughout the year. In Tanzania, *An. arabiensis* was common during the short rains and just before the long rains, whereas *An. gambiae* predominated during and just after the long rains

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(White et al. 1972). In Nigeria, White and Rosen (1973) found that *An. gambiae* is common during the long rains, with populations of *An. arabiensis* increasing as the rains receded. Whether these observations reflect differences in larval habitats, the rates of recruitment of adults, or mechanisms of dry season survival remains unknown. *Anopheles funestus* larvae are associated with permanent bodies of water with emergent vegetation (Gillies and DeMeillon 1968) and adult populations generally peak after those of *An. gambiae* s.l.

Larval habitats are important determinants of adult distribution and abundance. Although the transient habitats of *An. gambiae* may not be a reasonable target for vector control, an understanding of the dynamics and productivity of larval habitats is required if efforts to model and predict adult abundance are to succeed. In the current study, we used the technique of Scott et al. (1993) to define the larval habitats of *An. gambiae* and *An. arabiensis* in the context of other African anophelines, particularly *An. funestus.* On a finer scale, we determined whether *An. gambiae* and *An. arabiensis* segregate spatially or temporally among habitats.

Materials and Methods

The study was conducted in Asembo Bay, western Kenya, where intensive studies related to malaria transmission and control have been ongoing for >10 yr. This region consists of gently rolling hills and is drained by several permanent or semipermanent streams. Since January 1997, the Asembo area had been the site of a large-scale trial of permethrin-impregnated bed nets. Our collections were made in a part of Asembo Bay surrounded by 'control villages' that had not yet received bed net.

Collections were conducted from 7 March to 3 September of 1998, a period covered by one of two annual seasonal rainy periods that begin in late March or early April and end in late May or early June. Sampling began during the final part of a dry period when temporary larval habitats were difficult to find, continued throughout the rains, and was stopped after the rains ceased and most temporary larval habitats had dried. Collections were made twice per week along a 700-m transect that followed a semipermanent stream. The total sampling area was 0.2 km^2.

All pools of standing water within the study area were examined for larvae. Most larvae were collected with a standard 350-ml dipper. In small habitats, dippers were not effective and larvae were collected individually using plastic pipettes. Length, width, depth, and temperature were measured for each habitat. If the habitat was >3 m in length or width, these parameters were recorded as >3 m. Surface area was estimated from the length and width of the habitat. The presence/absence of aquatic vegetation, surface film, and mats of floating algae was noted. Algae were not classified further, but most mats were composed of filamentous algae. Surface films were usually a scum on the surface of the water that was likely caused by an active microbial layer. Often, organic debris was floating on the surface. A maximum of 20 anopheline larvae (five per instar) plus all pupae were placed in small tubes with water and transported to the laboratory. The remaining anopheline larvae and all culicine larvae were counted and returned to the habitat. Water samples (≈25 ml) were collected and transported to the laboratory where they were stored in the dark at 4°C.

In the laboratory, larvae from each habitat were placed in plastic cups and given a small amount of food. Third and fourth instars were identified immediately; first and second instars were allowed to develop before they were identified. Identifications were performed with a compound microscope by placing individual larvae in a depression slide with a small drop of water illuminated from above and below. Identification was done using the keys of Gillies and Coetzee (1987). All *An. gambiae* s.l. larvae were placed into individual 1.5-ml centrifuge tubes, dried over anhydrous calcium sulfate and stored at room temperature until identification by PCR using the methods of Scott et al. (1993). The pH of water samples was measured using an Orion pH/conductivity meter. Turbidity was measured using a Hach 2100A turbidity meter.

The association between the presence/absence of *An. gambiae*, *An. arabiensis*, and *An. funestus* and eight environmental parameters was tested by a repeated measures logistic regression. Each test was done using the GENMOD procedure in SAS assuming an autocorrelative structure. The eight environmental factors were surface area, temperature, pH, turbidity, number of days before the habitat dried out, the presence of aquatic vegetation, the presence of mats of algae, and the presence of a film on the water surface. For analysis, surface area was grouped into categories of <0.5 m^2, 0.5–2.5 m^2, 2.5–5 m^2, or >5 m^2, and turbidity was grouped into categories of <100 FTU, 100–200 FTU, 200–300 FTU, and >300 FTU. Factors initially were screened by univariate analysis. Those that were significant at $\alpha = 0.05$ were included in the multivariate analysis. Factors that were not statistically significant were removed from the model.

Additional statistical analyses tested the hypothesis that habitats with *An. gambiae*, *An. arabiensis*, or both species differed in the eight environmental parameters examined. Differences in continuous variables (temperature, pH, and the number of days before a habitat dried out) were tested by Wilcoxon rank-sum test. Differences in binary or categorical variables were examined using chi-square test. Habitats with neither species were excluded from these analyses. The degree of association between species based on the presence or absence of each species was tested by chi-square analysis.

Results

A total of 1,671 collections was made from 166 distinct habitats. The number of habitats sampled each day ranged from a high of 66 on 25 May to a low of 13 on 31 August and 3 September (Fig. 1). Habitat avail-

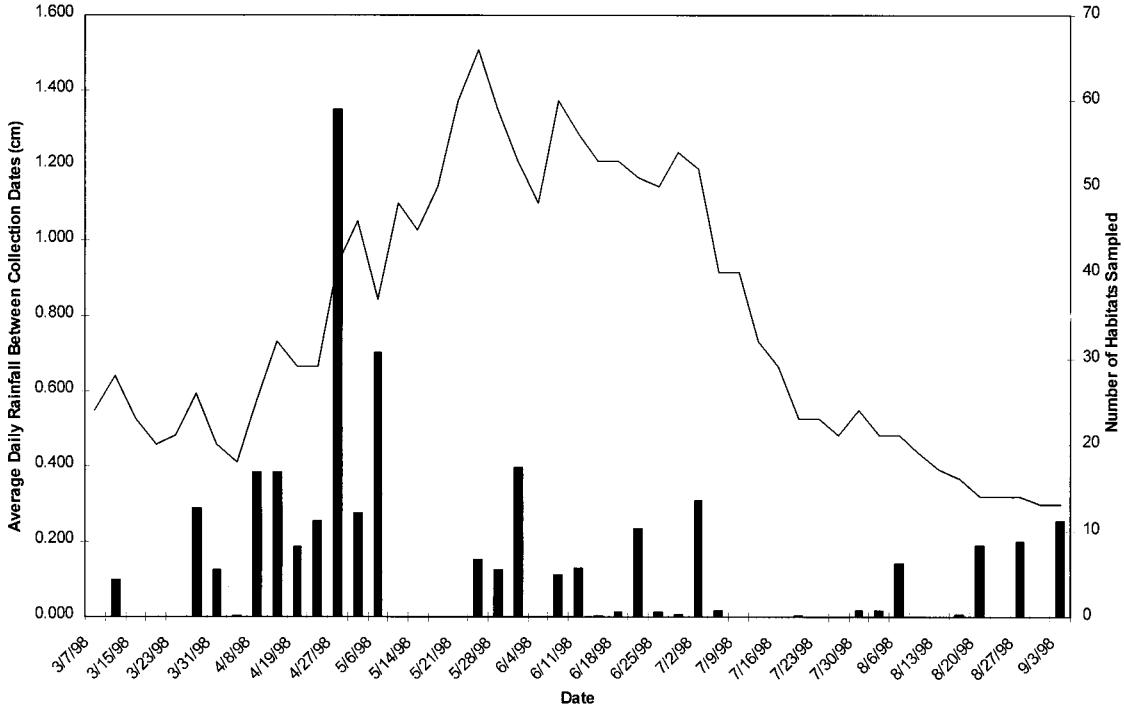

Fig. 1. Number of discrete habitats sampled on each date (line) and the average daily rainfall between sampling dates (bars).

ability corresponded to rainfall, although heavy rainfall flushed out some habitats. Habitat types included small sunlit pools, shallow grassy marshes, slow moving edges of the stream, large ponds, and artificial habitats such as bore holes and catch basins. No small container habitats were found.

A total of 8,588 anophelines was collected and identified morphologically. Of 1,671 collections, anophelines were present in 1,068 (63.9%). Of 166 habitats sampled, 135 (81.3%) were positive for anopheline larvae on at least one collection date. Table 1 shows numbers of each anopheline species collected. Our collections were designed to sample all available anopheline habitats in this area; nonetheless,

Table 1. Number of mosquitoes collected

Species	No. collected
Anopheles (Cellia) funestus Giles	2,784
Anopheles (Cellia) gibbinsi Evans	2,105
Ahopheles (Cellia) gambiae s.l.	1,751
Anopheles (Anopheles) coustani Laveran	779
Anopheles (Cellia) maculipalpis Giles	590
Anopheles (Cellia) rufipes (Gough)	276
Anopheles (Cellia) pharoensis/squamosus Theobald	158
Anopheles (Cellia) rivulorum Leeson	100
Anopheles (Cellia) pretoriensis (Theobald)	45
Culicines[a]	14,660

[a] Culicine mosquitoes were not identified routinely but included *Culex* (*Culex*) *quinquifaciatus* Say, *Culex* (*Lutzia*) *tigripes* Grandpre, *Mimomyia* (*Mimomyia*) *splendans* Theobald and two unidentified *Aedes* species.

two of the three most commonly collected species were malaria vectors, with *An. funestus* and *An. gambiae* s.l. being the first and third most common mosquitoes identified. Of the 1,751 *An. gambiae* s.l. collected, we attempted to identify 1,637 specimens to species by PCR. Of these, 240 (14.7%) were *An. gambiae* and 858 (52.4%) were *An. arabiensis.* PCR amplification failed for 539 (32.9%) specimens, probably due to problems in specimen processing.

The temporal distribution of numbers of *An. gambiae*, *An. arabiensis*, and *An. funestus* in our collections is shown in Fig 2. All three species were uncommon before the onset of the rains. As the rains began, the abundance of *An. gambiae* and *An. arabiensis* increased rapidly, with both species peaking on 25 May. In general, the temporal pattern of larval abundance was similar for these sibling species. However, the proportion of *An. gambiae* collected was lower before and after the rains. In contrast, *An. funestus* was collected infrequently before the onset of the rains, increased to a peak on 15 June then decreased in frequency until the end of the study.

Habitat characteristics of *An. gambiae* and *An. arabiensis* are summarized in Table 2. *Anopheles gambiae* was collected alone 41 times, whereas *An. arabiensis* was collected alone 193 times. The two species were collected from the same habitat 97 times. Both species were collected primarily from small, shallow habitats that persisted for 4–5 wk and lacked aquatic vegetation. Habitat characteristics of *An. gambiae* and *An.*

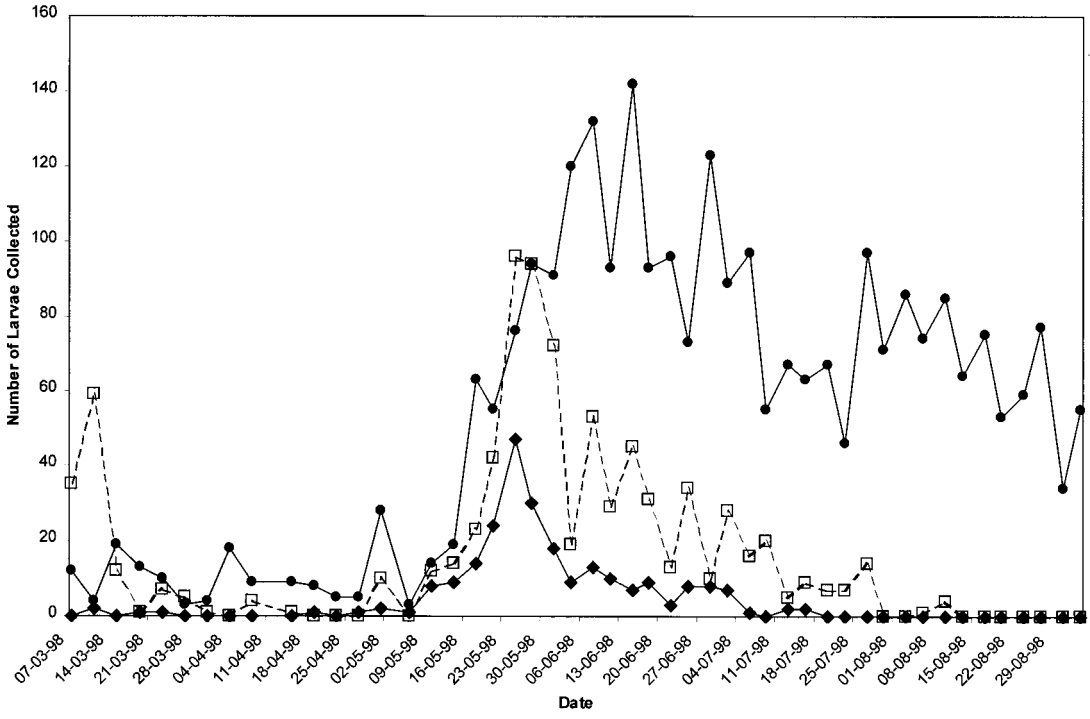

Fig. 2. Total number of *An. gambiae* (diamonds), *An. arabiensis* (open squares) and *An. funestus* (circles) identified during each sampling date.

arabiensis were similar, although *An. gambiae* habitats tended to be less persistent and more likely to have algae than *An. arabiensis* habitats. Habitat characteristics of *An. funestus* are summarized in Table 3 and were larger, deeper, cooler, more persistent, and less turbid than those of *An. gambiae* and *An. arabiensis. Anopheles funestus* habitats almost always had some form of aquatic vegetation.

The final models from the logistic regression analyses are given in Table 4. Both *An. gambiae* and *An. arabiensis* were associated positively with the presence of algae and increasing turbidity and negatively with the presence of aquatic vegetation, surface film and habitat persistence. In addition, *Anopheles arabiensis* was associated negatively with habitat surface area and positively associated with a higher pH. For both species, the odd ratios for persistence were statistically significant but very close to 1, indicating a weak relationship. *Anopheles funestus* was associated positively with habitat persistence, the presence of aquatic vegetation, large habitats, and the presence of algae. It was associated negatively with the presence of a surface film. As with *An. gambiae* and *An. arabiensis*, the odds ratio for habitat persistence was very close to 1, indicating a weak relationship.

Comparisons of habitat characteristics with *An. gambiae* only, *An. arabiensis* only, or both species were done by Wilcoxon rank-sum test for continuous variables and by chi-square analysis for categorical variables. Habitats with only *An. gambiae* differed from habitats with only *An. arabiensis* in the proportion of habitats with algae present; habitats with *An. gambiae* were more likely to have algal growth. Habitats with both species differed from habitats with either species

Table 2. Characteristics (±95% confidence intervals) of habitats with *An. gambiae* only, *An. arabiensis* only, both species or neither species

	An. gambiae only	*An. arabiensis* only	Both	Neither
No. of collections	41	193	97	1,340
Surface area (m^2)	3.68 ± 1.05	2.32 ± 0.33	1.79 ± 0.49	4.74 ± 0.16
Depth (cm)	29.4 ± 10.7	18.0 ± 3.5	9.7 ± 4.1	22.9 ± 1.3
Temp, °C	25.6 ± 0.81	24.9 ± 0.42	26.4 ± 0.7	24.5 ± 0.1
pH	7.19 ± 0.14	7.24 ± 0.06	7.19 ± 0.11	7.05 ± 0.02
Turbidity (FTU)	99.5 ± 33.6	94.3 ± 22.0	204.3 ± 45.6	58.0 ± 5.3
Persistence (days)	23.0 ± 6.7	38.2 ± 5.0	28.6 ± 6.7	68.9 ± 2,4
Habitats with aquatic vegetation (%)	56.1	47.2	26.8	80.0
Habitats with algae (%)	39.0	18.7	35.1	16.1
Habitats with surface film (%)	26.8	24.4	12.4	45.3

Table 3. Characteristics (±95% confidence intervals) of habitats with *An. funestus* present or absent

Parameter	*An. funestus* present	*An. funestus* absent
Number of times collected	442	1,229
Surface area (m^2)	5.55 ± 0.24	3.80 ± 0.22
Depth (cm)	33.3 ± 2.76	17.4 ± 1.50
Temp, °C	24.4 ± 0.27	24.8 ± 0.19
pH	7.03 ± 0.04	7.10 ± 0.03
Turbidity (FTU)	56.9 ± 14.4	77.0 ± 7.52
Persistence (days)	97.9 ± 4.61	55.4 ± 2.87
Habitats with aquatic vegetation (%)	89.1	66.6
Habitats with algae (%)	26.9	14.9
Habitats with surface film (%)	30.5	44.1

in temperature (warmer in habitats with both species), the presence of aquatic vegetation (less common in habitats with both species), and the presence of a surface film (less common in habitats with both species). Additionally, habitats with both species were more turbid and more likely to have algal growth than habitats with only *An. arabiensis* and they were smaller than habitats with only *An. gambiae.*

Anopheles gambiae and *An. arabiensis* were collected together 97 times. *Anopheles gambiae* was collected alone 41 times, whereas *An. arabiensis* was collected alone 193 times. Chi-square analysis indicated that *An. gambiae* and *An. arabiensis* were more likely to be present in the same habitat than would be expected by chance alone ($P < 0.001$). These two species were less likely to be collected with *An. funestus* than would be expected by chance alone ($P < 0.001$ for both comparisons).

Table 4. Repeated measures logistic regression models for *An. gambiae*, *An. arabiensis* and *An. funestus*

Parameter	Odds ratio	Lower CI	Upper CI	*P*
Anopheles gambiae s.s.				
Turbidity	1.86	1.53	2.27	<0.001
Habitat persistence	0.98	0.98	1.00	0.008
Presence of aquatic vegetation	0.38	0.22	0.67	0.001
Presence of algae	3.11	1.82	5.34	<0.001
Presence of surface film	0.54	0.32	0.93	0.026
Anopheles arabiensis				
Turbidity	1.40	1.14	1.72	0.001
Habitat persistence	0.99	0.99	1.00	0.040
Surface area	0.74	0.59	0.93	0.009
pH	1.67	1.16	2.40	0.006
Presence of aquatic vegetation	0.45	0.27	0.76	0.003
Presence of algae	1.67	1.05	2.64	0.029
Presence of surface film	0.60	0.39	0.93	0.021
Anopheles funestus				
Habitat persistence	1.01	1.00	1.01	0.001
Surface area	1.52	1.16	2.00	0.024
Presence of aquatic vegetation	1.79	0.99	3.27	0.056
Presence of algae	1.98	1.34	2.93	0.001
Presence of surface film	0.40	0.29	0.55	<0.001

An odds ratio greater than 1 indicates a positive association whereas an odds ratio less than 1 indicates a negative association.

Discussion

The larval habitats of *An. gambiae* and *An. arabiensis* were remarkably similar in our study area. Both *An. gambiae* and *An. arabiensis* were associated with habitats that were high in turbidity, persisted for short periods (3–5 wk), and were lacking in aquatic vegetation or surface film. Nearly one-third of all habitats that were positive for *An. gambiae* s.l. contained specimens of both species. Previous studies also have shown that these species may occur within the same habitat (Service 1970, White and Rosen 1973, Service et al. 1978, Charlwood and Edoh 1996, Minakawa et al. 1999). In the current study, chi-square analysis indicated that these species were more likely to be collected together than would be expected by chance alone and statistical analysis of habitat characteristics indicated only one difference between the habitats of *An. gambiae* and *An. arabiensis.* Charlwood and Edoh (1996) and Minakawa et al. (1999) also found few differences in the habitats of *An. gambiae* and *An. arabiensis.* In those studies, it was suggested that the distribution of each species in larval habitats might be related to the proximity of the preferred hosts of each species rather than to inherent differences in the larval environments; *An. gambiae* predominated in habitats near human dwellings, whereas *An. arabiensis* predominated in habitats near cattle. However, in our study area of western Kenya, cattle are kept very close to houses and, therefore, the host preference of these species is unlikely to have affected the larval distribution of these species.

Interestingly, several differences in habitat characteristics were observed between habitats with both species compared with habitats with only one of these species. Habitats with both species were warmer and less likely to have a surface film or aquatic vegetation than habitats with only *An. gambiae* or only *An. arabiensis.* Habitats with both species also were more turbid and more likely to have algae than habitats with only *An. gambiae* or *An. arabiensis*, although the differences between habitats with both species and *An. gambiae* alone were not statistically significant. With the exception of temperature, each of these variables was associated with the presence of *An. gambiae* and *An. arabiensis.* These observations indicated that habitats with characteristics that were associated strongly with either *An. gambiae* or *An. arabiensis* were more likely to be selected by both species.

The habitat characteristics of larval mosquitoes may be determined by the oviposition behavior of gravid females. However, few studies have been conducted on oviposition behavior. McCrae (1984) showed that *An. gambiae* females preferred to oviposit on turbid water rather than on clear water. Several factors contribute to turbidity including insoluble particles of soil, organics, microorganisms, and other materials (Hammer 1986). Which of these components, if any, are

attractive to *An. gambiae* and *An. arabiensis* remains to be determined. The microbial fauna of larval habitats likely release volatiles that may be used as oviposition cues or deterrents. It is not clear how other factors, such as the presence of vegetation or the persistence of the habitat, might influence the behavior of ovipositing females. It is unlikely that habitats are selected on the basis of these factors; rather, these factors may be correlated with other characteristics that act as cues for ovipositing females.

The temporal pattern of *An. gambiae* and *An. arabiensis* was similar with both species peaking on the same day. However, the proportion of *An. gambiae* was much greater during and immediately after the rains compared with more arid periods before and after the rains. Similar patterns have been observed in adult populations (White et al. 1972, White and Rosen 1973). These patterns may be due to enhanced survival of adult *An. arabiensis* under more arid conditions. However, little is known about the dry season survival mechanisms of these species. Beier et al. (1990) demonstrated that *An. gambiae* s.l. eggs could be found in dry soil and that eggs obtained from field-collected mosquitoes remained viable for up to 12 d. Alternatively, Omer and Cloudsley-Thompson (1970) demonstrated that *An. arabiensis* in the Sudan was capable of surviving extended dry periods through gonotrophic dissociation. Whether these are important dry season survival mechanisms for either species in western Kenya is unclear. However, in our small study area, larvae of both species were collected throughout most of the year, indicating that these species reproduce year round in this region.

Our study systematically documented the larval habitats of *Anopheles funestus.* This species was associated with large permanent bodies of water with aquatic vegetation. More than 85% of all habitats with *An. funestus* had some form of aquatic vegetation and in habitats with partial coverage of aquatic vegetation, *An. funestus* was always collected from thick stands of aquatic vegetation, never from areas of open water. *Anopheles funestus* exhibited a preference for clean fresh water, and this mosquito was rarely found in habitats with a film on the surface. As with *An. gambiae* and *An. arabiensis,* oviposition cues for gravid females are not known but are likely correlated to factors shown to be associated with the presence of larval *An. funestus.*

Studies of the ecology of larval anopheline mosquitoes are methodologically difficult; the current study had several limitations. First, consistent repeatable sampling of larval habitats to obtain estimates of abundance is difficult if not impossible with the methods available. Quantitative sampling was especially problematic in the current study because dipping was the method used initially but was unsuitable in small habitats. For this reason, we elected to use presence/absence as an outcome rather than mosquito abundance. Second, although the longitudinal design had the advantage of being able to detect temporal changes in larval habitats, it limited the size of the study area and, therefore, made it more difficult to generalize the results. However, our study generated results similar to Charlwood and Edoh (1996) and Minakawa et al. (1999) who employed cross-sectional designs covering large areas in Tanzania and western Kenya indicating that the results of our study may be applicable to much of east Africa. Finally, the failure rate of PCR identification of larvae was high in our study. Although this may have potentially biased some of the results, the proportion of *An. gambiae* was similar to that observed by Minakawa et al. (1999), indicating that failure rates were similar for *An. gambiae* and *An. arabiensis.*

The current study described the general habitat characteristics of the larval stages of *An. gambiae, An. arabiensis* and *An. funestus* in western Kenya. Although the general characteristics of the larval habitats are known, it is not understood well how individual habitats are selected or how these habitats contribute to adult populations. Further studies are required to better understand how ovipositing females select habitats and what factors influence the production of adults from those habitats.

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