PUPAL HABITAT PRODUCTIVITY OF ANOPHELES GAMBIAE COMPLEX MOSQUITOES IN A RURAL VILLAGE IN WESTERN KENYA

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Abstract. The productivity of larval habitats of the malaria vector Anopheles gambiae for pupae (the stage preceding adult metamorphosis) is poorly known, yet adult emergence from habitats is the primary determinant of vector density. To assess it, we used absolute sampling methods in four studies involving daily sampling for 25 days in 6 habitat types in a village in western Kenya. Anopheles gambiae s.s. comprised 82.5% of emergent adults and Anopheles arabiensis the remainder. Pupal production occurred from a subset of habitats, primarily soil burrow pits, and was discontinuous in time, even when larvae occupied all habitats continuously. Habitat stability was positively associated with pupal productivity. In a dry season, pupal productivity was distributed between burrow pits and pools in streambeds. Overall, these data support the notion that source reduction measures against recognizable productive habitats would be a useful component of an integrated management program for An. gambiae in villages.

INTRODUCTION

Larvae and pupae of Africa’s primary malaria vector, Anopheles gambiae, are usually found in small, temporary, sunlit, and turbid pools created by human or animal activity. The transient nature of these numerous habitats, coupled with the rapid development rate of larvae, makes difficult the process of sampling of larvae and pupae and interpretation of the meaning of sampling data. The consequent lack of quantitative information on habitat productivity has allowed to remain largely unchallenged the assumption that all habitats receiving eggs and occupied by larvae are productive for adults, an assumption that has been thoroughly examined and placed in epidemiologic context for population dynamics of Aedes aegypti and dengue virus transmission. Simulation modeling suggests that source reduction in the sense of habitat elimination would lower transmission.

Several lines of evidence are needed to assess the feasibility of larval control or source reduction for An. gambiae: 1) assessment of the abundance of different types of habitats, 2) measurement of the productivity of each habitat type, and 3) knowledge of the way in which habitats of different types are formed, and the social utility or lack thereof of each habitat type. The first two measures are quantitative sampling problems and are addressed in the current paper. The last question is primarily sociological and is addressed separately. The objective of the current study was to estimate habitat-specific pupal productivity of An. gambiae s.l. in a western Kenya village. From such empirical observations, it might be possible to determine the extent to which habitat productivity for An. gambiae can be predicted generally in a typical village. The feasibility of a source reduction program could logically be assessed based on such knowledge.

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Study site. The study was conducted in Kisian, a rural village located 15 km west of Kisumu town, western Kenya.

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Length, depth, and width measurements of each habitat were recorded daily. A record of whether the habitat was wet or dry at the time of the visit was also taken. In the laboratory, the pupae were held in paper cups to allow for emergence and were identified to species morphologically. Emerged *Anopheles gambiae* s.l. adults were killed by freezing, desiccated over anhydrous calcium sulfate, and stored at room temperature. A sample of emerged *An. gambiae* s.l. adults was identified to species using polymerase chain reaction (PCR).  

A third sampling series, here termed Study 3, involved 25 habitats sampled for 25 consecutive days during April–May, 2003, a wet period. In this study, larvae and pupae were collected from area samplers per habitat per day, regardless of habitat size. There was no separate habitat census for pupae. A fourth sampling series (Study 4) was identical to the third but was conducted in June 2003, after the rainfall had decreased from nearly daily to intermittent in frequency.  

**Data analysis.** Larval and pupal data were expressed both in terms of abundance per habitat and density per unit area of habitat or unit area of the sampling device. Pupal productivity per unit area is a measure of habitat efficiency to produce pupae, and the number of pupae produced per habitat estimates the production of adults from the aggregate of habitats in the village. For Studies 1 and 2, total productivity of habitats, as measured by the total larvae or pupae in samples, was compared among habitat types by repeated measures Poisson regression using SAS version 8.01. **Variables included in each model were habitat type, average habitat size during the sampling round, stability, and distance to the nearest house.** Habitat size, stability, and distance to the nearest house were categorized as dichotomous variables for analysis. The cutoffs for each variable were selected to maximize the number of habitats within each category and varied among the studies. Habitats were classified as large if their areas were greater than 5 m² for both studies. For stability, habitats were classified as stable if they were flooded for at least 18 days in Study 1 and 17 days for Study 2. For distance to the nearest house, habitats were classified as near if they were within 50 m of a human dwelling and far if they were greater than 50 m from a human dwelling. All pupal models as well as the model for larval abundance in Study 1 used an autoregressive correlation structure. The remaining model of larval abundance in Study 2 used an exchangeable correlation structure. The distribution was assumed to follow the Poisson. Linear regression of numbers of larvae or pupae per unit area on certain independent variables, including stability and distance treated as continuous (versus dichotomous as in Poisson regression above) was also done, with data transformed with log_{10} (x + 1). For the studies conducted in April–May and June 2003, larval and pupal densities in area samplers were summed over all 25 days and compared among habitat types using the Kruskal-Wallis nonparametric rank test. The nonparametric Spearman’s correlation coefficient was calculated to determine the extent to which larval and pupal densities were correlated.

**RESULTS**

**Habitat productivity, Studies 1 and 2.** Table 1 summarizes sampling effort and larval and pupal returns for Study 1 and Study 2. During Study 1, a total of 841 visits were made to 34 habitats during the 25-day period of the study, and a total of 4,603 *An. gambiae* s.l. larvae and 932 pupae was sampled. In Study 2, 450 visits were made to 18 habitats with 3,396 larvae and 309 pupae sampled (Table 1). Culicine larvae and pupae were frequently encountered but were not retained. Of the 1,241 anopheline pupae that were collected and allowed to emerge into adults, two were *An. coustani* Laveran, five were *An. funestus* Giles, and the rest were *An. gambiae* s.l. The sex ratio of *An. gambiae* s.l. based on adult emergences was 1.3 females per male, a statistically significant departure from a 1:1 ratio (χ² = 21.3, df = 1, P < 0.0001). Results of PCR for 286 emergent *An. gambiae* s.l. adults showed that 82.5% were *An. gambiae* s.s. and the remainder were *An. arabiensis*. The frequency distributions of total anopheline larva in area samples, and total pupae of *An. gambiae* s.l. censused from all habitats sampled for all 25 days in Study 1 and Study 2, are shown in Figure 1. Summary statistics and results of Poisson regression are shown for larvae in Table 2 and for pupae in Table 3.

Larvae were distributed at a range of densities across all habitat types and were typically present in habitats when water was present (Figure 1; Tables 1 and 2). Pupae, by contrast, were more variable in their densities and were commonly absent from many habitats despite intensive whole habitat
Mean daily larval production per area sample and pupal production per square meter in all the habitat types for Studies 1 and 2 are shown in Figure 3. These data show that habitats typically produced pupae only a few days within the 25-day sampling period, if they produced pupae at all. For example, burrow pits in Study 1 produced most of the pupae over a continuous 3-day period within the 25 consecutive day interval.

Habitat stability was defined as the number of days a habitat contained water during the 25-day sampling period. The mean number of days the habitats were found with water was 16.5 days (95% CI = 14.2 to 18.7) and 16.1 days (95% CI = 12.9 to 19.2) for Studies 1 and 2, respectively. In Poisson regression analysis, where stability was treated as a dichotomous variable, stable habitats had significantly more larvae/area sample, more pupae/habitat, and more pupae/m² than did unstable habitats during Study 1 (Tables 2 and 3). Linear regression of log₁₀(pupae + 1)/habitat census on log₁₀(larvae + 1)/area sample revealed a weak but positive relationship in Study 1 (R² = 0.38, r = 0.61, df = 32, P < 0.01) and a somewhat stronger positive relationship in Study 2 (R² = 0.56, r = 0.75, df = 16, P < 0.01) (Figure 2).

The size of the sampled habitats varied with study, habitat type, and visit. Mean habitat size in Study 1 was 4.2 m² (95% CI = 2.8 to 5.5) and the largest size recorded was 36 m² while in Study 2 it was 7.5 m² (95% CI = 4.3 to 10.8) and 33.8 m², respectively. Habitat area was very dynamic (data not shown) because on some days habitats were dry and on other days they were flooded and therefore relatively large. Poisson regression analysis showed that large habitats significantly had

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pupae/habitat</th>
<th>Pupae/m²</th>
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</thead>
<tbody>
<tr>
<td>Habitat type</td>
<td>Pupae/habitat</td>
<td>Pupae/m²</td>
</tr>
<tr>
<td>Burrow pits</td>
<td>31.00 ± 27.60</td>
<td>11.29 ± 13.81</td>
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<tr>
<td>Drainage channels</td>
<td>6.14 ± 3.71</td>
<td>0.73 ± 0.61</td>
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<tr>
<td>Hoof prints</td>
<td>2.04 ± 1.93</td>
<td>–</td>
</tr>
<tr>
<td>Rain pools</td>
<td>2.92 ± 3.15</td>
<td>–</td>
</tr>
<tr>
<td>Tire tracks</td>
<td>16.00 ± 14.61</td>
<td>9.22 ± 17.29</td>
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<tr>
<td>Stability</td>
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<tr>
<td>Small</td>
<td>14.33 ± 8.36</td>
<td>11.07 ± 11.00</td>
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<tr>
<td>Large</td>
<td>20.00 ± 19.26</td>
<td>2.15 ± 2.02</td>
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<td>Distance to nearest house</td>
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<tr>
<td>Near</td>
<td>15.86 ± 12.33</td>
<td>4.30 ± 4.27</td>
</tr>
<tr>
<td>Distant</td>
<td>18.00 ± 15.07</td>
<td>8.08 ± 9.17</td>
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</tbody>
</table>

* For each variable, the average number of larvae per area sampler over 25 days (area = 78.5 cm²) ± 95% confidence intervals is given. With columns for the same variable, different letters indicate statistically significant differences in Poisson regression analysis (P < 0.05).
more larvae than small habitats in Study 1, whereas in Study 2 small habitats had significantly more larvae (Table 2). In both studies, large habitats had statistically more pupae per habitat but less pupae per square meter than small habitats (Table 3).

The average distance between each habitat and the nearest house was 49.4 m (95% CI = 38.0 to 60.9, range 0 to 167 m) for Study 1 and 85.3 m (95% CI = 56.6 to 114.1, range 11 to 189 m) for Study 2. Poisson regression analysis for larval production in Study 1 indicated that habitats nearer to houses had more larvae and more pupae than distant habitats although the differences were not statistically significant. In Study 2, distant habitats were significantly more productive than habitats close to houses (Tables 2 and 3).

Habitat productivity, Studies 3 and 4. In Studies 3 and 4, larvae and pupae were sampled by area sampler only. During Study 3, a total of 15,259 larvae and 245 pupae were sampled. During Study 4, a total of 2,377 larvae and 18 pupae were sampled. Results are summarized as the mean total number of larvae and pupae collected per 25-day interval per habitat type (Figure 5). Larvae were far more abundant than pupae in area samplers, yet pupae were recovered by the area sampling method. In Study 3, there was no statistical difference among habitat types in larval density in area samplers (Kruskal-Wallis rank test, $\chi^2 = 3.9, df = 4, P = 0.42$). For pupae in Study 3, burrow pits were by far the most productive and were followed in rank order by much lower productivity in drainage channels, tire tracks, and close to nil production in rain pools and hoof prints (Figure 5A). There was a highly statistically significant difference among habitat types in pupal density in area samplers in Study 3 (Kruskal-Wallis rank test, $\chi^2 = 13.7, df = 4, P = 0.008$). There was no correlation between larval density and pupal density in Study 3 (Spearman rank correlation = 0.15, $P = 0.47$). In Study 4, overall populations were lower than in Study 3 (it was the identical set of habitats), but the pattern was similar. There was no statistical difference among habitat types in larval density in area samplers in Study 4 (Kruskal-Wallis rank test, $\chi^2 = 6.2, df = 4, P = 0.18$). There were too few pupae collected in Study 4 for statistical analysis, however, there was a trend toward greater production from burrow pits as 15 of the 18 total pupae were from that habitat type (Figure 5B).

**DISCUSSION**

We chose to measure pupal productivity, as pupae can be sampled from discrete habitats in the manner we described without interfering with oviposition, predation, flooding, input of wind-blown nutrients, larval feeding, or other natural phenomena that may affect production. The pupal stage represents the final step in metamorphosis of mosquitoes and is the transition from the aquatic, larval stage to the terrestrial, adult form. Estimates of pupal density are therefore the best proxy measure of adult productivity from natural habitats; our data indicate that larval density is not a good proxy for adult productivity. We sampled larvae with replacement so that density effects would be preserved. Intensive hand-labor, many worker-hours, and organized sampling teams were required to accomplish it in our study.

There are few published data on habitat productivity for *An. gambiae*, and studies tend to be qualitative in nature owing to the relative sampling method used and to the timing of sampling; these studies typically infer habitat productivity from larval abundance data.4,13–16 In simulated larval habitats placed in the field or held in greenhouses, pupal production diminished with increasing larval density and was stymied by shading, which reduced algae, an important larval food.18,19 Service15 observed that: “Small pools and puddles often appear to contain large numbers of *A. gambiae* larvae and are usually considered very productive, but this is not necessarily true . . . . In fact, in most habitats there are far less pupae than fourth-instar larvae, thus indicating a very high mortality.”

Service’s observations suggest that larval density and pupal

![Figure 1](image-url)
productivity are decoupled in many habitats, an observation supported by all four of our studies.

Owing to the size and location of the habitats, it was possible to select a subset of each type for periods of daily sampling, in longitudinal series, using a rigorously quantitative sampling method that allowed estimates of productivity per unit area of habitat, and per habitat. Our sampling method qualifies as an absolute sampling approach, which provides a direct reference to each habitat and to a common unit of habitat area, as opposed to relative sampling methods, which yield less reliable estimates, are referenced to the sampling device and not to a common unit of habitat, and are therefore subject to sampling bias.\(^{20,21}\) Measuring larval density and pupal productivity simultaneously with reference to unit area of larval habitat, to measure the relationship between the two variables, requires an absolute sampling method such as the area sampler used here; the drawbacks of relative sampling methods such as the standard mosquito dipper for shallow, small habitats are well-known and have been discussed elsewhere.\(^{21}\)

We are unaware of any previous study that quantified area-wide productivity toward a measure of efficiency of habitat productivity of An. gambiae pupae at the village scale or larger, in a longitudinal study where absolute (versus relative) sampling methods were used, although there have been several studies of distribution of An. gambiae immature stages among habitats in field settings and, in some cases, estimates of stage-specific survivorship.\(^{3,4,13–16}\) The productivity of adults from larval habitats is therefore the primary determinant of adult mosquito density in an area, barring immigration.\(^{21}\) Proximity to habitats is one determinant of malaria risk in sub-Saharan Africa.\(^{22–24}\) However, as we have already noted above, larval presence does not equate with pupal production from any given habitat.

The results of our four sampling studies, taken together, allow general conclusions. Most importantly, pupal productivity was confined to a small subset of the total array of habitats of various types that commonly harbored larvae. Larval density was not strongly correlated with pupal productivity, indicating that larval sampling or mere presence of larvae will not serve well as surrogates for pupal productivity. The most comprehensive sampling series here were Studies 1 and 2, because sampling was conducted with area samplers and with whole habitat censuses of pupae. During Study 1 (in a wet period), five types of habitat were present (streams were flowing, thereby obviating formation of pools in streambeds for the most part in Study 1). The greatest number of larvae and pupae was collected during this study. During Study 2, a relatively drier period, only streambed pools, drainage channels, and burrow pits were present, as the smaller, less stable types of habitats (hoof prints, rain pools, and tire tracks) had dried up. During the dry period, burrow pits and streambed pools contributed most of the pupal productivity but, surprisingly, during the first, wet sampling period when habitats proliferated, most pupal productivity was still confined to a single habitat type—burrow pits. Study 3 and Study 4, both smaller in scale owing to the restriction of pupal sampling to area samplers only, still supported the observations that burrow pits were the primary pupal producers. During the wet season, nearly one-third of habitats found were burrow pits, and just a few of these were by far the most productive for pupae. The mean pupal standing crop per habitat and habitat abundance taken together will dictate pupal productivity at the level of the village landscape. Taking into account average productivity of burrow pits and the abundance of this habitat relative to others, we estimate that these habitats accounted for about 85% of total pupal production during wet periods in this village. This finding is similar to that of Awono-Ambene and Robert\(^{22}\) and Robert and others\(^{23}\) who observed that market garden wells were the primary producer of An. arabiensis among a range of habitat types in urban Dakar, Senegal.

Aggregations of the “classic” hoof print An. gambiae habitats\(^{19}\) produced very few pupae during this study. Other habitats, such as drainage channels, tire tracks, and rain pools, were commonly occupied by larvae but did not produce pupae in large numbers. Comparisons of larval sampling and pupal productivity suggest that many habitats receive eggs, promote larval development to a certain degree, but fail largely to produce pupae. These habitats function in effect as egg sinks, where gravid females lay eggs but where the likelihood is greatly diminished that those habitats will support larval development to complete metamorphosis. Thus gravid females face a highly uncertain and risky set of oviposition choices and should be likely to distribute eggs across a range of habitats rather than deposit them in one location, to reduce the risk of all progeny failing to develop fully.

The biotic and abiotic characteristics of the variably productive and unproductive habitats were not known with certainty here. Habitat stability (i.e., the number of days in each 25-day sampling period that a habitat held water of any volume) was positively correlated with both larval density and pupal productivity, but stability explained less than half of the variation for both variables. Inspection of the axes and the distribution of the data points of the graph in Figure 2 indicates that of the 34 habitats sampled in that study, a small set of them were both stable and productive, another set was unstable and not productive, while yet another set was stable but not productive. Thus pupae failed to form in some stable
habitats, and likely these were habitats where populations of predators and parasites had formed. Service\textsuperscript{15,16} has shown that these natural enemies of \textit{An. gambiae} larvae are highly effective in suppressing production from aquatic environments and that they commonly occupy habitats. If stable but unproductive habitats could be better understood and constructed, they could satisfy the needs of villagers as water sources and drainage catchments\textsuperscript{8} while eliminating them as sources of malaria vectors. They might even serve as egg sinks. Currently, the biologic properties allowing these habitats to be stable but unproductive habitats are not known. When expressed as daily density of larvae and productivity of pupae as in Figure 3, the sampling data suggest that larval populations tend to perform as cohorts rather than as stably producing populations. Pupal productivity was highly discontinuous in all habitats sampled, when it occurred at all. These trends could be explained by cycles of flooding and drying of habitats owing to episodic rainfall.

The comparisons of habitat productivity between Study 1 and Study 2 deserve comment. Although habitats in both studies were similar in stability, yet this common element is deceiving because in the former study we included typically unstable habitats such as hoof prints and tire tracks, and average stability was calculated with data from those habitats during the wet period of Study 1, whereas in Study 2 those habitats along with rain pools were completely absent owing to less rain. Therefore, habitats in Study 2 were actually more stable, because the zero values of unstable habitats were not included in the calculation of average stability in this study, as they were in Study 1. Habitats in Study 2 were observed to have a more even distribution of pupae overall (Figure 1) than in Study 1, and habitats farther from human dwellings also tended to be more productive for pupae (see Table 3). These seemingly contradictory results are explicable on the basis of fewer habitats overall available for female oviposition and for sampling in the dry period of Study 2, and as a result, the fewer number of habitats categorized as close to dwellings. In both studies, larger and more stable habitats were somewhat less productive per unit area than were smaller and less stable habitats in terms of number of pupae per unit area. However, over time, larger habitats yield in total more pupae per habitat than smaller habitats and could therefore referred to as more productive, as opposed to the smaller habitats that could be described as more efficient. Also, as pre-

\textbf{Figure 3.} Mean \textit{An. gambiae} s.l. larvae per area sample per day and mean pupae per m\textsuperscript{2} per day in different larval habitat types in a western Kenya village.
viously noted for some large, stable habitats, pupal productivity was actually reduced possibly owing to establishment of predator and parasite communities in them. That pools in streambeds emerged as an important habitat in the dry period (Study 2) suggests that An. gambiae females are flexible in their oviposition site choice and that pools in streambeds might represent refuge habitats in dry periods when other habitats (rain pools, tire tracks, hoof prints) are unavailable for oviposition.

Our results are consistent with a theme resonating throughout malariology, namely with the idea that a source-reduction program targeting primarily burrow pits could have a substant-tial impact on malaria transmission in this village by reducing adult mosquito production immediately near human dwellings. Because villagers create all such pits, their location is known. All larval habitats are aggregated in the environment and most habitats occupy a small proportion of village area; most are near houses and thus easily accessible. Long-lasting, inexpensive, socially acceptable, and easily applied treatments to make them unproductive or procedures for stabilizing them and making them simultaneously unproductive while still useful to people are needed for these habitats. If the practical exigencies of such an effort seem surmountable, the effects of a stand-alone source-reduction program may very well interact synergistically with a high-coverage insecticide-treated bed nets (ITNs) or indoor residual spraying (IRS) program in an integrated fashion. Such integrated programs have been proposed for development against An. gambiae based on successes in certain locations, eradication efforts largely directed against larvae after introductions outside of the species’ normal geographic range, and predictions from simulation modeling. It may also be worthwhile to augment year-round source-reduction efforts in burrow pits with dry season targeting of streambed pools, as these habitats are relatively easy to find and, combined with burrow pits, produced a large proportion of pupae during dry periods. The habitats heretofore thought of as “typical” for rural Africa—tire tracks, hoof prints, and rain pools—are indeed unpredictable in occurrence in both space and time, but our results illustrate that these habitats contribute relatively little to overall adult productivity. The effort required to locate and treat such habitats is therefore not justified, even if larvae are present in them. The extent to which such a program would be effective outside of our particular study village remains to be seen. Kisian may be sufficiently representative of much of the Lake Victoria basin to allow fairly broad application; whether other parts of the east African savannah are sufficiently similar, environmentally and socially, will require further study.

Our results lead to quite different conclusions compared with those of Fillinger and others, who conducted weekly, longitudinal sampling of sets of habitats similar to those we

![Figure 4](image-url)  
**Figure 4.** Linear regression of number of An. gambiae s.l. larvae in area samples (A) or pupae in whole habitat census (B) on total number of days habitats held water (stability). Larval and pupal data were transformed with log_{10}(x+1).

![Figure 5](image-url)  
**Figure 5.** The mean number of total larval or pupal An. gambiae s.l. found in 78.5 cm² area samples taken daily for 25 days from 5 different habitat types (N = 5 per type) in Study 3 (A) and Study 4 (B) 2003 in a rural village in western Kenya.
studied, in an region about 100 km south of our study site. They used dippers for the sampling device and did not quantify pupal production but rather inferred that habitats occupied with *An. gambiae* larvae would be productive for pupae. Therefore, their methods were less likely to measure habitat productivity adequately, to capture short bursts of pupal production, or to associate variables such as stability with productivity, owing to the rapidity of habitat hydrological cycles. For example, they concluded that habitat stability was not associated with productivity, whereas in our study habitat stability clearly influenced pupal productivity (Figure 5). We would opine that weekly sampling is insufficient to associate stability and productivity, given that cohorts of larvae lead to pupation within 7 days. We would also disagree with their conclusion that a larval control program aimed at *An. gambiae* would require that all habitats occupied by larvae need to be treated. Our data indicate otherwise; indeed many habitats do not produce pupae, or produce so few that many habitats with larvae could be ignored; more likely, those few habitats closest to homes and producing pupae could be treated or eliminated to reduce pupal production meaningfully.

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