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# Survivorship of Immature Stages of *Anopheles gambiae* s.l. (Diptera: Culicidae) in Natural Habitats in Western Kenya Highlands

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**ABSTRACT**   We examined the survivorship of *Anopheles gambiae* s.l. Giles (Diptera: Culicidae) larvae and habitat productivity in three major habitat types in the western Kenya highlands. The age-specific distribution was determined for larvae and pupae, and survivorship curves were constructed. Larval-to-pupal survivorship was 6.8% in drainage ditches, 4.3% in cow hoofprints, and 1.8% in disused goldmines, respectively. High mortality rates were observed in all developmental stages. The estimated daily survival rate was highest in drainage ditches (0.74), followed by cow hoofprints (0.71), and it was lowest in disused goldmines (0.62). Productivity of emerging *An. gambiae* adults was generally low in these larval habitats (1.35, 1.55, and 1.84 mosquitoes per m$^2$ per wk in drainage ditches, disused goldmines, and cow hoofprints, respectively). In total, seven families of larval mosquito predators were identified from the larval habitats, including Hydrophilidae, Dytiscidae, Corixidae, Nepidae, Notonectidae, Belostomatidae, and Cordulidae. Predator density in disused goldmines was significantly higher than that of other habitat types. Determination of the relative importance of predation, habitat stability and food contents on natural mosquito habitat productivity would help to design cost-effective vector control methods specifically targeted at the productive habitats.

**KEY WORDS**   malaria, *Anopheles gambiae* larvae, habitat productivity, African highland

Malaria is a major public health problem in sub-Saharan Africa. In the East African highlands, the threat is mounting as malaria outbreaks become more frequent in areas where malaria was previously rare (Malakooti et al. 1998; Lindblade et al. 1999; Shanks et al. 2000, 2005; Hay et al. 2003; Abeku et al. 2004; John et al. 2005; Shanks et al. 2005). Because no malaria vaccine is available, and parasite resistance to antimalarial drugs is developing rapidly, vector control is an important method for reducing malaria transmission in developing countries (Biard 1998, Trape et al. 2002, Killeen et al. 2004). A potential important target of malaria vector control is the immature stages of anopheline mosquitoes (Killeen et al. 2002, Fillinger et al. 2003). Determination of larval mosquito survivorship and the sources of mortality in natural habitats can provide useful information for developing effective larval control methods (Campos and Lounibos 2000).

Habitat types influence development and survivorship of larvae of *Anopheles gambiae* s.l. Giles (Diptera: Culicidae), the primary malaria vector in Africa (Service 1977, Jacob et al. 2003, Edillo et al. 2006, Minakawa et al. 2006, Mutuku et al. 2006). Recent studies in western Kenya revealed positive relationships between habitat stability and pupal occurrence (Minakawa et al. 2005a, Mutuku et al. 2006). *An. gambiae* larvae mainly occur in small temporary, sunlit pools such as borrow pits, cow hoofprints, tire tracks, drainage ditches, and small puddles (Gillies and De Meillon 1968, Minakawa et al. 1999, Gimnig et al. 2001). The potential risk of using such habitats is high to female mosquitoes because the larvae must survive, develop into pupae, and emerge as adults before the habitat desiccates. However, it has been suggested that larval mortality is lower in such habitats than it is in large, permanent habitats where the predation rate is high (Sunahara et al. 2002). In this study, we examined age-specific mortality and habitat productivity of *An. gambiae* adults in three major habitat types in natural conditions in the western Kenya highlands. We also determined abundances of potential larval predators in larval habitats. Information on the contribution of different types of larval habitats to adult vector abundance is useful to the development of rational environmental management and larval control methods for reducing malaria transmission.

## Materials and Methods

**Study Area.** The study site is located in the Iguhu area (34° 45′ E, 0° 10′ N) in Kakamega district, Western Province, Kenya. The 4- by 4-km$^2$ study site has an

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altitude ranging from 1,420 to 1,600 m above sea level. The total rainfall and mean daily temperature from July 2003 to July 2004 were 1,730 mm and 20°C, respectively. Mean daily temperature during the experiment between 4 June and 3 July 2005 was 19.0°C. Relative humidity and total amount of rainfall during the same period was 84.5% and 180 mm, respectively. Peak rainfall usually occurs between April and June, followed by a short rainy season in October and November. Steep hills and undulating topography characterize the area.

Drainage ditches, disused goldmine pits and cow hoofprints were the major larval habitats of *An. gambiae* s.l. larvae in this study area (Minakawa et al. 2005b). Drainage ditches were created to draw water from a stream for farming. The width of ditches was usually ≈0.5 m, and water in the ditches was often stagnant. Goldmine pits were created by local residents during the process of searching for gold. The pits were roughly round, and the diameter was usually <3 m. During the rainy season, water accumulated in the pits where the water depth seldom exceeded 1 m. Cow hoofprints, which were usually <0.15 m in diameter, were often found near the stream edges and in wetlands in the valley bottom.

**Vertical Life Tables.** We determined *An. gambiae* mortality and survivorship of mosquito immature stages by constructing vertical life tables by using natural habitats with overlapping generations. A horizontal life table is appropriate for cohorts that can be followed over time, whereas a vertical life table is applied to populations with completely overlapping generations (Service 1971, 1977, 1993; Reisen and Siddiqui 1979; Edillo et al. 2004). Seven disused goldmine pits and seven drainage ditches were randomly selected from *An. gambiae*-positive larval habitats. Daily, one hundred (100) dips were made from each habitat by using a standard dipper (350 ml) for a period of 30 d in June–July 2005. All *An. gambiae* s.l. larvae sampled in each habitat were first transferred to a white tray from which the larvae were carefully picked using a wide-mouthed plastic pipette. The larvae were scored into instars (Gillies and Coetzee 1987), counted, and placed back into the habitats. Furthermore, we studied the survivorship and mortality rates of *An. gambiae* s.l. in 26 larvae-positive cow hoofprints for the same period. For cow hoofprints, mosquito larvae were first picked directly from the habitats by using a plastic pipette, and then the habitat was further examined for remaining anopheline larvae in each developmental stage by using a standard dipper. All water and mosquito larvae were returned to the habitat.

**Mosquito Larval Predators.** We identified macroinvertebrates in disused goldmine pits, drainage ditches and cow hoofprints during larval dipping sampling. The collected macroinvertebrates were identified to family or genus, and the abundance of each taxon was recorded before being returned to the original habitats. Predatory status of collected macroinvertebrates was inferred from previous studies conducted in western Kenya (Service 1973, 1977; Carlson et al. 2004).

**Habitat Productivity.** We determined the productivity of emerging adult *An. gambiae* in disused goldmines, drainage ditches, and cow hoofprints. We defined productivity of malaria vectors as the number of emerging adult mosquitoes per $m^2$ per wk in the aquatic habitats. Habitat productivity was examined in 10 randomly selected habitats in each of the three habitat types by using emergence traps (one trap per habitat). Emerging mosquitoes were collected daily for a period of seven consecutive days each month during June 2003 to June 2004. For logistical reasons, habitat productivity was not monitored in February 2004. All anopheline adults were identified using morphological keys (Gillies and Coetzee 1987) and rDNA-polymerase chain reaction (PCR) (Scott et al. 1993) for members of the *An. gambiae* complex.

The emergence traps prevented adult mosquitoes from ovipositing in the area covered by the trap and immature mosquitoes from entering into the trap; therefore, we relocated the emptied trap daily within the same habitat. In the case when a habitat was flushed out by heavy rains or desiccated during sampling days, the data for that habitat were discarded. Over the study period, the total number of emergence trap collection data points at daily interval was 743, 736, and 615 for disused goldmines, drainage ditches, and cow hoofprints, respectively. Although the traps may not provide an absolute estimate of mosquito productivity of a habitat, they are suitable for comparing the relative productivity of different larval habitat types (WHO 1975, Service 1993). We used two sizes of the trap, 1 and 0.5 $m^2$ (horizontal dimension) and 1 m in height, depending on the habitat size. When an aquatic habitat was smaller than the trap, the water surface area covered by the trap was measured. Water temperature was recorded hourly using Stowaway Tidbit data loggers (Onset Computers, Bourne, MA).

**Statistical Analysis.** The data collected over the 30-d period were pooled for each larval stage in each habitat for vertical life table analysis. We used mean stage-specific larval development duration from our previous studies in seminatural habitats in the same study area and same rainy season to calculate survivorship curves (1.54, 3.47, 2.93, 3.21, and 1.67 d for first, second, third, and fourth instars, and pupae, respectively; Munga et al. 2006). The larval developmental durations estimated from seminatural habitats are reasonable approximations to those in natural habitats because of similar water temperature reported in Munga et al. (2006) and the current study. A negative exponential growth model was used to fit the relationship between observed abundance and cumulative developmental time for each developmental stage. This model assumes constant daily survival rates for all larval developmental stages and a stable age distribution. To determine whether the age distribution over the 30-d sampling period was stable, we plotted the 5-d moving average of the percentage of larvae in different developmental stages and pupae. Larvae in the first two instars and in the last two instars were pooled. We then divided the sampling period into three 10-d
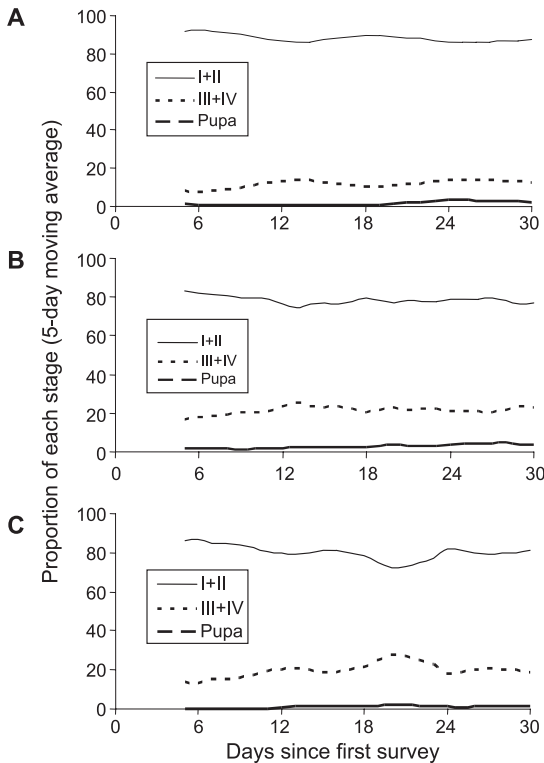
**Fig. 1.** Temporal stability of age distribution of *An. gambiae* s.l. larvae and pupae in drainage ditch (A), disused goldmine (B), and cow hoofprint (C) habitats in western Kenya highlands. Five-day moving average in the percentage of larval or pupal stages is plotted.



**Fig. 2.** Age distribution and survivorship curves of *An. gambiae* s.l. larvae (instars I, II, III, and IV, and pupae) in drainage ditch (A), disused goldmine (B), and cow hoofprint (C) habitats in western Kenya highlands. The dots that are not connected show the observed values, whereas the solid lines show model fitting.

intervals and analyzed the variation in the percentage of these life stages in each interval by using one-way analysis of variance (ANOVA). The daily mortality rate is estimated for each habitat type by regression of logarithm of number of individuals in each developmental stage against the total time to develop into the particular developmental stages. A chi-square test was used to detect significant differences in larval-to-pupal survivorship among the three habitat types.

ANOVA with repeated measures was used to detect significant variation in productivity of *An. gambiae* s.l. among the three habitat types. When significant variation was detected, we used Tukey–Kramer honestly significant difference (HSD) test to compare the differences between means, adjusted with the Bonferroni correction for multiple comparisons. We added all predators from each larval habitat, and used one-way ANOVA with repeated measures to compare the differences in density of larval predators among the three habitat types. The density of larval predators was calculated by dividing the total number of predators found in each habitat at each sampling occasion by the total number of dips (100 dips for drainage ditch and disused goldmine habitats, and one dip for cow hoofprints).
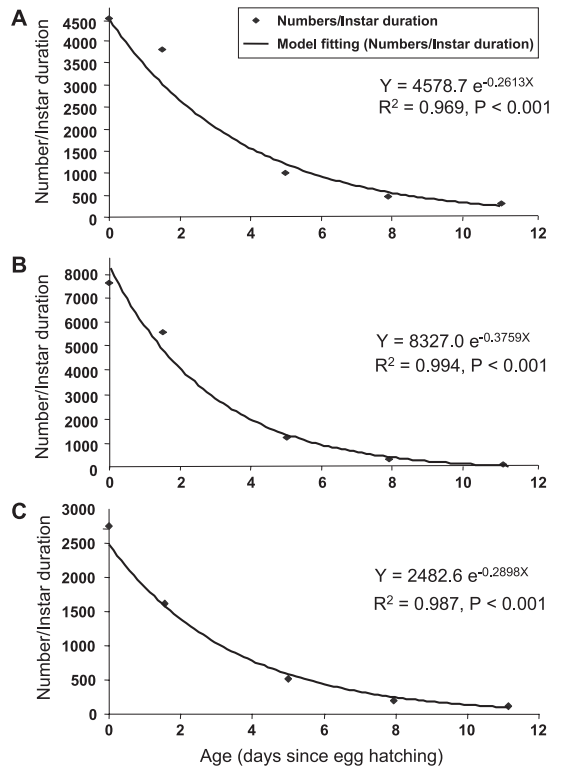
## Results

**Vertical Life Tables.** The 5-d moving average of the percentage of larvae and pupae in the three larval habitat types showed that the age distribution of *An. gambiae* s.l. larvae over the study period was relatively stable (Fig. 1). Thus, we used the negative exponential growth model to fit the relationship between observed abundance and cumulative developmental time for each developmental stage, and we subsequently determined *An. gambiae* s.l. larval survivorship curves for drainage ditch (Fig. 2A), disused goldmine (Fig. 2B) and cow hoofprint habitats (Fig. 2C). The estimated daily survival rate was highest in drainage ditches (0.739), followed by hoofprints (0.711), and it was lowest in disused goldmines (0.624). Stage-specific life tables showed a high mortality rate at every developmental stage, particularly the second and third instars. In the disused goldmines, the fourth instars also suffered a high mortality (Table 1). Overall, only 6.8, 1.8, and 4.3% first instars survived to the pupal stage in drainage ditches, disused goldmines, and cow hoofprints, respectively (Table 1; $\chi^2 = 194.64$, df = 2, $P < 0.001$). The daily mortality rate of the different developmental stages ranged from 10.9 to 31.9% in the drainage ditches, 18.7–45.6% in the disused goldmines and

Table 1. Developmental stage-specific survivorship of immature *An. gambiae* in different habitat types in western Kenya highlands

| Developmental stage ($i$) | Duration of each stage ($d$) | No. entering stage $i$ ($S_i$) | No. deaths in stage $i$ ($D_i$) | Relative proportion dying ($D_i/S_i$) | Daily mortality rate in stage $i$, $[1 - (S_{i+1}/S_i)^{1/d}]$ |
|---|---|---|---|---|---|
| Drainage ditches | | | | | |
| Instar I | 1.54 | 4,535 | 736 | 0.162 | 0.109 |
| Instar II | 3.47 | 3,799 | 2,797 | 0.736 | 0.319 |
| Instar III | 2.93 | 1,002 | 536 | 0.535 | 0.314 |
| Instar IV | 3.21 | 466 | 158 | 0.339 | 0.121 |
| Pupa | 1.67 | 308 | | 0.451 | 0.301 |
| | | $S = 0.068$ | | | |
| Disused goldmines | | | | | |
| Instar I | 1.54 | 7,616 | 4,597 | 0.454 | 0.187 |
| Instar II | 3.47 | 5,533 | 4,294 | 0.776 | 0.350 |
| Instar III | 2.93 | 1,239 | 879 | 0.709 | 0.456 |
| nstar IV | 3.21 | 360 | 221 | 0.614 | 0.257 |
| Pupa | 1.67 | 139 | | 0.137 | 0.084 |
| | | $S = 0.018$ | | | |
| Cow hoofprints | | | | | |
| Instar I | 1.54 | 2,742 | 1,126 | 0.411 | 0.291 |
| Instar II | 3.47 | 1,616 | 1,092 | 0.676 | 0.277 |
| Instar III | 2.93 | 524 | 320 | 0.611 | 0.372 |
| Instar IV | 3.21 | 204 | 86 | 0.422 | 0.157 |
| Pupa | 1.67 | 118 | | 0.754 | 0.568 |
| | | $S = 0.043$ | | | |

$S$ is the overall survivorship from first instar to pupae.

15.7–56.8% in the cow hoofprints (Table 1). Daily pupal mortality rate was highest in cow hoofprint habitats (56.8%) and lowest in disused goldmines (8.4%).

**Habitat Productivity.** We collected a total of 1,182 mosquitoes using emergence traps in the three habitat types. Of these, 181 (14.5%) were *A. gambiae* s.l., and culicines were more abundant than anophelines (81.5%) (Table 2). Other anopheline species collected were *Anopheles coustani* Laveran (0.1%), *Anopheles implexus* Theobald (1.1%), *Anopheles maculipalpis* Giles (0.3%), and *Anopheles squamosus* Theobald (2.5%). Among the 181 *An. gambiae* s.l., 161 individuals (88.9%) were identified as *An. gambiae* s.s. by rDNA-PCR analysis. The remaining 20 specimens of *An. gambiae* s.l. were not identified to species because of PCR amplification failure.

Productivity of emerging *An. gambiae* s.l. adults between the three habitat types was not statistically significant ($F = 0.10$; df = 2, 34; $P > 0.05$). The estimated mean productivity was 1.35, 1.55, and 1.84 mosquitoes per m$^2$ per wk in drainage ditches, disused goldmine, and cow hoofprints, respectively. Of a total

Table 2. Abundance of anopheline mosquito species and unidentified culicines collected by emergence traps in drainage ditches, cow hoofprints, and disused goldmines in western Kenya highland

| Species | Drainage ditches | Cow hoofprints | Disused goldmines |
|---|---|---|---|
| *An. gambiae* s.l. | 58 | 15 | 108 |
| *An. coustani* | 0 | 0 | 1 |
| *An. implexus* | 7 | 1 | 5 |
| *An. maculipalpis* | 0 | 4 | 0 |
| *An. squamosus* | 4 | 11 | 15 |
| Culicines | 471 | 468 | 24 |
| Total | 540 | 499 | 153 |

of 2,094 trap records during the 13-mo study period, the percentages of daily observations with one or more *An. gambiae* adults were 5.4% in drainage ditches, 8.7% in disused goldmines, and 4.3% in cow hoofprints. Cow hoofprints produced *An. gambiae* adults only during June and July 2003. The mean water temperature was similar among the three habitat types over the study period (21.9°C for drainage ditches, 22.0°C for disused goldmines, and 22.0°C for cow hoofprints). The average daily minimum and maximum temperatures did not vary markedly among the three types of habitat (minimum temperature: 18.3°C for drainage ditches, 19.3°C for disused goldmines, and 18.2°C for cow hoofprints; maximum temperature: 27.8°C for drainage ditches, 26.3°C for disused goldmines, and 28.4°C for cow hoofprints).

**Larval Mosquito Predators.** We identified seven families of larval mosquito predators from the three habitats: Hydrophilidae, Dytiscidae, Corixidae, Nepidae, Notonectidae, Belostomatidae, and Cordulidae (Table 3). Disused goldmine pits and drainage ditches had all seven families, whereas cow hoofprints had only three. The most prevalent predator was Hydrophilidae in drainage ditches and disused goldmines, and Hemiptera in cow hoofprints. Overall, predator density in disused goldmines (2.51 predators per dip) was significantly higher than in the drainage ditches (1.13 predators per dip) and cow hoofprints (0.95 predators per dip) ($P < 0.01$; Tukey HSD test); however, the predator density between drainage ditches and hoofprints was not significantly different ($P = 0.61$; Tukey HSD test).

## Discussion

In this study, we found mortality rates of *An. gambiae* larvae exceeding 93, 95, and 98% in drainage ditch,

Table 3.  Mean density (number of predators per dip) of *An. gambiae* larval predators in drainage ditch, disused goldmine, and cow hoofprint natural habitats

| Insect order | Family | Drainage ditches | Disused goldmines | Cow hoofprints |
|---|---|---|---|---|
| Coleoptera | Hydrophilidae | 0.27 (0.03) a | 0.62 (0.03) b | 0.00 (0.00) c |
| Coleoptera | Dytiscidae | 0.10 (0.01) a | 0.29 (0.02) b | 0.00 (0.00) c |
| Hemiptera | Corixidae | 0.09 (0.01) a | 0.11 (0.01) a | 0.00 (0.00) b |
| Hemiptera | Nepidae | 0.23 (0.04) a | 0.30 (0.03) a | 0.83 (0.17) b |
| Hemiptera | Notonectidae | 0.02 (0.01) a | 0.05 (0.01) a | 0.23 (0.09) b |
| Hemiptera | Belostomatidae | 0.15 (0.01) a | 0.21 (0.02) b | 0.00 (0.00) c |
| Odonata | Cordulidae | 0.05 (0.01) a | 0.07 (0.01) a | 0.06 (0.05) a |

The numbers in parentheses indicate standard errors. The lowercase letters after the numerical values indicate the results of Tukey honestly significant differences multiple comparison tests. The values with the same letter were not statistically significant at $P = 0.05$ level after adjustment with the Bonferroni correction.

cow hoofprint, and disused goldmine habitats, respectively. Our results are similar to the findings of previous studies in lowland areas in East Africa by Service (1971, 1973) and Aniedu et al. (1993) who reported larval mortality of between 95 and 100% in small pools and ponds near the Kisumu and Baringo districts of western Kenya. Several factors influence the survival and immature stages of *An. gambiae.* An important factor is predation in relatively stable habitats (Service 1977). Indeed, we found abundant predators such as coleopterans and hemipterans in disused goldmines and drainage ditches. The importance of predation is evidenced by much higher larval survivorship (35–51%) in artificial, seminatural habitats where predators had not yet been colonized in the same study area (Munga et al. 2006).

Another critical factor influencing mosquito larval survivorship is habitat stability (Minakawa et al. 2005a, Mutuku et al. 2006). Habitat stability may be a particularly severe problem for small habitats such as cow hoofprints. Due to high evaporation rates in tropical regions, cow hoofprints may dry out before larvae complete their development, although the warmer water temperature in cow hoofprints may reduce mortality rate and shorten the developmental time. In addition, heavy rain may flush out larvae and eggs during the rainy season. Thus, mosquito larval survivorship was low in cow hoofprints despite low predator density. The third important factor is nutritional conditions (Tuno et al. 2005, Kaufman et al. 2006, Munga et al. 2006). Food resources may be limiting factors in small unstable habitats. Frequent disturbances such as drought and floods may limit production of algae that are an important food source for *An. gambiae* (Kaufman et al. 2006). Moreover, high canopy cover limits algal production in larval habitats (Tuno et al. 2005, Munga et al. 2006). Poor food resources and crowding may induce cannibalism among *An. gambiae* larvae. For example, Koenraadt and Takken (2003) reported first instars of *An. gambiae* were eaten by fourth instars of *An. gambiae* and *Anopheles arabiensis* Patton larvae, and the presence of fourth instars significantly reduced the survivorship of first instars. Thus, interactions between the larvae may have caused disproportionately higher stress on the younger instars. Perhaps cannibalism is more severe in small habitats with a high larval density (Koenraadt et al. 2004).

Two methods for estimating larval habitat productivity have been reported in the literature. One method is pupal counting (Mutuku et al. 2006), and the other method is emergence traps (Munga et al. 2006). Each method has its advantages and disadvantages. For example, pupal counting yields a direct measurement of pupal production of a habitat, but it may be logistically difficult in large habitats, or substantial errors may occur when habitats contain aquatic vegetation in which mosquito pupae are concealed. In addition, the number of pupae being recovered or counted depends on how thorough the investigator examines the habitat; thus, attention should be given to standardization across different investigators. Emergence traps are easy to deploy, and the number of adults recovered can be readily standardized. However, it only provides a relative measurement of habitat production. Aggregated distribution of mosquito larvae within a large habitat may introduce sampling bias in the estimation of adult mosquito production when limited number of emergence traps is placed in a habitat. Therefore, comparison between these two methods for habitat productivity estimation is valuable. In this study, the emergence trap method revealed that the productivity of *An. gambiae* adults was low in the highlands in the major larval habitats. Despite significant variation in the survivorship of mosquito larvae among the three habitat types, the adult productivity was similar. This may be partly due to differences in the number of first instars in each habitat type. In disused goldmine habitats, for example, the abundance of first instars was much higher than in drainage ditches. Higher abundance of first instars in disused goldmines may have compensated, to some degree, for the effect of reduced larval survivorship.

In summary, we found high larval mortality and low pupal productivity in larval habitats in the highlands. High larval mortality contributed to low adult mosquito abundance and low malaria transmission in the area (Ndenga et al. 2005, Koenraadt et al. 2006). Determination of the relative importance of predation, habitat stability, and food contents in natural mosquito habitat productivity will enhance our understanding of the mechanisms of mosquito larval population regulation and help with identification of productive habitats and development of vector control methods specifically targeted at the productive habitats.

## Acknowledgments

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