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# Egg hatching, larval movement and larval survival of the malaria vector *Anopheles gambiae* in desiccating habitats

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## Abstract

**Background:** Although the effects of rainfall on the population dynamics of the malaria vector *Anopheles gambiae* have been studied in great detail, the effects of dry periods on its survival remain less clear.

**Methods:** The effects of drying conditions were simulated by creating desiccated habitats, which consisted of trays filled with damp soil. Experiments were performed in these trays to (i) test the ability of *An. gambiae sensu stricto* eggs to hatch on damp soil and for larvae to reach an artificial breeding site at different distances of the site of hatching and (ii) to record survival of the four larval stages of *An. gambiae* s.s. when placed on damp soil.

**Results:** Eggs of *An. gambiae* s.s. hatched on damp soil and emerging larvae were capable of covering a distance of up to 10 cm to reach surface water enabling further development. However, proportions of larvae reaching the site decreased rapidly with increasing distance. First, second and third-instar larvae survived on damp soil for an estimated period of 64, 65 and 69 hrs, respectively. Fourth-instar larvae survived significantly longer and we estimated that the maximum survival time was 113 hrs.

**Conclusion:** Short-term survival of aquatic stages of *An. gambiae* on wet soil may be important and adaptive when considering the transient nature of breeding sites of this species in sub-Saharan Africa. In addition, the results suggest that, for larval vector control methods to be effective, habitats should remain drained for at least 5 days to kill all larvae (e.g. in rice fields) and habitats that recently dried up should be treated as well, if larvicidal agents are applied.

## Background

Soon after the start of the rains, populations of the malaria vector *Anopheles gambiae sensu lato* increase explosively in sub-Saharan Africa. The immature stages of this vector can then be found in numerous transient habitats created by the rains, such as hoof prints, car tracks, borrow pits and ditches [1]. The relationship between rainfall on the one hand and mosquito population dynamics and malaria risk on the other is well established and has been

modelled accordingly [2–4]. In contrast, the effects of a period of drought on mosquito survival remain less clear.

Previous studies have suggested that adult members of the *An. gambiae* complex survive the dry season either as aestivating females [5,6] or as populations in hidden *refugia* [7,8]. These dry season survival strategies facilitate rapid recolonisation of the area at the onset of the rainy season. During a short spell of drought, Charlwood *et al.* [9] found a complete cessation of recruitment of young adults to the population and a drastic decline in adult *Anopheles arabiensis* in Tanzania. This was ascribed to mass larval mortality due to the rapid drying up of the numerous breeding sites, normally present during the wet season. Once the main rains had started, the populations began to build up again.

Recently, Minakawa *et al.* [8] showed that *An. gambiae s.s.* oviposits on moist soil, if no better alternative, e.g. flooded soil, is available. This was also true for *Anopheles melas*, a salt-water breeding member of the *An. gambiae* complex [10]. Since eggs of *An. gambiae s.l.* remain viable for 12–16 days under dry conditions [11,12], egg dormancy has been proposed as a short-term survival mechanism of this species. Field observations, whereby soil from larval habitats that had dried up was investigated for the presence and viability of immature stages, support this hypothesis [8,11,13]. Similar observations were made with other malaria vectors, such as *Anopheles albimanus* [14] and *Anopheles balabacensis* [15].

During the rainy season, the immature stages of *An. gambiae* may experience desiccation, due to drying up of their habitats after a few days without rainfall. In addition, eggs may have been laid on damp soil as a result of the female's oviposition choice. The unpredictable rainfall pattern will select those females that make optimal habitat choices for their offspring. Investigating the trade-off between female oviposition choice and optimal larval survival is of key importance in understanding mosquito population dynamics [16].

In this study, we looked at the consequences of a female's choice by testing the ability of eggs to hatch on damp soil and the extent to which emerging first-instar larvae were able to reach a nearby artificial breeding site at varying distances from a simulated oviposition site. Second, we investigated the potential of the four larval development stages to survive on damp soil.

## Methods

### *Mosquitoes*

For the egg hatching experiment, we used eggs of *An. gambiae s.s.* (Kisumu strain), maintained at the Centre for Vector Biology and Control Research (CVBCR) of the Kenya Medical Research Institute (KEMRI) in Kisumu, Kenya. For the larval survival experiment, we used larvae of *An. gambiae s.s.* (Ifakara strain), maintained at the Mbita Point Research and Training Centre (MPRTC) of the International Centre of Insect Physiology and Ecology (ICIPE), Mbita, Kenya. Adults of both strains were maintained in 30 cm cubic cages with *ad libitum* access to a 6% glucose solution. The Kisumu strain was maintained on rabbits, while the Ifakara strain was fed on a human arm twice a week.

### *Egg hatching and larval movement*

Plastic plates (18 cm ∅, 4 cm deep) or trays (40 × 30 × 11 cm) were filled to a depth of 4 cm with local, black cotton soil, which was first saturated with river water. The topsoil layer was smoothed and in the middle of the plates or trays, breeding sites were simulated by making circular depressions (5 cm ∅, 2 – 3 cm deep), which were filled with river water. Fifty eggs of *An. gambiae* s.s., that had been incubated for one day on wet filter paper, were placed on a small piece of filter paper (0.5 × 1.0 cm). This filter paper was placed directly in the site with water (control) or on the damp soil at 0, 2, 5 or 10 cm from the edge of the artificial breeding site (Figure 1). Each distance experiment was replicated 5 times, each replicate (plate or tray) receiving one batch of 50 eggs. The control, 0 and 2 cm distance experiments were carried out in the plates, while the 5 and 10 cm distance experiments were done in the trays. Plates and trays were placed in a laboratory of the CVBCR, without controlled environmental conditions. During the experiment, mean minimum indoor temperature was 19°C and mean maximum indoor temperature 30°C. Plates and trays were not exposed to direct sunlight.

At 8 am and at 6 pm the water level in the artificial breeding sites was topped up. At the same times, the soil was dampened by spraying water with a plant sprayer from a height of approximately 1 meter. We made sure there was no water run-off or collection of water on the damp soil.

The number of larvae that was recovered in the artificial breeding site was counted and removed daily for 3 consecutive days. For each replicate, the number of larvae reaching the water surface was expressed as the proportion of the total number of eggs tested. After 3 days, the number of hatched and unhatched eggs was determined by counting the number of opened and closed eggs on the filter paper under a stereomicroscope. For each replicate, the number hatched was expressed as the proportion of the total number of eggs tested. The filter paper was then placed directly in the artificial breeding site, and the number of larvae that hatched overnight was scored the next day.

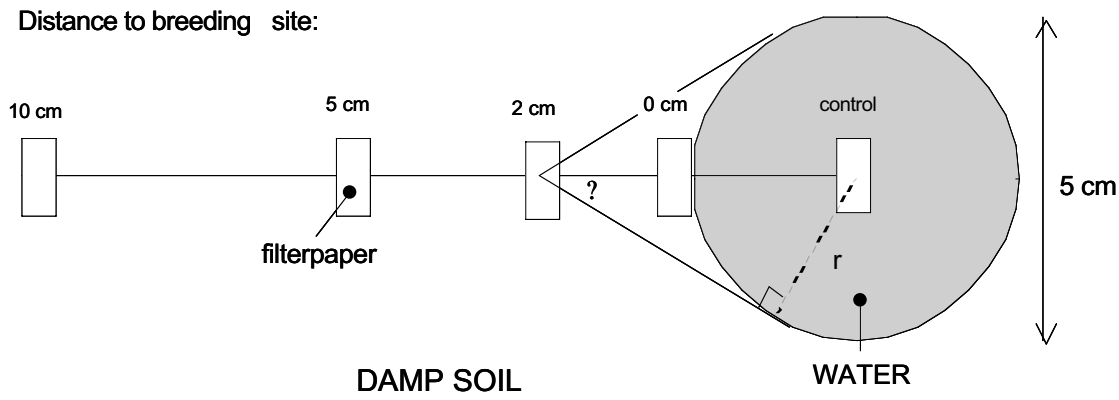


**Figure 1**
Experimental set-up of the egg hatching experiment showing the positions of the filter paper relative to the simulated breeding site.

To test for differences between distances, the observed proportions of first-instar larvae in the artificial breeding site were arcsine square root transformed [17]. Analysis of variance (ANOVA) was done on transformed data, followed by Tukey HSD *post hoc* tests for pairwise comparisons between treatments to determine significance levels. All calculations were carried out with SPSS 11.0 software.

For each replicate, the proportion of larvae expected to reach the site was calculated with the following two equations:

$p_{exp} = h * (2 * \alpha / 360)$ (1)

$\alpha = \text{invsin}((r + d + 0.25) / r)$ (2)

whereby $p_{exp}$ is the proportion expected in breeding site, $h$ the proportion hatched, $r$ the radius of the breeding site (in cm), $d$ the distance between the placement of eggs and the breeding site (0, 2, 5 or 10 cm) and 0.25 the distance between the centre and the edge of the filter paper (1.0 × 0.5 cm) (see Figure 1).

Calculations of $p_{exp}$ assumed that larvae move randomly in all directions upon hatching and that mortality during movement is negligible. To test these assumptions, we compared observed and expected proportions of larvae present in the breeding site. Proportions were arcsine square root transformed [17], after which paired t-tests were done. Calculations were carried out with SPSS 11.0 software.

### *Larval survival*

Volcanic soil from within the compound of MPRTC was sun-dried, filtered through wire mesh (3 mm) and saturated with water from Lake Victoria (1 litre of soil : 0.38 litre of water). For each of the four instars tested, 12 metal trays (26 × 26 × 9 cm) were filled with the saturated soil and four depressions (4 cm ∅, 8 cm deep) were evenly distributed in the soil of each tray. At the beginning of the experiment, 20 first, second, third or fourth-instar An. gambiae s.s. larvae were placed on the damp soil at the bottom of each depression. Six hours after the beginning of the experiment, 4 depressions per instar were filled completely with water to simulate rainfall. Larvae that appeared alive at the water surface were counted and removed, and the depressions were re-examined 6 and 12 hours later. Twelve hours after the beginning of the experiment, another 4 depressions per instar were filled with water and larvae appearing at the water surface counted. This procedure was continued at 6 hour intervals up to 72 hours after the start of the experiment. All metal trays were placed inside a greenhouse in a large basin of water to prevent ants from disturbing the experiments. During the experiment, trays were exposed to ambient conditions and allowed to dry out. Outdoor temperatures during the experiment ranged from 17.9°C to 28.7°C and averaged 23.2°C.

**Table 1: Mean proportions (± s.e.) of *An. gambiae s.s.* eggs hatched on damp soil at different distances from a simulated breeding site and mean proportions (± s.e.) of eggs that hatched after 3 days after placement in water. Different letters within the second column indicate significant differences ($P < 0.05$) between the treatments as determined by Tukey HSD *post hoc* comparisons.**

| Treatment | Proportion hatched on damp soil within 3 days | Proportion additionally hatched in water on the 4th day |
|---|---|---|
| 0 cm | 0.79 ± 0.14 a | 0.012 ± 0.027 |
| 2 cm | 0.30 ± 0.12 b | 0.120 ± 0.121 |
| 5 cm | 0.67 ± 0.20 a | 0.024 ± 0.026 |
| 10 cm | 0.54 ± 0.18 ab | 0.075 ± 0.034 |
| Control (in water) | 0.85 ± 0.05 a | |

For each replicate, the total number of larvae appearing at the water surface within 12 hours was expressed as the proportion of the total number of larvae tested (20 per replicate) and these proportions were used as a measure of survival. The proportions were arcsine square root transformed [17], after which a linear regression model was fitted through the data to evaluate survival through time for each instar. All calculations were done with the SPSS 11.0 software

## Results

### *Egg hatching and larval movement*

At all distances from the breeding site, eggs of *An. gambiae s.s.* hatched on filter paper placed on damp soil, but the proportion that hatched within 3 days at 2 cm distance of the artificial breeding site was significantly lower than the control, 0 and 5 cm treatments (Table 1). When, after the experiment, the unhatched eggs were placed directly in the water, the highest proportion additionally hatching originated from the 2 cm treatment, followed by the 10, 5 and 0 cm treatment (Table 1). Although ANOVA showed a significant difference between these proportions ($P = 0.039$), Tukey HSD *post hoc* tests could not detect significant differences when proportions were compared pairwise.

The proportion of newly emerged larvae that reached the site differed significantly between distances (ANOVA, $P < 0.001$; Figure 2). Eighty-five percent of the eggs that were placed directly in the water, i.e. the control treatment, hatched within 3 days. A significantly lower proportion of larvae was recovered from the site, when eggs had been placed at the edge of the water surface (Tukey HSD; $P < 0.001$). At 2, 5 and 10 cm again a significantly lower proportion of larvae was recovered in the site, but no differences in proportions were observed between these three distances (Tukey HSD, $P > 0.05$).

The observed proportion of free-swimming larvae in the artificial breeding site, that were placed during the egg stage on the edge of the artificial breeding site (0 cm treatment), was significantly greater than expected (paired t-test, $P < 0.05$), while at 2 and 5 cm no significant differences were found between observed and expected proportions (paired t-test, $P > 0.05$; Figure 2). At 10 cm distance, the proportion reaching the site was significantly lower than expected (paired t-test, $P < 0.05$).

### *Larval survival*

Figures 3 and 4 show the average proportion of larvae that appeared at the water surface, and thus survived, after exposure to different periods of drought for all four larval stages. The estimated regression parameters of the linear models that were fitted through the data after arcsine square root transformation are shown in Table 2. By comparing the 95% confidence intervals of regression coefficient $a$ of the fourth-instar with that of the other three instars, we found that survival of fourth-instar larvae was significantly higher than of the other three instars ($P < 0.05$). Using the model, we predicted that 50% of first, second and third-instar larvae had died after 31, 29 and 33 hours, respectively, while 50% of the fourth-instar larvae had died after 53 hours (Table 2). We estimated that first, second and third-instar larvae could survive up to 64, 65 and 69 hours, respectively, while fourth-instar larvae survive up to 113 hours (4.7 days) under the experimental conditions.

## Discussion

When natural larval habitats of *An. gambiae s.l.* dry up, their contribution towards the population dynamics of the adult stage is often neglected, since mass larval mortality is assumed to occur [9]. We found that eggs of *An. gambiae s.s.* hatched and that emerging larvae showed limited capability of reaching a nearby breeding site, when placed on damp soil. With this experiment we simulated a natural situation whereby eggs remain on damp soil when the water level drops or eggs are oviposited deliberately on damp soil after desiccation of the habitat [8,10]. In addition, we found that larvae of *An. gambiae s.s.* survive for several days in sites that are drying up, depending on the larval stage.

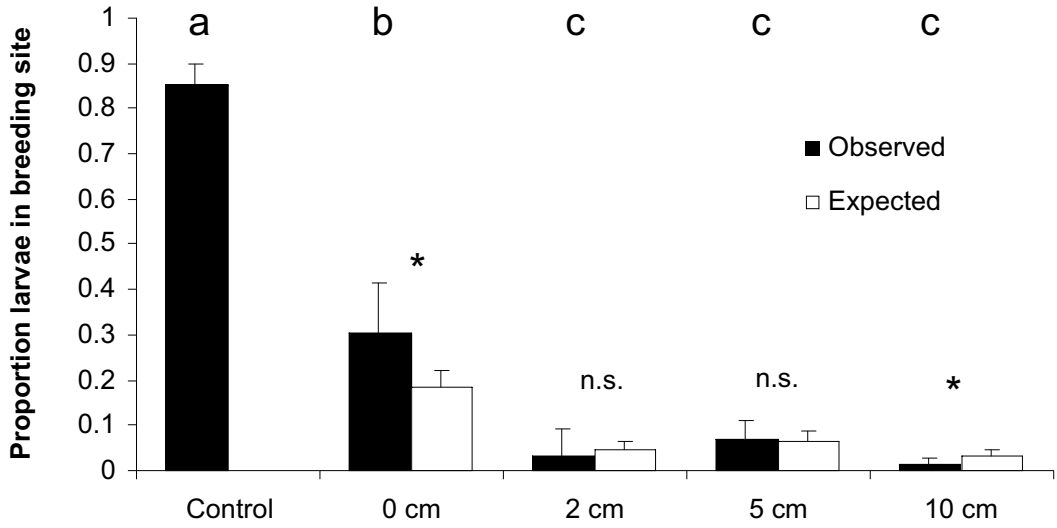


**Figure 2**
Observed and expected proportions of larvae in the simulated breeding sites, placed during the egg stage at different distances from the site. Different letters indicate significant differences ($P$ <0.05, Tukey HSD) between the observed proportions at the different distances. Significant differences ($P$ <0.05, paired t-test) between observed and expected proportion of a distance are indicated by '*'; n.s. = not significant

Larvae that hatch from eggs on damp soil were able to reach a breeding site within a distance of 10 cm, albeit in low numbers. We induced hatching of the eggs by keeping the soil of our experimental sites damp by daily spraying of water, but during spraying we made sure that no water ran off or that a film of water was created. Surprisingly, the proportion that hatched at 2 cm distance was significantly lower than at 0 and 5 cm distance. During the experiment we noticed that the plates with the 2 cm treatments dried out more quickly than the other plates. This may be explained because the 2 cm plates were placed closest to the door of the experimental room, and could have dried faster as a result of draught. The observation that the highest proportion of additional hatching occurred in the 2 cm treatment, supports the idea that these eggs may have been exposed to excessively dry conditions during their egg stage and may not have hatched during the first 3 days as a result.

Assuming random movement of newly hatched larvae, we found that more larvae than expected reached the artificial breeding site, when hatched directly on the edge of the site. We observed that a ring of water film (of ~1 cm) was present, directly around the edge of the artificial site, which may explain the higher than expected numbers in the water at this distance. At 2 and 5 cm, there was no difference between observed and expected, while at 10 cm fewer than expected larvae reached the site. This can be explained by assuming that at greater distances mortality during movement towards the site becomes important. Energy reserves may get depleted and larvae may die before reaching the water.

All different larval instars of *An. gambiae s.s.* survived on damp soil, but the duration of survival depended on the developmental stage of the larvae. From our data we estimated that first, second and third-instar larvae could survive 64–69 hours maximum (2.7 – 2.9 days), while

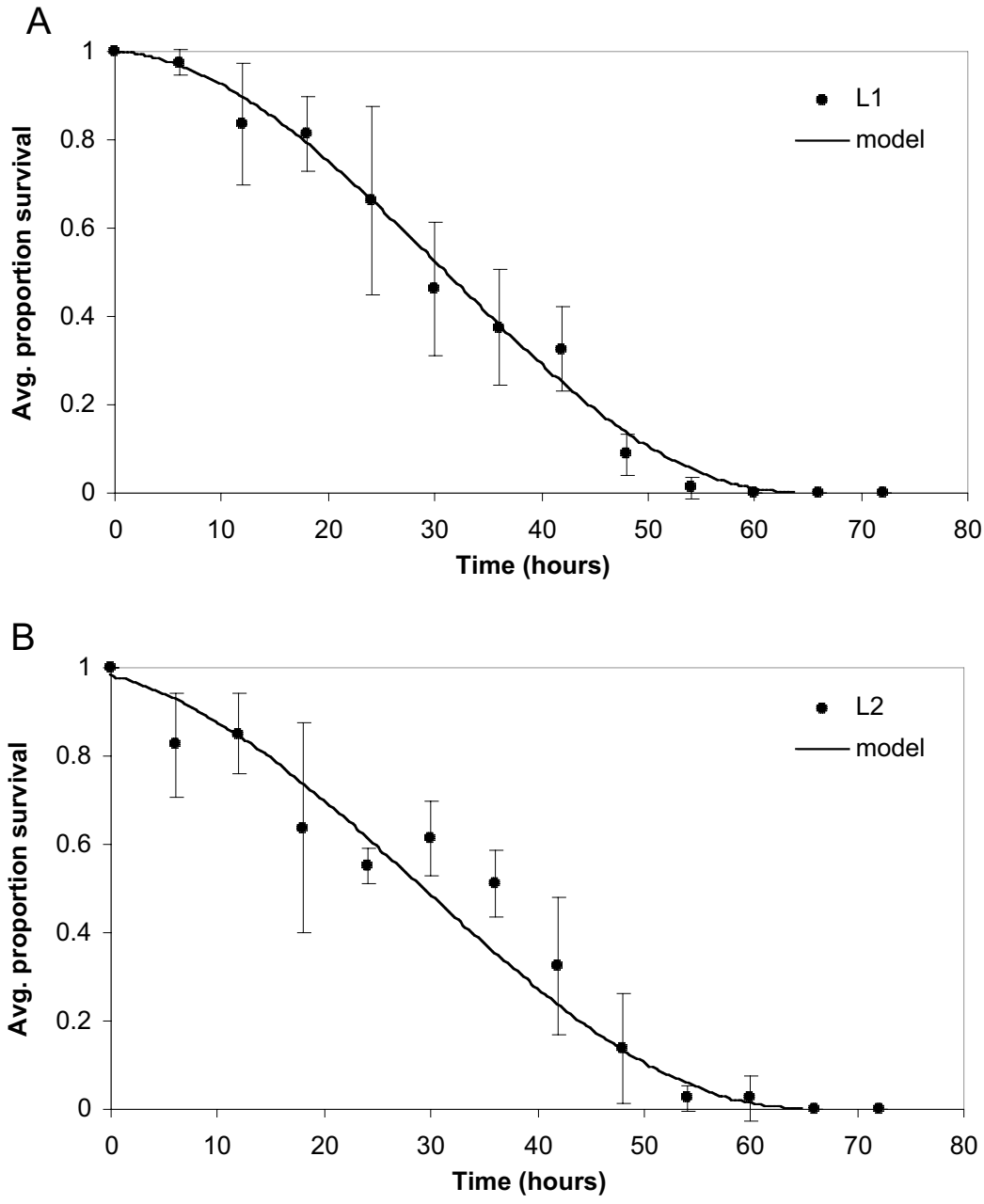


**Figure 3**
Average proportion (± s.e.) of larvae of *An. gambiae s.s.* surviving on damp soil after exposure to different periods of drought. The lines show the models after back-transformation of the linear model (arcsine($\sqrt{p}$) = $a$ * time + $b$) obtained after arcsine square root transformation of the original data. Model descriptions are given in Table 2. A: first-instar larvae, B: second-instar larvae.

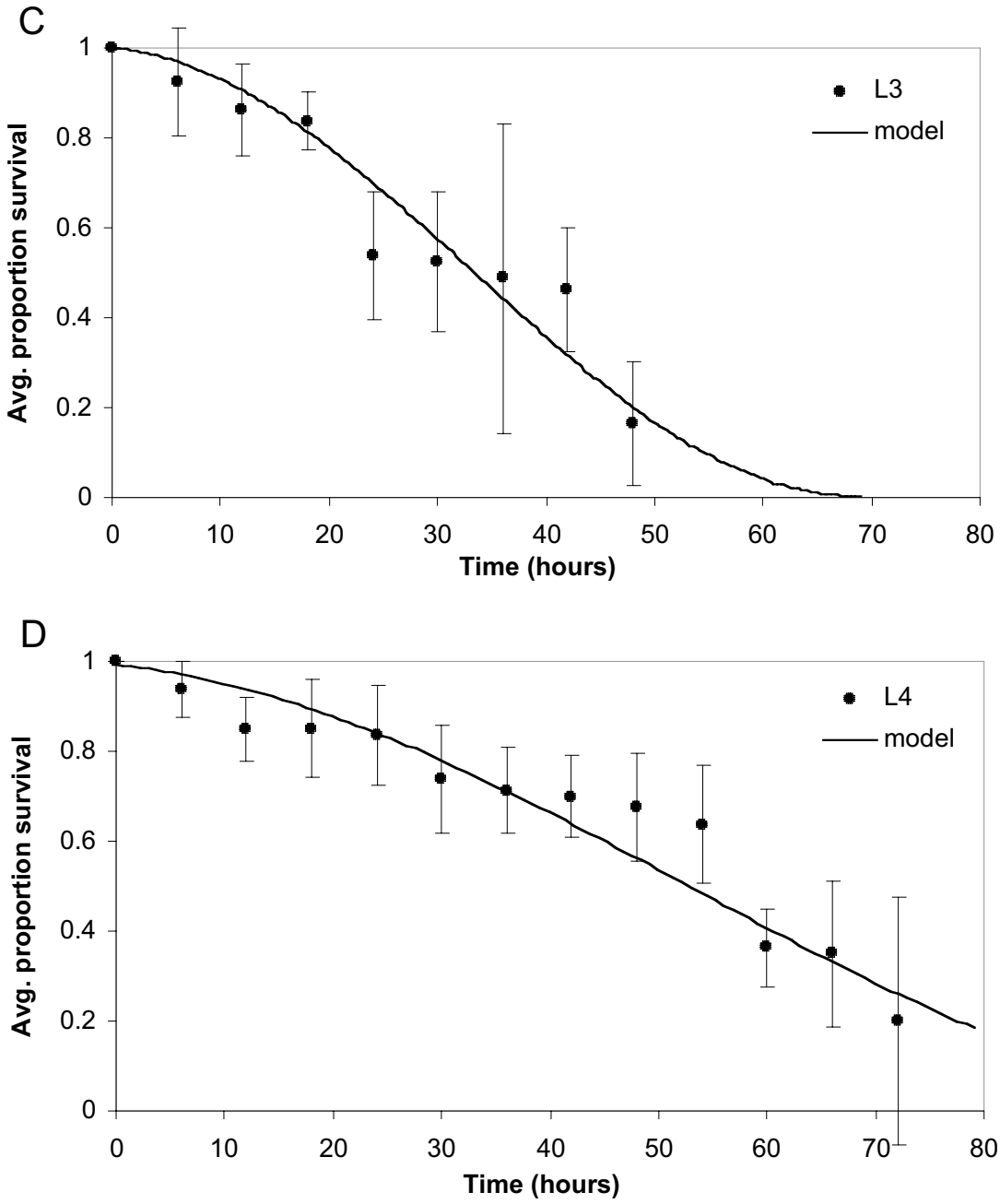


**Figure 4**
Average proportion (± s.e.) of larvae of *An. gambiae s.s* surviving on damp soil after exposure to different periods of drought. The lines show the models after back-transformation of the linear model (arcsine($\sqrt{p}$) = $a$ * time + $b$ ) obtained after arcsine square root transformation of the original data. Model descriptions are given in Table 2. C: third-instar larvae (observed for 48 hours only), D: fourth-instar larvae

**Table 2: Regression coefficients (with 95% confidence intervals (C.I.)) after arcsine square root transformation of survival proportions for the four different instars of *An. gambiae s.s.*. Model predictions of 50% and 0% survival are given in hours.**

| | Model: arcsine ($\sqrt{p}$) = $a$ * time + $b$ | | | | | Model predictions | |
|---|---|---|---|---|---|---|---|
| instar | $a$ | 95 % C.I. | $b$ | 95 % C.I. | $R^2$ | 50 % survival (hours) | 0 % survival (hours) |
| L1 | -0.024 | -0.026 – -0.022 | 1.53 | 1.46 – 1.61 | 0.931 | 31 | 64 |
| L2 | -0.022 | -0.024 – -0.019 | 1.43 | 1.34 – 1.52 | 0.895 | 29 | 65 |
| L3 | -0.022 | -0.027 – -0.018 | 1.52 | 1.39 – 1.66 | 0.727 | 33 | 69 |
| L4 | -0.013 | -0.015 – -0.011 | 1.47 | 1.38 – 1.56 | 0.748 | 53 | 113 |

fourth-instar larvae could survive markedly longer up to 113 hours (4.7 days). However, this latter estimate should be considered with caution, since the model was based on data up to 72 hours (3 days) only. Other published experimental tests with larval survival of *An. gambiae* in drying habitats are few. Muirhead-Thomson [13] found that young larvae could survive for two days on damp soil, while older instars 'were more susceptible to drying up'. Unfortunately, no precise maximum survival time was given. Our study, however, suggests the opposite for survival of fourth-instar larvae, since they survived significantly longer than larvae of the first 3 instars. Holstein [12] observed that larvae (instar undetermined) still developed into pupae after desiccation on damp clay for 4 days. Other evidence of survival on damp soil is anecdotal and comes from personal observations of larvae appearing after adding water to recently desiccated natural habitats.

Female *An. gambiae* mosquitoes are assumed to use semiochemicals to select suitable aquatic habitats for oviposition [18,19]. Interestingly, they take a high risk when they select damp soil sites for oviposition, as has been shown for this species [8]. This behaviour is not uncommon since other malaria vector species, such as *An. balabacensis* [20], *An. albimanus* [21] and the closely related *An. melas* [10] express this behaviour as well. Probably, the high risk of laying eggs on damp soil pays off when the sites are flooded, since these sites will then not have been colonised by predators, parasites and/or pathogens [22,23].

Although eggs of *An. gambiae* can survive for 12–16 days under dry conditions [11,12] and larvae for a few days on humid soil (this study, [12,13]), we argue that these strategies may not be sufficient to survive throughout the dry season, which generally lasts 6–8 months in sub-Saharan Africa. Other mosquito species, such as *Aedes* spp. [24] or other Diptera, such as *Pylopedilum vanderplanki* (Chironomidae) [25], have long dormant immature stages of a few months and several years, respectively, which are adaptive for surviving long-term adverse conditions. Nevertheless, the short-term survival of eggs and larvae of *An. gambiae* in drying habitats may be adaptive for rapid exploitation of extremely dynamic habitats. Sites may dry up relatively quickly, even during the wet season, but a rain shower normally follows within a few days and development can then resume immediately. We did not investigate, however, to what extent the desiccation period affected the fitness of the adult stages, but this will be addressed in future studies. Long-term survival throughout the dry season is most likely restricted to the adult stages, either as aestivating females [5,6] or as populations in hidden *refugia* [7,8].

Although the World Health Organization considers larval control to have limited applicability in Africa [26], strategies directed towards the aquatic stages of *An. gambiae*, such as biological control with *Bacillus thuringiensis* var. *israelensis* (Bti) or *Bacillus sphaericus* [27–29] and drainage of larval habitats, may provide important tools for the Roll Back Malaria Initiative in Africa [30,31]. The data resulting from this study suggest that, if larviciding is applied, recently desiccated habitats should be treated as well. If not, larvae in these desiccated habitats may survive and the habitats may become an important source of rapid re-infestation. As a consequence, control attempts may be frustrated.

Apart from having direct implications for larval control operations, the results will also affect environmental management practices, in particular intermittent drainage of irrigated rice fields. Although rice irrigation is associated with an increased malaria risk in some areas and a decreased risk in others (reviewed in [32]), there is general concensus that proper water management is required to obtain high rice yields with low water consumption and no additional malaria risk (reviewed in [33]). Therefore, intermittent irrigation seems to be the best choice [34,35], but if the dry field period is too short, the larval stages will survive, as shown in this study. Within three dry days, most of the young instar larvae will have been killed, but only after five dry days will the older instars have been killed. Iterative drainage for a period of several days seems feasible for rice irrigation schemes, but only where climate conditions favour rapid drying [33].

## Conclusions

Results of this study showed that eggs of *An. gambiae s.s.* hatch on damp soil. Emerging larvae, albeit in low numbers, are capable of reaching a nearby artificial breeding site within a range of 10 cm. In addition, larvae of *An. gambiae s.s.* survive on damp soil for at least a few days, depending on the larval stage. The short-term survival, as shown in this study, may be important and adaptive for the transient nature of the breeding sites of this species in sub-Saharan Africa. In addition, the results may have important implications for larval vector control methods: habitats should remain drained for at least 5 days to kill all larvae and recently desiccated habitats should be treated as well if larvicidal agents are applied.

## Authors' contributions

CJMK developed and carried out the experiments together with KPP. AKG, BGJK and WT supervised the experiments and actively contributed to the interpretation of the findings. All authors read and approved the final manuscript.

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## References

1. Gillies MT and DeMeillon B: *The Anophelinae of Africa South of the Sahara South African Institute for Medical Research, Johannesburg*; 1968.
2. Gill CA: **The relationship between malaria and rainfall** *Ind J Med Res* 1920, **37:**618-632.
3. Patz JA, Strzepek K, Lele S, Hedden M, Greene S, Noden B, Hay SI, Kalkstein L and Beier JC: **Predicting key malaria transmission factors, biting and entomological inoculation rates, using modelled soil moisture in Kenya** *Trop Med Intl Hlth* 1998, **3:**818-827.
4. Craig MH, Snow RW and le Sueur D: **A climate-based distribution model of malaria transmission in sub-Saharan Africa** *Parasitol Today* 1999, **15:**105-111.
5. Omer SM and Cloudsley-Thompson JL: **Survival of female *Anopheles gambiae* Giles through a 9-month dry season in Sudan** *Bull World Health Organ* 1970, **42:**319-330.
6. Taylor CE, Touré YT, Coluzzi M and Petrarca V: **Effective population size and persistence of *Anopheles arabiensis* during the dry season in west Africa** *Med Vet Entomol* 1993, **7:**351-357.
7. Charlwood JD, Vij R and Billingsley PF: **Dry season refugia of malaria-transmitting mosquitoes in a dry savannah zone of east Africa** *Am J Trop Med Hyg* 2000, **62:**726-732.
8. Minakawa N, Githure JI, Beier JC and Yan G: **Anopheline mosquito survival strategies during the dry period in western Kenya** *J Med Entomol* 2001, **38:**388-392.
9. Charlwood JD, Kihonda J, Sama S, Billingsley PF, Hadji H, Verhave JP, Lyimo E, Luttikhuizen PC and Smith T: **The rise and fall of Anopheles arabiensis (Diptera: Culicidae) in a Tanzanian village** *Bull Entomol Res* 1995, **85:**37-44.
10. Giglioli MEC: **Oviposition by *Anopheles melas* and its effect on egg survival during the dry season in the Gambia, West Africa** *Ann Entomol Soc Am* 1965, **58:**885-891.
11. Beier JC, Copeland R, Oyaro C, Masinya A, Odago WO, Oduor S, Koech DK and Roberts CR: **Anopheles gambiae complex egg-stage survival in dry soil from larval development sites in western Kenya** *J Am Mosq Assoc* 1990, **6:**105-109.
12. Holstein MH: *Biology of Anopheles gambiae – Research in French West Africa Geneva: World Health Organization*; 1954.
13. Muirhead-Thomson RC: **Studies on the breeding places and control of *Anopheles gambiae* and *A. gambiae* var. *melas* in coastal districts of Sierra Leone** *Bull Entomol Res* 1945, **36:**185-252.
14. Stone WS and Reynolds FHK: **Hibernation of anopheline eggs in the tropics** *Science* 1939, **90:**371-372.
15. Rajapaksa N: **Field and laboratory observations in Sabah, East Malaysia on the proportion of *Anopheles balabecensis balabacensis* eggs hatching after holding in a humid atmosphere** *Bull World Health Organ* 1971, **45:**263-265.
16. Roberts D: **Mosquitoes (Diptera:Culicidae) breeding in brackish water: female ovipositional preferences or larval survival?** *J Med Entomol* 1996, **33:**525-530.
17. Sokal RR and Rohlf FJ: *Biometry – The principles and practice of statistics in biological research* 3rd edition. *New York: W.H. Freeman and Company*; 1995.
18. Takken W and Knols BGJ: **Odor-mediated behavior of Afrotropical malaria mosquitoes** *Annu Rev Entomol* 1999, **44:**131-157.
19. Blackwell A and Johnson SN: **Electrophysiological investigation of larval water and potential oviposition chemo-attractants for *Anopheles gambiae s.s*** *Ann Trop Med Parasitol* 2000, **94:**389-398.
20. Wilkinson RN, Gould DJ, Boonyakanist P and Segal HE: **Observations on *Anopheles balabacensis* (Diptera: Culicidae) in Thailand** *J Med Entomol* 1978, **14:**666-671.
21. Breeland SG, Pletsch DJ and Quarterman KD: **Laboratory studies on the suitability of mud as an oviposition substrate for *Anopheles albimanus* Wiedemann** *Mosq News* 1970, **30:**81-88.
22. Service MW: **Mortalities of the immature stages of species B of the *Anopheles gambiae* complex in Kenya: comparison between rice fields and temporary pools, identification of predators, and effects of insecticidal spraying** *J Med Ent* 1977, **13:**535-545.
23. Service MW: **Mortalities of the larvae of the *Anopheles gambiae* Giles complex and detection of predators by the precipitin test** *Bull Entomol Res* 1973, **62:**359-369.
24. Sota T and Mogi M: **Interspecific variation in desiccation survival time of *Aedes* (Stegomyia) mosquito eggs is correlated with habitat and egg size** *Oecologia* 1992, **90:**353-358.
25. Hinton HE: **Cryptobiosis in the larva of *Polypedilum vanderplanki* Hint. (Chironomidae)** *J Ins Physiol* 1960, **5:**286-300.
26. WHO: **Global fund to fight AIDS, tuberculosis and malaria – The global malaria situation: current tools for prevention and control** *A55/INF.DOC./6* .
27. Majori G, Ali A and Sabatinelli G: **Laboratory and field efficacy of *Bacillus thuringiensis* var. *israelensis* and *Bacillus sphaericus* against *Anopheles gambiae s.l.* and *Culex quinquefasciatus* in Ouagadougou, Burkina Faso** *J Am Mosq Control Assoc* 1987, **3:**20-25.
28. Barbazan P, Baldet T, Darriet F, Escaffre H, Djoda DH and Hougard JM: **Impact of treatments with *Bacillus sphaericus* on *Anopheles* populations and the transmission of malaria in Maroua, a large city in a savannah region of Cameroon** *J Am Mosq Control Assoc* 1998, **14:**33-39.
29. Fillinger U, Knols BGJ and Becker N: **Efficacy and efficiency of new *Bacillus thuringiensis* var. *israelensis* and *Bacillus sphaericus* formulations against Afrotropical anophelines in Western Kenya** *Trop Med Intl Hlth* 2003, **8:**37-47.
30. Killeen GF, Fillinger U and Knols BGJ: **Advantages of larval control for African malaria vectors: Low mobility and behavioural responsiveness of immature mosquito stages allow high effective coverage** *Malaria J* 2002, **1:**8.
31. Killeen GF, Fillinger U, Kiche I, Gouagna LC and Knols BGJ: **Eradication of Anopheles gambiae from Brazil: lessons for malaria control in Africa?** *The Lancet Infectious Diseases* 2002, **2:**618-627.
32. Ijumba JN and Lindsay SW: **Impact of irrigation on malaria in Africa: paddies paradox** *Med Vet Entomol* 2001, **15:**1-11.
33. Keiser J, Utzinger J and Singer BH: **The potential of intermittent irrigation for increasing rice yields, lowering water consumption, reducing methane emissions, and controlling malaria in African rice fields** *J Am Mosq Control Assoc* 2002, **18:**329-340.
34. Klinkenberg E, Takken W, Huibers F and Touré YT: **The phenology of malaria mosquitoes in irrigated rice fields in Mali** *Acta Trop* 2003, **85:**71-82.
35. Mutero CM, Blank H, Konradsen F and Hoek Wvd: **Water management for controlling the breeding of *Anopheles* mosquitoes in rice irrigation schemes in Kenya** *Acta Trop* 2000, **76:**253-263.