

Semi-field evaluation of the effect of cow and chicken dung on life history traits of *Anopheles funestus* in rural south-eastern Tanzania

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Abstract

Background

Anopheles funestus is a major malaria vector in south-eastern rural Tanzania. Larval development and survival are influenced by environmental conditions, including livestock manure present in breeding habitats. While previous studies on *An. arabiensis* and *An. gambiae* have documented that nutrient-rich substrates like manure can affect mosquito life history traits, the impact of substrates on *An. funestus* remains poorly understood. Therefore, this study assessed the effect of cow and chicken dung on selected life history traits of *An. funestus* under semi-field system.

Methods

First-instar *An. funestus* larvae were exposed to cow or chicken dung leachate at four concentrations (0.25, 0.5, 0.75 and 1.0 g/100mL) and compared with clean-water controls under semi-field conditions. For each dung type, 20 larvae were introduced per concentration per replicate across five replicate rounds. Larval survival, adult emergence, development rate, and wing length were recorded.

Results

Chicken dung caused concentration-dependent reductions in larval survival and adult emergence ($p < 0.001$), increased larval development rate ($p = 0.004$), and reduced adult body size, while cow dung produced weaker effects. Physicochemical parameters, including pH, electrical conductivity, temperature and algae presence, varied with dung concentration for both dung types and were associated with larval mortality. However, these associations should be interpreted cautiously because they were correlated with dung concentration; ammonia and nitrate also increased progressively with concentration for both dung types.

Conclusions

Under the semi-field conditions and concentrations tested, livestock manure differentially affected *Anopheles funestus* life-history traits. Chicken dung showed strong concentration-dependent negative effects, whereas cow dung produced comparatively milder effects. Field validation is required before drawing operational conclusions about manure management and malaria vector control.

Background

Malaria remains a major global public health challenge, with cases rising to 282 million cases globally in 2024, representing an increase of about 9 million cases (3%) compared with 2023 [1]. Despite ongoing control efforts, progress in reducing malaria transmission has slowed in recent years, particularly in

resource-limited settings [1]. The WHO African Region continues to bear the greatest burden, accounting for approximately 94% of global malaria cases and 95% of malaria deaths [1]. Within this region, Tanzania is among the ten countries with the highest malaria burden, contributing about 3.3% of global malaria cases and 4.3% of malaria deaths [1]. The persistence of malaria transmission is driven by multiple interacting factors, with one of the most significant being human activities, particularly agricultural practices, which can modify aquatic habitats and affect mosquito breeding, survival, and population dynamics [2–5].

Agriculture contributes to economic development and food security. In sub-Saharan Africa, agriculture is the backbone of the economy and major source of food and livelihood [6]. However, some agricultural practices can inadvertently increase the risk of malaria transmission by altering mosquito habitats [5]. Irrigation systems and water management practices associated with farming create persistent standing water bodies such as canals, puddles, furrows and irrigated fields, which provide stable, shallow aquatic environments stable breeding sites for *Anopheles*, supporting larval development and allowing mosquito populations to persist and reproduce outside of natural rainfall cycles [2, 4, 7, 8]. Studies from irrigated agro-ecosystems in sub-Saharan Africa show that irrigated landscapes have significantly more diverse and abundant mosquito breeding habitats than non-irrigated areas, with irrigation infrastructure producing a higher number of larval sites that can sustain malaria vectors throughout both dry and wet seasons [7–11]. Furthermore, habitat modification through deforestation and conversion of natural vegetation to agricultural land alters local microclimates, reduces habitat complexity and predator assemblages, increases water temperature and sunlight exposure in breeding sites, and thereby enhances *Anopheles* larval survival and development, increasing vector abundance and malaria transmission potential [5, 12–15]

Beyond creating physical aquatic breeding sites, agricultural practices in smallholder farming systems also introduce organic inputs particularly livestock manure, that chemically alter aquatic environments in ways that directly favour *Anopheles* larval development [16–19]. Livestock manure, particularly cow and chicken dung are among the most commonly used organic fertilizers (OFs) in both traditional and urban agricultural systems [20–22]. In Tanzania, the widespread use of these fertilizers is supported by the country's large livestock population. According to the National Sample Census of Agriculture 2019/20, Tanzania has approximately 87.7 million chickens and 33.9 million cattle, most of which are owned by smallholder farmers [23]. However, the contamination of aquatic habitats by livestock manure is not limited to its use as a crop fertilizer. Improper disposal and storage of manure near water bodies, surface runoff from manured fields during rainfall events, and free-range pastoral systems where cattle deposit dung directly into or adjacent to standing water all represent key pathways through which nutrient-rich organic matter enters larval habitats [19, 24–26]. However, the effects of livestock manure on mosquito larval habitats are not always positive and may depend on the level of organic enrichment. At low to moderate concentrations, manure-derived nutrients stimulate microbial growth, increasing the availability of bacteria, algae, and fungi that serve as primary larval food resources [17, 25, 27]. At higher loading, however, rapid decomposition may alter physicochemical conditions including pH, electrical conductivity and nitrogenous compounds, potentially creating chemically stressful or oxygen-limited

conditions. Therefore, the net effect of manure on mosquito fitness is expected to depend on dung type, concentration, exposure duration and local habitat conditions [16, 19].

Although livestock manure enriches larval habitats, its effects on mosquito life history traits are not uniform across dung types. Cow and chicken dung differentially affect *Anopheles* mosquitoes; chicken dung reduces larval survival and adult production in *An. gambiae* s.s. and *An. arabiensis*, whereas cow dung is associated with enhanced larval development rates [28]. Further evidence indicates that both cow and chicken dung act as oviposition deterrents to *An. arabiensis*, with chicken dung producing stronger deterrence than cow dung, suggesting that manure-based organic fertilizers may also modulate the spatial distribution of egg-laying and therefore the production of new vector cohorts within agroecosystems [29]. These distinct effects illustrate the complex interactions between organic fertilizers and mosquito populations, which vary based on the type of fertilizer used, its concentration and the specific mosquito species involved [28–30]. However, despite the evidence of the effects of cow and chicken dung on *An. gambiae* s.s and *An. arabiensis* [28], its potential effects on *Anopheles funestus* remain unexplored.

Anopheles funestus, is a dominant malaria vector in eastern and southern Africa [31]. In south-eastern Tanzania and specific districts around Lake Victoria, this species is responsible for more than 90% of all malaria transmission events [32, 33], largely due to its strong anthropophilic (i.e. strong preference for blood meals from humans) and endophilic (i.e. strong preference for resting indoors than outdoors) [34, 35]. Its larvae develop mainly in permanent or semi-permanent aquatic habitats, including spring-fed pools, ponds, and slow-moving river tributaries typically clear, vegetated, deeper than 0.5 m, and located near human settlements and agricultural land [36], making these habitats particularly susceptible to contamination by livestock manure through surface runoff, improper disposal, and free-range livestock access. Understanding the ecological and biological factors that influence the population dynamics of *An. funestus* is crucial for effective malaria control strategies. Therefore, this study assessed the effects of cow and chicken dung on selected life history traits of *An. funestus* specifically larval survival, development rate, adult emergence and adult body size in a semi-field system (SFS), to better understand the implications of livestock manure inputs for malaria vector populations.

Methods

Study area

The study was conducted between May and December 2025 in Kining'ina village, Kilombero District, south-eastern Tanzania (8.11417°S, 36.67484°E) (Fig. 1). The area lies at an average altitude of 270 m above sea level, with annual rainfall ranging from 1200 to 1800 mm, temperature ranging between 20 and 32°C, and relative humidity ranging from 51 to 71%. The area experiences a tropical climate characterized by distinct wet and dry seasons and supports extensive smallholder farming and livestock keeping, with cattle and poultry commonly raised by local households. Experiments were carried out within the semi-field system (SFS) of Ifakara Health Institute (IHI), a large screened enclosure designed

to simulate natural environmental conditions while allowing controlled mosquito experiments. Laboratory-reared colonies of *An. funestus* originating from south-eastern Tanzania and maintained at the institute's insectary were used in the experiments. A detailed description and validation of the SFS for entomological research have been published previously [37].

Mosquito strain used

First-instar larvae of *An. funestus* were obtained from the fully susceptible FUTAZ strain maintained at the insectary of IHI. The FUTAZ strain was originally established from wild mosquito populations collected in Ifakara, south-eastern Tanzania, and is known to be susceptible to commonly used pyrethroid insecticides. The colony has been maintained under controlled insectary conditions since 2018. Mosquitoes were reared at $27 \pm 2^\circ\text{C}$, 70–80% relative humidity, and a 12:12 h light-dark photoperiod. Larvae were maintained in trays containing dechlorinated water and fed TetraMin® fish food (Tetra GmbH, Germany) twice daily, while adult mosquitoes were held in cages and provided with a 10% sucrose solution *ad libitum*. For colony maintenance, adult females were periodically blood-fed using a membrane feeding system with warmed bovine blood.

Larvae were transported from the IHI insectary to the SFS in aerated containers designed to preserve viability during transport. Throughout the experiments, larvae in both treatment and control groups received the same standardised TetraMin® (Tetra GmbH, Germany) feeding regime twice daily. This standardised feeding was used to maintain baseline larval nutrition and ensure that differences between groups reflected dung-associated changes in the aquatic environment rather than nutritional limitation.

Cow and chicken dung collection and preparation

Freshly deposited cow dung was collected from cow paddocks at the Kining'ina semi-field site using a clean stainless-steel scoop to avoid contamination. The cows were freely grazing within the semi-field facility compound and fed exclusively on grasses growing naturally on the ground, free from pesticides and herbicides. No medication, including ivermectin, had been administered to the cows in the three months prior to the study period to ensure that residual compounds did not confound the experimental outcomes. Freshly deposited chicken dung was collected from domestic chickens (*Gallus gallus domesticus*) maintained in enclosures at the IHI Vector Sphere facility. These chickens had not been treated with any medications except for a single prophylactic dose of a coccidiostat administered during the chick stage and were fed exclusively on chicken bran throughout the study period. A fresh batch of dung was collected separately for each of the five experimental rounds, so no batch was reused or stored across rounds. Fresh manure varies considerably in water content, depending on the animal, its diet, how long ago it was deposited, and the surrounding environmental conditions [38]. If used fresh, this variation would make it impossible to know whether a fixed wet mass of dung represented the same amount of manure solids from one round to the next. To remove this source of variation, each batch was dried in a drying oven at 60°C for 24 hours to remove moisture and stabilize the dung before use. Low-

temperature drying (55–60°C) is commonly used for biological materials because it effectively removes moisture while minimizing chemical alterations compared with higher drying temperatures [28, 39]. The dried dung was then coarsely ground using a mortar and pestle to achieve a uniform particle texture and used immediately for concentration preparation.

Different concentrations of cow and chicken dung were prepared based on a weight-to-volume (w/v) ratio, expressed as grams of dried dung per 100 mL of water (g/100 mL). Dried dung was weighed using a portable digital pocket scale (200 g × 0.01 g precision). The required amount of dung for each treatment concentration was calculated by scaling the desired g/100 mL concentration to a final preparation volume of 1 L (1000 mL) of filtered well water. Since 1 L of water is equivalent to ten units of 100 mL, the required dung masses were 2.5 g, 5.0 g, 7.5 g, and 10.0 g for preparing concentrations equivalent to 0.25, 0.5, 0.75, and 1.0 g/100 mL, respectively. Each weighed dung amount was thoroughly mixed with 1 L of filtered well water measured using a graduated cylinder to produce the respective treatment concentrations. The control consisted of filtered well water without dung addition. To prevent dung dispersal and maintain visibility of larvae during observations, the dung was placed in permeable tea bag-like filter material prior to submersion, allowing nutrients to diffuse into the water while keeping solid particles contained. Because dried dung was enclosed in permeable filter material, the experiment assessed the effect of dung leachate rather than direct exposure to loose manure particles. This approach allowed soluble nutrients and chemical products to diffuse into the water while maintaining visibility of larvae, but it may not fully represent natural manure contamination where solid particles, microorganisms and habitat structure also influence larval ecology. The prepared mixtures were left to hydrate for 24 hours before introducing larvae to allow soluble nutrients and other leachates to diffuse into the water and establish stable treatment conditions.

Experimental design in the semi-field system

The experiment was conducted in two semi-field chambers (28.8 m × 7 m each), with one chamber assigned to cow dung treatments and the other to chicken dung treatments. Chamber assignment by dung type was fixed for the entire duration of the experiment; chambers were not rotated or alternated between dung types across replicate rounds. Consequently, any chamber-specific environmental variation, such as differences in shading, airflow, or microclimate between the two chambers, could not be statistically separated from the effect of dung type, and this is acknowledged as a limitation of the experimental design.

Within each chamber, five shallow ground depressions were prepared, each fitted with a 6 litre plastic basin to serve as an aquatic larval habitat. Four habitats containing dung solutions at concentrations of 0.25, 0.5, 0.75, and 1.0 g/100 mL served as treatment group, while one habitat containing untreated water served as the control (Fig. 2). Larvae were allocated to basins from the same cohort of first-instar larvae, and observations were conducted using a standardized order across treatments. Temperature and relative humidity were monitored continuously using a data logger (Tinytag TV-4500, manufactured by Gemini Data Loggers UK Ltd) programmed to record measurements at 30-minute intervals.

Aquatic habitats were filled with different dung concentrations 24 hours before adding the larvae, allowing the dung to fully mix with water and release nutrients. All habitats were covered with nylon nets to trap emerging adults and limit the introduction of other insects in the habitats.

The experiment had a total of five replicates. Five replicates were used to account for biological and environmental variability between experimental runs and to improve the reliability and precision of treatment-effect estimates [40]. In each replicate round, twenty first-instar larvae of *An. funestus* (24 to 36 hours old) were introduced into each aquatic habitat.

Assessment of larval survival

Larval survival was monitored every 24 hours by counting and recording the number of live and dead larvae in each habitat across both treatment and control groups. Dead larvae were removed immediately upon detection to maintain water quality and ensure accurate recording of larval survival throughout the experiment. Observation continued from the first instar until all larvae have either died or pupated.

Assessment of larval development rate and adult emergence

Development rate was assessed by recording the time (days) taken for individual first-instar larvae to develop into pupae. Because larvae pupated at different times within the same habitat, pupae were collected daily using a Pasteur pipette immediately after pupation and transferred into plastic cups containing 50 mL of water from their original habitats to maintain the same exposure conditions. The cups were then placed inside 15×15×15 cm net cages and monitored daily until adult emergence. Adult emergence was determined by recording the number of mosquitoes that successfully reached the adult stage, expressed as a proportion of the total number of first-instar larvae initially introduced into each habitat.

Mosquito wing size measurement

Adult mosquitoes emerging from each treatment and control habitat were killed by freezing at – 20°C [41, 42]. Dead mosquitoes were collected and stored in Eppendorf tubes containing silica gel and cotton wool, then transported from the SFS to the IHI Insectary for wing measurement. In the Insectary, a single wing from each mosquito was placed on a microscope slide and a drop of distilled water was used for sticking it to the slide. Wing length was measured, using a micrometer ruler under a microscope, from the alula notch to the wing tip following procedures described elsewhere [43, 44]. Wing length was used as a proxy for mosquito body size.

Measuring the physicochemical parameters of dung treated water

Each concentration of cow and chicken dung was monitored daily to assess changes in physicochemical parameters. A NOYafa NF-EZ9908 4 In 1 pH Meter Water Tester was used to measure pH (0–14), Total Dissolved Solids (ppm), Electrical Conductivity ($\mu\text{S}/\text{cm}$) and Temperature ($^{\circ}\text{C}$). The presence or absence of algae in each treatment was recorded daily through visual observation.

A Digital Spectrophotometer (Hach DR 2800) was used to measure nutrient concentrations in the dung treated water, specifically nitrogen (N) in the form of ammonium ($\text{NH}_3\text{-N}$) and nitrate ($\text{NO}_3\text{-N}$). Ammonium was determined by the Nessler method (0.02–2.50 mg/L $\text{NH}_3\text{-N}$) with absorbance readings at 425 nm, while nitrate was quantified using the NitraVer 5 reagent via the cadmium reduction method, HR(0.3–30.0 mg/L $\text{NO}_3\text{-N}$) with absorbance at 500 nm. Samples yielding nutrient concentrations above the analytical range of either method were diluted with filtered water and re-analysed to obtain readings within the validated calibration range. Final $\text{NH}_3\text{-N}$ and $\text{NO}_3\text{-N}$ concentrations were calculated by multiplying the measured values by the corresponding dilution factors. Instrument calibration and quality control checks were conducted in accordance with the manufacturer's recommended procedures prior to sample analysis.

Data from physicochemical parameters measurements from each dung concentration were then statistically correlated with *An. funestus* life history traits, to assess how dung induced changes in water quality influenced larval survival.

Statistical analysis

Data were analysed using open-source statistical software, R version 4.5.2 [45].

Effect of cow and chicken dung concentrations and physicochemical parameters on larval survival of *An. funestus*

Survival analysis was conducted using the *survival* package [46] with survival defined as time to death (days); larvae that died before pupation were recorded as events, while those that pupated were right-censored on the day of pupation. Kaplan-Meier survival estimators were used to compare survival across concentrations via the log-rank test, and curves were visualized using the *survminer* package [47].

A separate multivariable Cox proportional hazards models were fitted for cow and chicken dung to estimate hazard ratios (HRs, 95% CI) for larval mortality, with concentration and physicochemical parameters (pH, electrical conductivity, temperature, and algae presence) as fixed effects and experimental round as a frailty term.

Ammonia (NH_3) and nitrate (NO_3^-) were measured once per round rather than daily, precluding their inclusion in the survival models; these were instead summarized descriptively by concentration and dung type.

Effect of cow and chicken dung on development rate

Development time (for larvae that survived to pupation) was calculated as the pupae-weighted mean pupation day, and development rate as its inverse (1/day). Dung concentration effects were analysed using linear mixed-effects models (concentration as a fixed effect, experimental round as a random effect); estimated margin means and 95% CIs were obtained using *emmeans* [48] and plotted with *ggplot2* [49].

Effect of cow and chicken dung on adult emergence

Adult emergence was analysed using binomialGLMMs in the *lme4* package [50], with dung concentration as fixed effects and experimental round as a random effect; Results were reported as odds ratios (ORs, 95% CI). Because no adults emerged at 0.75 and 1.0 g/100 mL chicken dung in any round, these concentrations caused complete separation and were excluded from the model, with treatment instead reported as observed emergence proportions (exact binomial 95% CI).

For larval survival, development rate, and adult emergence, Tukey-adjusted pairwise comparisons between concentrations were obtained using *glht* (survival models) or *emmeans* (development rate and emergence models); these adjusted *p*-value are distinct from the unadjusted *p*-values reported for individual model coefficients, and both are labelled explicitly where relevant.

Effect of cow and chicken dung on wing size

Mean differences in wing size were analysed using an estimation statistics using the *dabestr* package [51]. Wing size (mm) was the continuous response variable and concentration (g/100 mL) as a categorical predictor. Mean differences between each treatment and control were estimated via bootstrap resampling (95% CI) and visualized using Gardner-Altman estimation plots [51]

All plots were generated using the *ggplot2* package [49]. Model diagnostics using the *DHARMa* package [52] were performed for the development-rate linear mixed model; other models were evaluated using standard model-summary diagnostics. Statistical significance was assessed at $p < 0.05$.

Results

Effect of chicken dung on *An. funestus* larval survival

There was a statistically significant difference in larval survival of *An. funestus* across chicken dung concentrations overall (log-rank test, $\chi^2 = 192$, $df = 4$, $p < 0.001$, Fig. 3 & Table 1). Post hoc pairwise comparisons further showed that all treatment concentrations were associated with significantly reduced larval survival compared with the control ($p < 0.001$, Figure provided in additional **file 1**). Among the higher concentration, larval mortality risk did not differ significantly between 0.75 and 0.5g/100ml ($p = 0.082$) or between 1.0 and 0.75 g/100ml ($p = 0.997$), but was significantly higher at 1.0 g/100ml than at 0.5 g/100ml ($p = 0.032$, additional **file 1**).

Table 1
Hazard ratios for larval mortality of *An. funestus* across different chicken dung concentrations relative to the control.

Concentrations (g/100ml)	HR [95% CI]	p-value	Median survival, days [95% CI]
Control	1		16 [14, NA]
0.25	3.86 (2.52, 5.92)	< 0.001	9 [8, 11]
0.5	9.59 (6.28, 14.64)	< 0.001	5 [5, 7]
0.75	14.03 (9.21, 21.37)	< 0.001	5 [4, 5]
1.0	14.74 (9.63, 22.56)	< 0.001	4.5 [3, 5]

Effect of chicken dung on adult emergence

There was a significant effect of chicken dung concentration on adult emergence overall (Type III Wald, $\chi^2 = 43.43$, $df = 2$, $p < 0.001$). Adult emergence of *An. funestus* was significantly lower at 0.25 and 0.5 g/100 mL compared with the control ($p < 0.001$, Table 2). No adult emergence was observed at 0.75 and 1.0 g/100 mL, because complete separation prevented reliable odds ratio estimation for these concentrations, they were reported descriptively using exact binomial 95% confidence intervals (observed emergence = 0%, 95% CI: 0-3.6% for both concentrations). Tukey-adjusted pairwise comparisons similarly showed significant differences among all concentrations (all $p < 0.05$).

Table 2
Adult emergence success (%) of *An. funestus* across chicken dung concentrations, from a generalized linear mixed-effects model.

Concentrations (g/100mL)	Adult Emergence (%)	Odds Ratios (ORs)	95% CI	p-value
Control	54	1		
0.25	31	0.35	0.19–0.64	0.001
0.5	6	0.05	0.02–0.12	< 0.001
0.75	0	–	–	–
1	0	–	–	–

(–) Not estimable because no adult emergence occurred; these concentrations are reported descriptively only (observed emergence = 0%, exact binomial 95% CI: 0–3.6%).

Effect of chicken dung on development rate

Development rate varied significantly among chicken dung concentrations, overall ($F = 19.11$, $df = 2$, $p = 0.004$). Larvae exposed to higher chicken dung concentrations developed significantly faster than those in the control group (Table 3). The estimated development rate was lowest in the control group (0.084 day^{-1}) and increased significantly at 0.25 g/100mL (0.096 day^{-1} ; $p = 0.003$) and 0.50 g/100mL (0.097 day^{-1} ; $p = 0.007$). Tukey-adjusted pairwise comparisons showed no significant difference between the two concentrations ($p = 0.993$). The estimate at 0.5 g/100ml should be interpreted with caution, as it was based on only two of the five replicate rounds; no larvae survived to pupation in the remaining three rounds.

Table 3 Estimated development rate of <i>An. funestus</i> larvae across chicken dung concentrations, from a linear mixed-effect model.			
Concentrations (g/100 mL) (n)	Estimated development rate (1/day)	95% CI	P-value
Control (5)	0.0845	0.0801–0.0889	
0.25 (5)	0.0962	0.0918–0.1006	0.003
0.5 (5)	0.0965	0.0899–0.1032	0.007

n = number of replicate rounds. P-values are unadjusted comparisons to the control

Effect of chicken dung on mosquito body size

Wing length of adults emerging from larvae exposed to chicken dung decreased with increasing dung concentration (Fig. 4). Mean wing length in the control group was 3.02 mm (95% CI: $2.99\text{--}3.05$), while those from the 0.25 g/100 mL concentration had a mean wing length of 3.03 mm (95% CI: $2.98\text{--}3.07$), with the mean difference not statistically significant from the control, as its 95% CI crossed zero. In contrast, adults from the 0.5 g/100 mL concentration had a lower mean wing length of 2.85 mm (95% CI: $2.61\text{--}3.09$). The bootstrap mean difference from the control was -0.167 mm [CI: $-0.352, -0.020$], which excluded zero; however, this result should be interpreted with caution given the small sample size ($n = 6$) at this concentration. No adults emerged at 0.75 and 1.0 g/100 mL concentrations.

Association of physicochemical parameters with larval survival in chicken dung treatments

After accounting for dung concentration and variation among experimental rounds, pH, electrical conductivity (EC), and algae presence were significantly associated with larval survival. A one standard deviation increase in pH ($HR = 0.31$, $p < 0.001$) and EC ($HR = 0.79$, $p = 0.015$) was associated with reduced larval mortality. Temperature had no significant effect on larval survival ($HR = 1.00$, $p = 0.99$). Compared with habitats without algae, the presence of algae was associated with a lower mortality hazard ($HR = 0.29$, $p < 0.001$) (Table 4). However, pH, EC, and algae presence were themselves strongly

associated with dung concentration (all $p < 0.001$), which together explained approximately 43% of the variance in these water-quality measures.

Table 4
Association of water quality parameters with *An. funestus* larval mortality in chicken dung concentrations.

Variable	HR	95% CI	<i>p</i> -value
pH	0.31	0.24–0.39	< 0.001
EC (µS/cm)	0.79	0.65–0.95	0.015
Temperature (°C)	1.00	0.85–1.17	0.99
Algae present (vs absent)	0.29	0.17–0.49	< 0.001

Effect of chicken dung concentration on ammonia and nitrate levels.

Chicken dung treatments resulted in progressive concentration-dependent increases in both ammonia (NH₃) and nitrate (NO₃⁻) levels across the concentration gradient and across all five replicates (additional **file 2**). Ammonia levels ranged from 0.01mg/L in the control to 12.84 mg/L at higher concentration (1.0g/100mL), while nitrate concentrations increased more steeply, ranging from 0.1mg/L to 253.2 mg/L across the same gradient. Nitrate consistently showed a steeper concentration-dependent accumulation compared to ammonia, suggesting differential release and transformation of inorganic nitrogen forms with increasing chicken dung input.

Effect of cow dung on *An. funestus* larval survival

Larval survival of *An. funestus* did not differ significantly across cow dung concentrations overall (log-rank test, $\chi^2 = 9.4$, $df = 4$, $p = 0.052$, Fig. 5). However, Cox proportional hazards regression, accounting for variation among experimental rounds, indicated that larvae exposed to all cow dung concentrations had a significantly higher hazard of mortality than those in the control (Table 5). Nevertheless, Tukey-adjusted pairwise comparisons did not reach statistical significance for any cow dung concentration relative to control ($p > 0.05$, additional **file 3**), nor among the cow dung concentration themselves (all $p > 0.05$, additional **file 3**).

Table 5
Hazard ratios for larval mortality of *An. funestus* across cow dung concentrations relative to the control

Concentrations (g/100ml)	HR [95% CI]	P-value	Median survival, days [95% CI]
Control	1		13 [12, 14]
0.25	1.74 (1.15, 2.62)	0.009	11 [10, 13]
0.5	1.70 (1.13, 2.58)	0.011	11 [10, NA]
0.75	1.66 (1.11, 2.50)	0.014	11 [10, NA]
1.0	1.59 (1.07, 2.38)	0.023	12 [11, 15]

Effect of cow dung on adult emergence

Adult emergence of *An. funestus* did not differ significantly across cow dung concentrations overall (Type III Wald, $\chi^2 = 3.79$, $df = 4$, $p = 0.435$). Although the estimated probability of adult emergence decreased progressively from 50% in the control to 37% at 1.0 g/100 mL, none of the cow dung concentrations differed significantly from the control (all $p > 0.05$). Pairwise comparisons similarly showed no significant differences between any concentration levels (all adjusted $p > 0.05$) (Table 6).

Table 6
Adult emergence success (%) of *An. funestus* across cow dung concentrations, from a generalized linear mixed-effects model.

Concentrations (g/100ml)	Adult emergence (%)	Odds Ratios (ORs)	95% C I	P-value
Control	50	1		
0.25	46	0.85	0.49–1.48	0.571
0.5	45	0.82	0.47–1.43	0.479
0.75	42	0.72	0.41–1.27	0.257
1.0	37	0.59	0.33–1.03	0.065

Effect of cow dung on development rate

Development rate did not vary significantly across cow dung concentrations overall ($F = 2.03$, $df = 4$, $p = 0.139$). The estimated development rate was lowest in the control group (0.086 day⁻¹) and highest at 0.25 g/100mL and 0.50 g/100mL (0.105 day⁻¹ for both), declining at 0.75 g/100mL (0.095 day⁻¹) and 1 g/100mL (0.093 day⁻¹). Tukey-adjusted pairwise comparisons between all concentration pairs were not statistically significant ($p > 0.05$) (Table 7).

Table 7
Estimated development rate of *An. funestus* larvae across cow dung concentrations, from a linear mixed-effect model.

Concentration (g/100 mL) (n)	Estimated development rate (1/day)	95% CI	P-value
Control (5)	0.0857	0.0723–0.0991	
0.25 (5)	0.1052	0.0917–0.1186	0.034
0.50 (5)	0.1053	0.0919–0.1188	0.033
0.75 (5)	0.0953	0.0818–0.1087	0.270
1.00 (5)	0.0926	0.0791–0.1060	0.425

n = number of replicate rounds. P-values are unadjusted comparisons to the control.

Effect of cow dung on mosquito body size

Wing length did not differ significantly between cow dung treatments and the control (Fig. 6). Mean wing length was 3.03 mm (95% CI: 2.99–3.06) in the control and ranged from 2.99 to 3.01 mm across treatments, with bootstrap mean differences of -0.015 to -0.034 mm relative to control; all 95% CIs crossed zero, indicating no significant effect of cow dung concentration on adult wing length.

Association of physicochemical parameters with larval survival in cow dung treatments

After accounting for dung concentration and variation among experimental rounds, pH and electrical conductivity (EC) were significantly associated with larval survival. A one standard deviation increase in pH (HR = 0.32, $p < 0.001$) and EC (HR = 0.04, $p < 0.001$) was associated with reduced larval mortality. Temperature was not significantly associated with larval survival (HR = 0.88, $p = 0.24$), and algae presence showed no significant association with larval survival (HR = 1.16, $p = 0.67$) (Table 8).

Table 8
Association of water quality parameters with *An. funestus* larval mortality in cow dung concentrations.

Variable	HR	95% CI	p-value
pH	0.32	0.25–0.42	< 0.001
EC (μS/cm)	0.04	0.02–0.06	< 0.001
Temperature (°C)	0.88	0.71–1.09	0.24
Algae present (vs absent)	1.16	0.59–2.27	< 0.67

Effect of cow dung concentration on ammonia and nitrate levels

Cow dung treatments produced concentration-dependent increase in both ammonia and nitrate levels (additional **file 4**). Ammonia ranged from 0 to 8.63 mg/L, while nitrate rose more steeply, from 0.1 to 54.9 mg/L, across the same gradient. This progressive enrichment indicates increasing nutrient accumulation in the aquatic environment with higher cow dung input.

Discussion

Malaria vector control remains a cornerstone of malaria prevention and control efforts globally. Among available vector control interventions, larval source management, which involves targeting mosquitoes during their aquatic stages, is widely recognized as an important supplementary approaches [53]. This study evaluated the effects of cow and chicken dung on selected life-history traits of *An. funestus* under semi-field conditions. Overall, the findings indicate that livestock-derived organic matter can influence the suitability of aquatic habitats for *An. funestus*, with the observed responses varying according to dung type and concentration. The stronger adverse responses observed in the chicken-dung experiment may reflect greater organic and nutrient loading and associated changes in water quality, which could create increasingly stressful conditions for larval development. In contrast, the comparatively limited effects observed in the cow-dung experiment suggest that, at the concentrations tested, cow dung caused less severe alteration of larval habitat conditions. These findings are broadly consistent with previous studies showing that different manure types can have contrasting effects on malaria-vector development [28] and highlight the potential importance of livestock waste and associated physicochemical changes in shaping *An. funestus* population dynamics in agricultural environments.

The absence of significant effects of cow dung on larval survival, development rate, and adult wing length indicates that, at the concentrations tested, cow dung did not impose strong developmental stress on *An. funestus*. One possible explanation is that cow dung functioned primarily as an organic subsidy rather than as a toxic stressor, thereby increasing detrital substrates that can enhance microbial growth and larval food availability. This interpretation is consistent with previous evidence showing that organic enrichment of aquatic habitats can stimulate microbial and algal production that serves as food for *Anopheles* larvae [25]. Previous studies have likewise reported neutral or relatively minor effects of cattle manure on mosquito development, including minimal effects on larval survival and adult fitness or, in some cases, accelerated larval development [28, 30]. Similarly, a study conducted in Kenya documented no significant reduction in larval survivorship following cow dung exposure, though differences in development time and adult body size were observed [54].

However, findings from other studies on the effects of cow dung on mosquito larvae have not been entirely consistent. Some studies have reported increased larval mortality and reduced abundance of *Anopheles* larvae in habitats containing cow dung [55, 56]. These differences across studies may be explained by variations in experimental conditions, including differences in dung type and age, nutrient

composition, concentration used, water conditions, microbial communities, mosquito species tested, and livestock feeding practices that may influence dung composition [28]. This suggests that the effects of cow dung on mosquito development are highly dependent on environmental and experimental conditions.

In contrast to cow dung, chicken dung had pronounced negative effects on the life history traits of *An. funestus*. Increasing chicken dung concentrations significantly reduced larval survival and adult emergence, while larvae that survived developed more rapidly, and produced smaller adults among the mosquitoes that successfully emerged. These findings suggest that chicken dung created increasingly unfavourable conditions for larval development under the semi-field conditions. The negative effects observed in chicken dung treatments were accompanied by marked changes in water quality. Increasing chicken dung concentrations resulted in greater accumulation of nitrogenous compounds, particularly nitrate and, to a lesser extent, ammonia, indicating substantial nutrient enrichment of larval habitats. Poultry manure is known to contain high concentrations of nitrogen-rich compounds and decomposes rapidly in aquatic environments, releasing nutrients and metabolic by-products into surrounding water [57, 58]. The close association between rising nutrient levels and declining larval performance observed in this study suggests that elevated organic loading progressively reduced habitat suitability for *An. funestus* development.

Changes in physicochemical conditions may also have contributed to the observed biological effects. Water quality parameters such as pH, electrical conductivity and temperature influence mosquito larval development by affecting physiological processes, food availability, and habitat suitability [59]. The observed variation in physicochemical parameters among chicken dung treatments suggests that poultry manure modified the aquatic habitat, and these changes were associated with differences in larval survival. Beyond the measured physicochemical changes, additional processes may have contributed to the observed effects. Poultry manure is rich in nitrogen, phosphorus, and potassium and is highly soluble compared to many other livestock wastes [57]. As decomposition occurs, compounds such as ammonia and other metabolic by-products accumulate in the aquatic environment, potentially altering oxygen availability and creating chemically stressful conditions for mosquito larvae and other aquatic organisms [57, 60]. Chicken dung also contains high levels of uric acid and ammonia [58], and its microbial breakdown by bacteria such as *Corynebacterium*, *Nocardia*, and *Pseudomonas* can generate additional by-products that may further reduce habitat suitability for aquatic organisms [58, 61].

Although relatively few studies have directly investigated the effects of raw chicken dung on *Anopheles* mosquitoes, findings from studies on poultry manure are consistent with the patterns observed in the present study. Chicken manure has been reported to cause high larval mortality and substantially reduce adult emergence in *An. gambiae* s.s. and *An. arabiensis* [28]. These patterns closely mirror those observed in *An. funestus*, where increasing chicken dung concentrations resulted in severe larval mortality and near complete inhibition of adult emergence. In addition to chemical changes, microbial factors may also provide a possible explanation for the observed effects. Previous studies have reported the presence of *Bacillus* species in chicken faeces [57, 62, 63]. Some *Bacillus* species, including *Bacillus*

thuringiensis, produce toxins that are harmful to mosquito larvae [64, 65]. However, the microbial composition and activity of the chicken dung were not assessed in the present study; therefore, the contribution of these bacteria to the observed larval mortality cannot be established. Future studies should investigate the microbial composition and activity of chicken dung to determine whether microbial processes contribute to its effects on *An. funestus* larvae.

The reduction in adult wing length observed in the chicken dung treatments further supports the view that chicken dung created suboptimal conditions for larval development. Adult body size in mosquitoes is strongly influenced by the nutritional and environmental conditions experienced during the larval stage, and exposure to stressful environments can limit growth and accumulation of energy reserves before pupation [43, 66]. Accelerated development under stressful conditions may represent an adaptive response that allows larvae to escape deteriorating aquatic habitats more rapidly, reducing prolonged exposure to environmental stress and increasing the probability of successful adult emergence [67]. However, when the larval period is shortened, less time is available for feeding and for accumulating the lipid and protein reserves required to support adult survival, flight, reproduction, and host-seeking behaviour. Consequently, mosquitoes that emerge under such conditions are often smaller and possess reduced energetic reserves, potentially resulting in lower survival, reduced fecundity, and diminished dispersal capacity [43, 66].

In the context of malaria transmission, these carry-over effects from the larval stage may be epidemiologically important. Smaller adult females generally possess fewer nutritional reserves and lower reproductive potential than larger females [43, 66]. Reduced adult size may also be associated with lower survival, which could decrease the probability that a mosquito survives long enough to complete the extrinsic incubation period of the malaria parasite and successfully transmit infection [66].

However, not all studies have reported reduced adult size following manure exposure. Some studies observed increased adult size in mosquitoes developing in cow and chicken dung-enriched habitats [28, 30]. These conflicting results likely stem from differences in the dung itself (what the animals ate, whether the dung was fresh or old), how much dung was used, how long larvae were exposed, the water conditions, and even the mosquito species tested. In the present study, the reduction in wing length in chicken dung concentrations suggests that the negative effects associated with high organic loading outweighed any potential nutritional benefits.

Comparison of the two manure types demonstrated markedly different effects on the life-history traits of *An. funestus*. Cow dung produced relatively low concentrations of nitrate and ammonia and had limited effects on larval survival, development rate and adult emergence. Importantly, cow dung did not produce a significant reduction in adult wing length under the concentrations tested. In contrast, chicken dung was associated with substantially greater accumulation of nitrogenous compounds and caused significant reductions in larval survival and adult emergence, accelerated development among surviving larvae, and reduced adult body size. These contrasting responses suggest that the biological effects of livestock manure on mosquito development depend not only on the presence of organic matter but also

on the extent to which manure alters the physicochemical characteristics of larval habitats. Under the experimental conditions, the chicken dung treatments showed stronger negative effects on mosquito development than the cow dung treatments, a pattern that was accompanied by greater nutrient enrichment and organic loading in the chicken dung treatments. Similar findings indicate that cattle manure has relatively minor effects on survival, whereas chicken manure substantially reduced larval survival and adult emergence in *Anopheles* mosquitoes [28].

The implications of these findings for malaria transmission are not completely straightforward, but they indicate that livestock manure can influence mosquito population dynamics in meaningful ways. Larval survival, emergence success, and adult body size are key determinants of vector abundance and the proportion of mosquitoes that reach infectious age. The strong reductions in larval survival and adult emergence observed at higher chicken dung concentrations therefore indicate a likely decrease in the number of adult mosquitoes. Chicken dung exposure was also associated with reduced adult wing size, indicating a shift toward smaller-bodied individuals that typically exhibit reduced survival, lower energy reserves, and diminished fecundity [43, 66]. Collectively, these effects may reduce both mosquito abundance and individual fitness, thereby lowering the probability that mosquitoes survive long enough to complete the extrinsic incubation period and transmit malaria. However, these patterns should be interpreted cautiously within the controlled semi-field setting of this study, where high chicken dung concentrations created conditions that appear less suitable for *An. funestus* development under experimental exposure.

Importantly, these findings also provide ecological insight into *An. funestus*, a major African malaria vector for which very little is known about how livestock manure affects its early life stages. By using semi-field conditions, findings of this study offer a realistic view of how livestock manure inputs may shape larval habitats in farming environments where livestock keeping and agriculture occur alongside malaria transmission. This adds valuable information to the limited literature on livestock manure and mosquito fitness, and highlights that manure type, concentration, and local environmental conditions can create very different outcomes for mosquito fitness. Nevertheless, several limitations should be considered when interpreting the findings. The cow and chicken dung experiments were conducted in separate semi-field chambers, and although environmental conditions were standardized, chamber-specific effects cannot be completely excluded when comparing dung types. In addition, laboratory-reared FUTAZ larvae were used and were provided with standardized TetraMin feed, which may not fully reflect the nutritional conditions experienced by wild larvae. The use of dried dung enclosed in filter material represented exposure to dung leachates rather than direct contact with naturally deposited manure, and dung was collected from a single location, which may not capture variation associated with different livestock diets and management practices. Furthermore, dissolved oxygen, microbial communities, and aquatic predators were not quantified, preventing direct assessment of the ecological mechanisms underlying the observed responses, while ammonia and nitrate concentrations were measured only once per experimental round, limiting their incorporation into the survival analyses. Consequently, although these findings demonstrate that livestock manure can substantially alter the suitability of *An. funestus* larval habitats under semi-field conditions, further field-based studies

incorporating a broader range of environmental conditions and ecological measurements are needed to determine whether similar patterns occur in natural breeding habitats and across different agro-ecosystems. This knowledge is essential for determining whether livestock waste could unintentionally reduce vector populations or whether, under certain circumstances, it may still create suitable breeding sites.

Conclusion

This study demonstrates that, under the semi-field conditions and concentrations tested, chicken dung leachate reduced *An. funestus* larval survival, adult emergence, and adult body size among surviving mosquitoes, whereas comparatively weaker effects were observed in the cow dung experiment. These findings show that livestock-derived organic matter can alter the suitability and productivity of *An. funestus* larval habitats, with responses varying across manure treatments and concentrations. In agricultural settings where livestock keeping, manure use, surface runoff, and mosquito breeding habitats overlap, these ecological interactions may be important for understanding larval habitat productivity and for informing malaria vector surveillance and control. Further field studies are needed to determine whether these findings are consistent under natural environmental conditions and to clarify their implications for malaria vector ecology and control.

Abbreviations

°C	Degree Celsius (Centigrade)
CIs	Confidence Intervals
EC	Electrical Conductivity
FUTAZ	<i>Anopheles funestus</i> strain from Tanzania
GLMMS	Generalized Linear Mixed Models
HRs	Hazard Ratios
IHI	Ifakara Health Institute
IRB	Institutional Review Board
LMMs	Linear Mixed-Effects Models
NH ₃	Ammonia
NH ₃ -N	Ammonium nitrogen
NO ₃	Nitrate ion

NO₃-N Nitrate nitrogen

OFs Organic Fertilizers

ORs Odds Ratios

pH Potential of Hydrogen (measure of acidity/alkalinity)

SFS Semi-Field System

TDS Total Dissolved Solids

WHO World Health Organization

Declarations

Ethics approval

Ethical approval for the study was obtained from the Ifakara Health Institute Institutional Review Board (IHI/IRB/No: 29-2025). The permission to publish this study was obtained from National Institute for Medical Research (NIMR) (Ref No. BD.242/437/01D/202). The study did not enrol human participants or collect human biological samples. All mosquito experiments were conducted under semi-field containment procedures approved by the institute.

Competing interests

The authors declare no competing interests.

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Author Contribution

MDM and EWK conceived the study. MDM, HHM, LLM, YAM, ATM developed the methodology. MDM, HHM and EWK organized the semi-field experiments. MDM collected data and wrote the first draft of the manuscript. MDM, LLM, YAM and HSN analysed and interpreted the data. HHM, LLM, ATM, YAM, DSM, HSN, SK and EWK reviewed the manuscript. SK and EWK reviewed and supervised the study. All authors reviewed and approved the final version of the manuscript for submission.

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Availability of data and materials

The data presented in this study are available on request from the corresponding authors.

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Figures



Figure 1

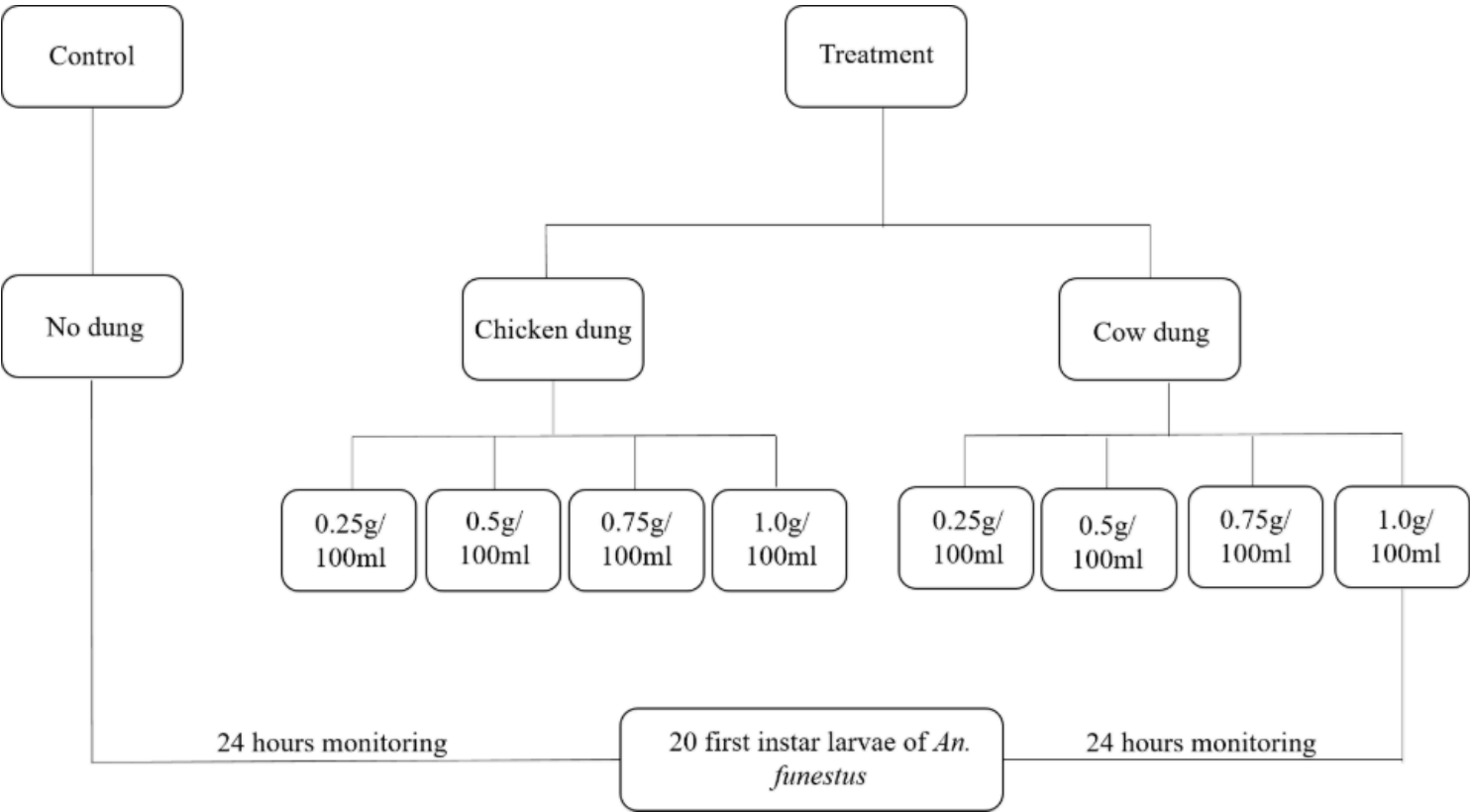


Figure 2

Schematic diagram of experimental setup in the semi-field environment.

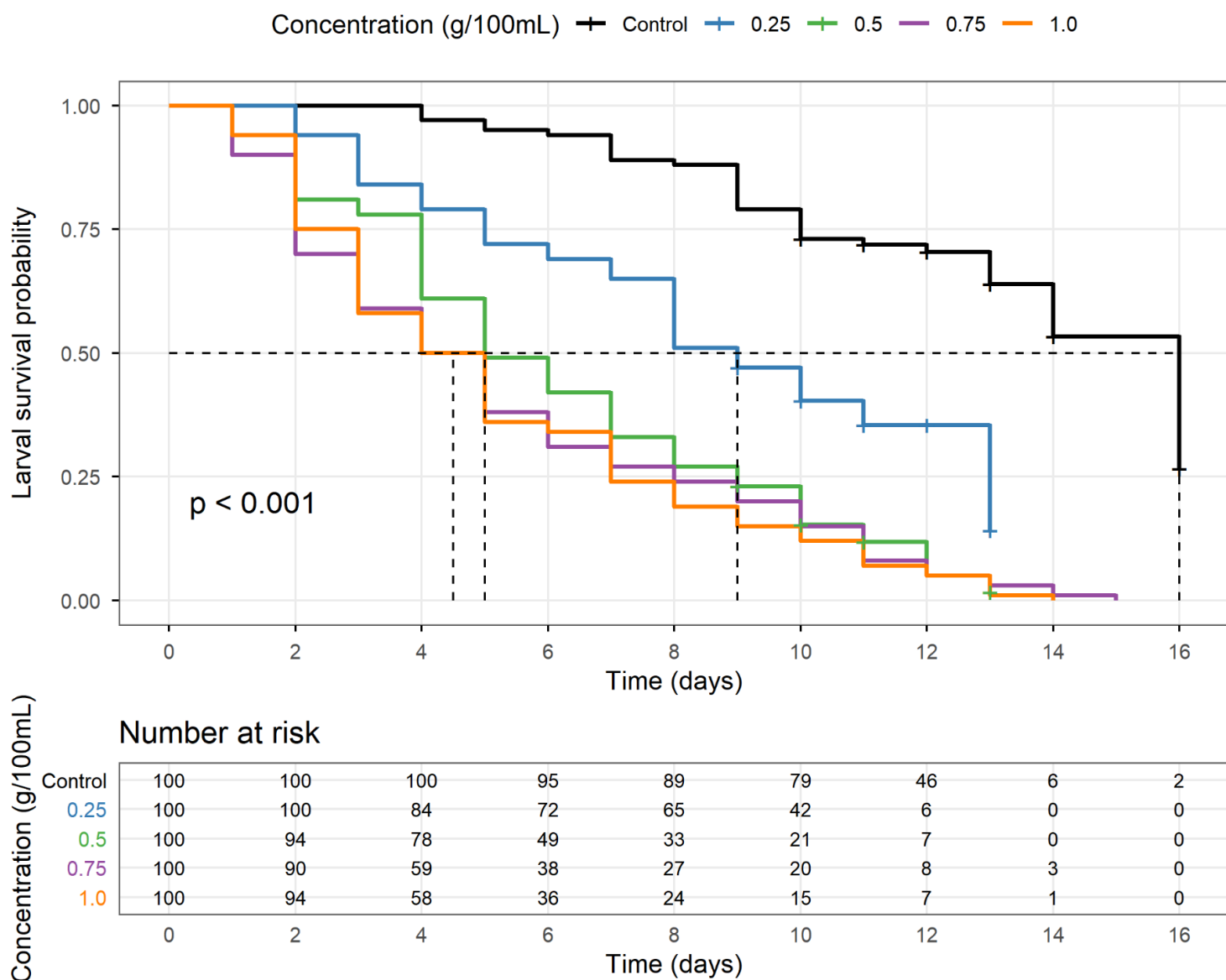


Figure 3

Kaplan-Meir survival curves for *An. funestus* larvae exposed to chicken dung at five concentrations. Larvae that died were treated as events; larvae that pupated were right-censored. Dashed lines indicate median survival time for each concentration. The table below the plot shows the number of larvae remaining at risk at each time point.

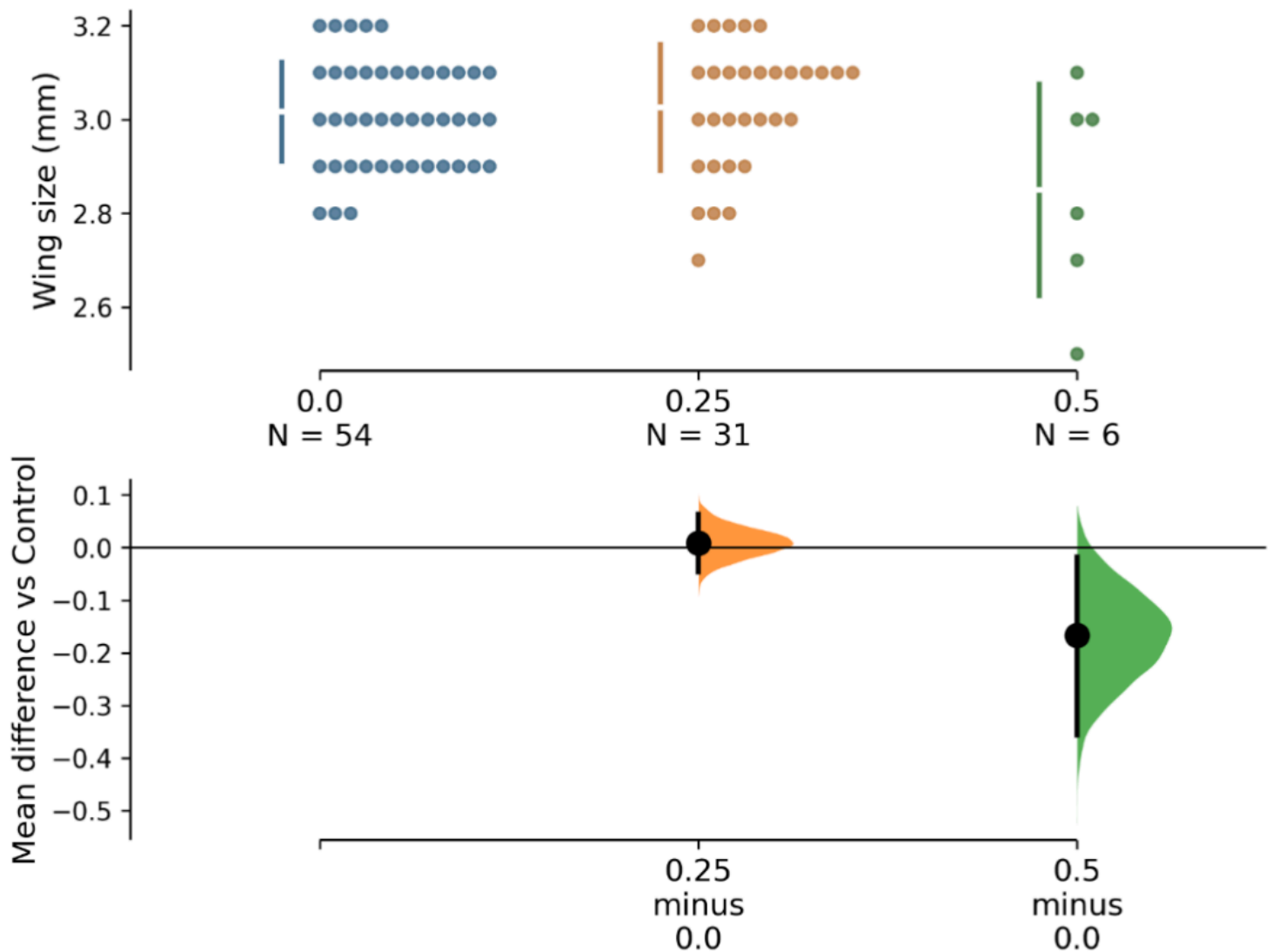


Figure 4

Wing length of *An. funestus* adults emerging from larvae exposed to chicken dung, shown for control, 0.25 and 0.5 g/100 mL. **Top panel:** raw measurements (points) with group means and 95% CIs; **Bottom panel:** bootstrap mean difference in wing length relative to control, with 95% CI (Gardner-Altman estimation plot).

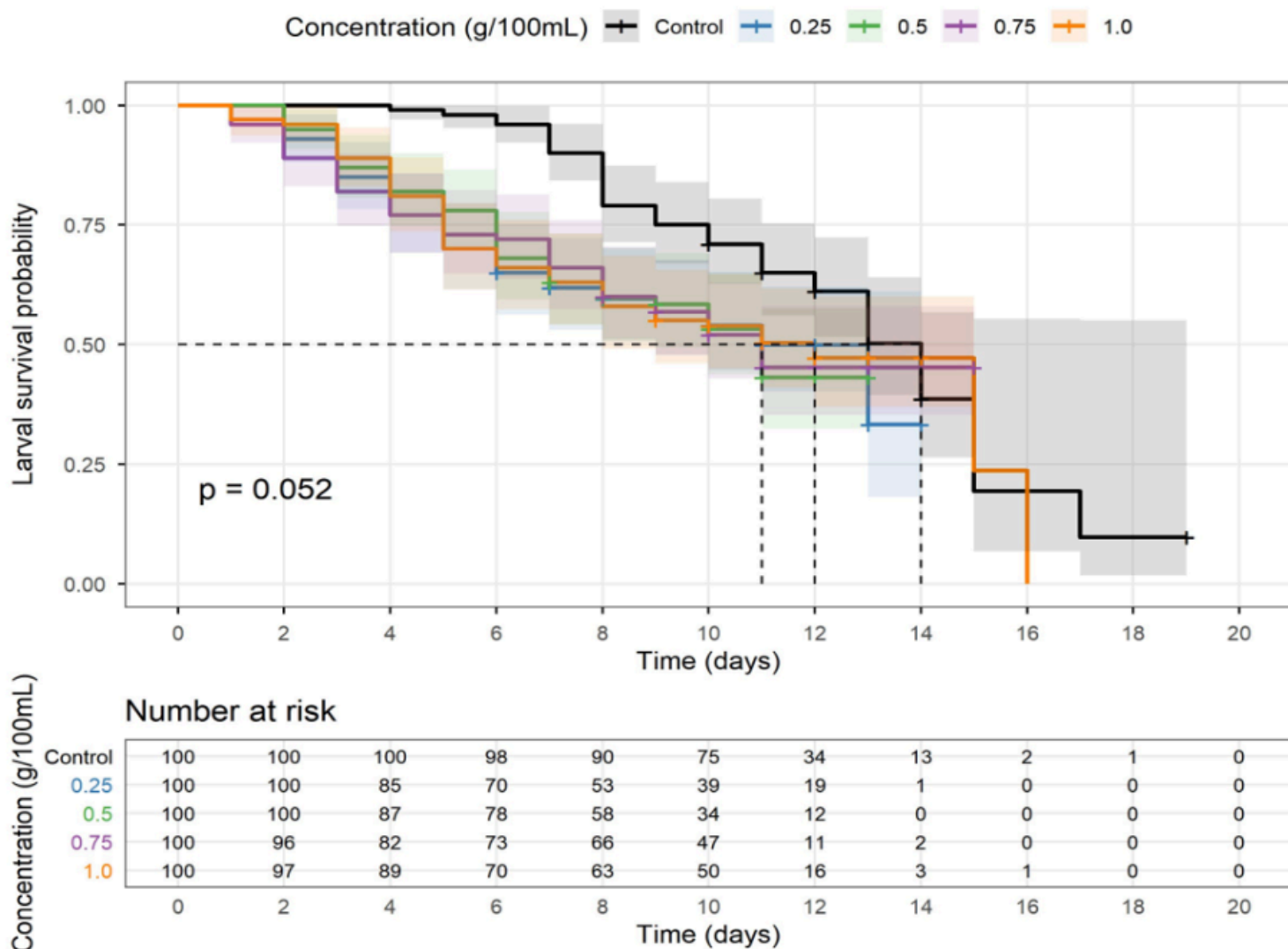


Figure 5

Kaplan-Meier survival curve for *An. funestus* larvae exposed to cow dung at five concentrations. Larvae that died were treated as events; larvae that pupated were right-censored. Dashed lines indicate median survival time for each concentration.

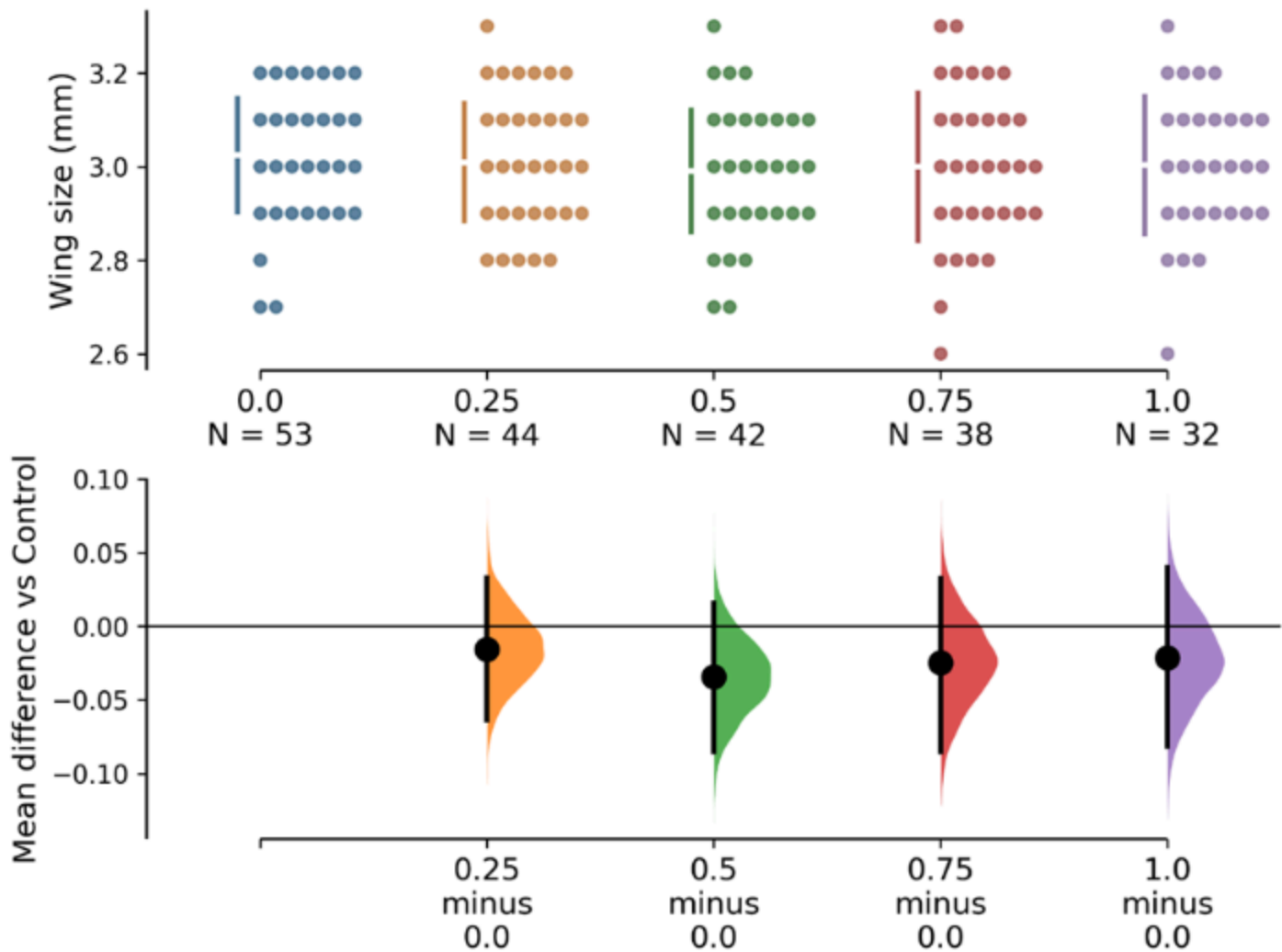


Figure 6

Wing length of *An. funestus* adults emerging from larvae exposed to cow dung, shown for control, 0.25, 0.5, 0.75 and 1.0 g/100 mL. **Top panel:** raw measurements (points) with group means and 95% CIs; **Bottom panel:** bootstrap mean difference in wing length relative to control, with 95% CI (Gardner-Altman estimation plot).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Additionalfile1.docx](#)
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