The influence of manure-based organic fertilisers on the oviposition behaviour of *Anopheles arabiensis*

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Abstract

The rice agroecosystem provides suitable breeding habitat for many malaria vector species, and rice-adjacent communities are consequently exposed to a greater malaria transmission risk than non-rice-associated communities. As part of efforts to expand rice production in Africa, sustainable and climate-adapted practices such as the System of Rice Intensification (SRI) are being promoted. SRI encourages the use of organic fertilisers (OFs) such as cow and chicken dung, as opposed to inorganic industrially produced fertilisers, due to their lower resource cost, apparent benefit to the rice agroecosystem and as a means to reduce the greenhouse gas emissions associated with the production of industrial fertilisers. However, the impact of OFs on mosquito fauna is not well documented and may have knock-on consequences on malaria transmission risk. Here, we demonstrate, using dual choice egg count assays, that both cow and chicken dung modulate the oviposition behaviour of *Anopheles arabiensis*, a major malaria vector in Sub-Saharan Africa. A significantly reduced proportion of eggs were laid in water treated with either cow or chicken dung compared to untreated water, with higher dung concentrations resulting in further reduced proportions. When presented in competition, significantly fewer eggs were laid in water treated with chicken dung than with cow dung. Moreover, there was no evidence of egg retention in any experiment, including in no-choice experiments where only dung-containing dishes were available. These results suggest both cow and chicken dung may act as oviposition deterrents to malaria vector species and that the application of manure-based OFs in rice agriculture may modulate the oviposition behaviour of *An. gambiae s.l.* within agroecosystems. Quantification of the ammonia present in dung-infused water showed higher concentrations were present in the chicken dung infusion, which may be one contributing factor to the difference in observed deterrence between the two dung types. Deterrence of mosquito oviposition in OF-treated farms may potentially affect the overall production of malaria vectors within rice fields and their contribution to local malaria transmission.

Keywords: Organic fertilisers, oviposition, *Anopheles arabiensis,* rice cultivation, malaria vectors.

1. Introduction

The relationship between rice cultivation and the proliferation of malaria vector mosquitoes is well recognised (Service, 1989; Muturi et al., 2008). The rice agroecosystem provides a conducive breeding habitat for many mosquito species through an abundance of standing water, whilst rice plants themselves emit volatiles that attract some gravid mosquito species (Jarju et al., 2009; Wondwosen et al., 2016). The development of rice agroecosystems has been implicated in increases in mosquito vector populations (Sissoko et al., 2004; Muturi et al., 2006) and malaria transmission (Chan et al., 2022), however, the epidemiology of malaria is complex and is also modulated by socioeconomic factors (Ijumba and Lindsay, 2001).

Rice cultivation typically requires additional inputs to increase yields, such as controlled irrigation, fertilisers, herbicides, and pesticides. The System of Rice Intensification (SRI), a rice cultivation practice developed to aid resource-poor farmers in improving rice yield and quality (Thakur and Uphoff, 2017), promotes the application of organic fertilisers (OFs) in place of or supplementary to industrially produced inorganic fertilisers. Importantly, OF application is also a climate-adapted practice that reduces greenhouse gas emissions associated with inorganic fertiliser use (Wang et al., 2018), and is therefore desirable to become more commonplace. However, the composition of OFs in SRI is variable and often comprises readily available waste material (Katambara et al., 2013), such as animal manures, composted vegetative matter, or a mix of these two (Stoop, Uphoff and Kassam, 2002). The presence of OFs in irrigated fields, and the volatiles they may emit, may play an important role in modulating how gravid malaria vectors respond to these as potential oviposition sites.

Anopheles arabiensis is one of the dominant malaria vector species of Africa (Sinka et al., 2012) and is commonly found breeding in irrigated rice agroecosystems as the principal vector species (Muturi et al., 2013; Ijumba, Mosha and Lindsay, 2002). Over the past decade, *An. arabiensis* has become a more prominent vector species as long-lasting insecticidal nets (LLINs), deployed widely across Africa, more effectively control the two other dominant malaria vectors, *An. gambiae s.s.* and *An. funestus* (Kitau et al., 2012; Perugini et al., 2020). Further, *An. arabiensis* exhibits a higher degree of plasticity in its behavioural modalities, such as a higher degree of zoophagy or generalist host preferences, an increased degree of exophagy and exophilly, and behavioural resistance to common control measures such as LLINs (Clements, 1999; Tirados et al., 2006; Muriu et al., 2008; Takken and Verhulst, 2013; Killeen et al., 2016). This behavioural adaptability makes *An. arabiensis* difficult to control with conventionally effective strategies and is an increasing challenge with the spread of insecticide resistance in many populations (Kafy et al., 2017; Messenger et al., 2017; Kabula et al., 2014; Kawada et al., 2011), which can be enhanced by agricultural inputs such as fertilisers and herbicides (Samuel, Brooke and Oliver, 2020; Jeanrenaud, Brooke and Oliver, 2019; Oliver and

Brooke, 2018). Given the association between irrigated rice agriculture and *An. arabiensis* breeding and the increasing challenge the species pose to malaria elimination and residual transmission, it is important that we seek to understand how alternative agricultural practices, such as SRI, impact their bionomics.

The oviposition behaviour of malaria vectors determines the spatiotemporal distribution of larvae and can influence both juvenile and adult fitness traits, so it is therefore critical in the context of understanding the epidemiology and control of malaria. Oviposition site choice by gravid female mosquitoes is modulated by a wide range of environmental information detected from various sources and several different sensory pathways, including tactile, gustatory, olfactory, and visual (Day, 2016). Biotic and abiotic factors, such as moisture (Okal et al., 2013), salinity, surrounding vegetation (Wondwosen et al., 2016, 2017; Asmare et al., 2017; Eneh, Okal, et al., 2016), bacterial or fungal growth (Eneh et al., 2019; Eneh, Saijo, et al., 2016; Sumba et al., 2004), the persistence of the aquatic habitat (Eneh et al., 2019), the presence of predators (Blaustein, Blaustein and Chase, 2005; Albeny-Simões et al., 2014), and the presence and density of conspecifics (Mwingira et al., 2019) all modulate how attractive a potential oviposition site is. The larval habitat, and the resources and challenges they face, are determined by the behaviour of the mother in response to these factors, reflecting trade-offs that ultimately affect the survival and fitness of the next generation. Therefore, oviposition site choice is considered non-random and highly discriminatory (Yoshioka et al., 2012).

Despite the widespread use of OFs, such as cow and chicken dung, in rice cultivation more broadly, and in SRI across Africa specifically (Latif et al., 2005; Rahman, 2013; Meertens, 2001), relatively little attention has been paid to how they may affect the oviposition behaviour of *Anopheles* mosquitoes. Most published studies have focused on Culicine mosquitoes, which are typically more tolerant of highly nutrient-polluted water (Opoku and Ansa-Asare, 2009). In a semi-field trial in Botswana, cattle dung containing larval mesocosms were found to promote colonisation by *Culex pipiens pipiens* and an unidentified *Anopheles* species (Buxton et al., 2020). In contrast, a laboratory study found *An. gambiae s.s.* did not lay a significantly different number of eggs in cow dung polluted water compared to distilled water but laid significantly fewer eggs compared to sites containing water from lake Victoria (Otienoburu et al., 2007). However, there is limited data on anopheline mosquito oviposition responses concerning the presence of cow dung in potential egg-laying sites.

No published literature on the effects of chicken dung on anopheline mosquito oviposition has been identified, although there has been some work on Culicine mosquitoes. In dual choice assays, a 1% chicken dung infusion in distilled water was found to attract gravid *Culex quinquefasciatus* whilst repelling *Cx. tarsalis*, an observation the authors suggested was due to the former species typically inhabiting more organically polluted habitats than the latter (Kramer and Mulla, 1979). Earlier work reported similar observations for *Cx. peus*, later synonymised with *Cx. thriambus* (Eldridge and Harbach, 1992), which assumed a similar response to *Cx. quinquefasciatus* towards chicken dung-infused water (Ikeshoji and Mulla, 1970). Moreover, attempts to identify whether chicken dung-induced oviposition responses are microbially mediated were partly elucidated by exposing *Cx. quinquefasciatus* and *Cx. tarsalis* to infusions of chicken dung that had been aged undisturbed, and infusions that had been autoclaved post-fermentation. *Cx. quinquefasciatus* responded positively to the aged treatment whilst showing no significant response to the autoclaved treatment (Kramer and Mulla, 1979).

Given the mix of responses of Culicine mosquitoes to OFs, it seems clear that the presence of cow or chicken dung in a potential larval habitat may influence *An. gambiae s.l.* oviposition behaviour. However, their stimulatory or inhibitory nature remains uncertain and may be significantly affected by the intrinsic ecological preferences of a given species. Further, such cues may interact with other important cues used to determine oviposition site quality. Recent research has demonstrated that dung-based OFs influence *An. gambiae s.l.* life history traits. Larval exposure to cow dung results in increased development rates, adult size, adult longevity, and insecticide tolerance (Jeanrenaud, Brooke and Oliver, 2019; Hardy et al., 2022); whilst chicken dung exposure has also been found to increase adult size it also induces significant larval mortality, reduces the production of adults, and slows larval development (Hardy et al., 2022). Considering the relative fitness advantages and disadvantages presented to *Anopheles* larvae when exposed to cow or chicken dung respectively, further research is warranted to elucidate whether these substances impact the oviposition behaviour of *An. arabiensis,* a significant malaria vector of Africa (Sinka et al., 2010) which is frequently reported to breed in rice paddies (Mwangangi et al., 2006; Mwangangi et al., 2006; Muturi, Shililu, et al., 2008; Wondwosen et al., 2017). Such an investigation may reveal how the use of dung-based OFs in SRI, and rice cultivation more widely, may alter vector ecology and in turn the local burden of malaria.

In this study the effect of cow and chicken dung presence in potential egg-laying sites, and the concentration of dung, on *An. arabiensis* oviposition behaviour was investigated. The possibility of egg retention due to cow or chicken dung presence was examined, as was the proportion of eggs laid in potential oviposition sites containing either cow or chicken dung when no other alternatives are available. The concentration of ammonia present in cow and chicken dung-infused water was determined, to investigate one possible factor in the oviposition behaviour observed.

2. Methods

2.1 Mosquitoes

Mosquitoes were maintained in a climate-controlled insectary at 27.5 ± 2°C and 50 ± 10% relative humidity, with a 12:12 light-dark photoperiod. *An. arabiensis*, Dongola strain, was obtained from the International Atomic Energy Association (Austria). Larvae were reared in a solution of 0.1% aquarium salts and deionised water and fed Tetramin© fish flakes *ad libitum*. Stock colony adults were allowed to feed *ad libitum* on a 10% sucrose solution and females were offered a blood meal for egg production at seven to ten days old.

2.2 Sourcing of dung and preparation of dung infusions

Chicken dung was sourced from domestic chickens (*Gallus gallus var. domesticus*) kept by a private hobbyist in Kent, UK. No medications had been administered to the chickens, except for a single dose of coccidiostat two years prior. The chickens were fed on pesticide-free feed, comprised of chicken pellets, corn, and vegetable scraps. Cow dung was sourced from an organic farm in Kent, UK, from Irish Holstein-Friesian cows (*Bos taurus*) that had not been treated with ivermectin for at least six months. The cow diet consisted of non-pesticide treated feed, comprising grass, bean sprouts, and barley in summer, and silage and protein powder in winter. Before experimentation, both dung types were dried for 24 hrs at 60°C, then ground coarsely with a spice grinder (Wahl®). They were then refrigerated at 4°C separately in airtight jars, with a small amount of silica gel, kept separate from the dung.

Infusions of both cow and chicken dung were prepared at concentrations of 0.125, 0.25, 0.5, and 1 g/100 ml. For each concentration, the dung was mixed into deionised water and incubated for 24 hours at 27.5 ± 2°C and 50 ± 10% relative humidity. Before their use, the dung infusions were filtered through Fisherbrand™ QL100 filter paper.

2.3 Preparation of gravid mosquitoes

Mosquitoes to be used for experimentation were held in 30x30x30 cm BugDorm™ cages at a density of 400 individuals, at an approximately 50:50 sex ratio. Adult females, aged five to nine days old, were offered two blood meals of defibrinated horse blood, one hour after scotophase, separated by 48 hours. After 48 hours following the second blood meal, fully gravid females, as determined by abdomen appearance (Detinova, 1962), were isolated from the colony by gently capturing them individually in Thermo Scientific™ universal containers.

2.4 Assay arena

Assay arenas consisted of a 30x30x30 cm BugDorm™ insect cage in which two oviposition dishes, a test and control dish, were placed in opposite corners. The oviposition dishes comprised of a glass petri dish (10 cm Ø) with a cotton pad (approx. 8 cm Ø) placed inside and a filter paper (9 cm Ø) placed on top, to which 10 ml of either deionised water (control) or dung infusion was added. All Petri dishes were cleaned and autoclaved before use. The position of oviposition dishes were systematically altered (Fig. 1) between cages to minimise any spatial bias that may occur, as previously described (Okal et al., 2015; Eneh, Okal, et al., 2016). The first test dish was placed in a random corner and subsequent dishes were placed in the next clockwise corner, with the control dish placed in the opposite corner to the test dishes in each instance. A sugar feeder, consisting of a cotton ball soaked in 10% sucrose solution, was placed in the centre of each cage to allow *ad libitum* feeding throughout the experiment. Cages were placed inside new polyethene bags to prevent contamination of volatiles emanating from outside the bioassay arena and adjacent cages and to maintain humidity and consistency between trials. Between trials, the cages were cleaned by soaking in a 0.4% sodium hypochlorite solution for 45 minutes, then thoroughly rinsed before being air dried.

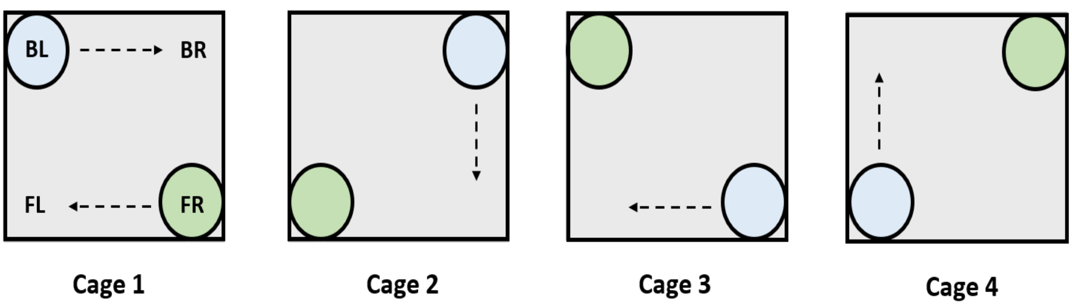

Fig 1: Oviposition dish arrangement in dual-choice assays. Blue circles represent the test dishes, which are rotated in a clockwise fashion. The green circles represent the control dishes, which are positioned in the diagonally opposite corner to the test dish. Dish positions are (FL) – front left, (FR) – front right, (BL) – back left, (BR) – back right. Image redrawn and adapted from Okal *et al*, 2015.

2.5 Behavioural bioassays

To investigate the oviposition behaviour of *An. arabiensis* in response to water infused with cow or chicken dung, a series of dual choice oviposition assays were conducted. These assays sought to examine how mosquitoes distributed their eggs across two potential oviposition sites when offered a treatment dish containing dung-infused water and a control of deionised water, and if eggs were retained in response to cow or chicken dung infusions. For each replicate of each behavioural bioassay, a single female *An. arabiensis* mosquito was placed into a cage one hour before scotophase and allowed 24 hours to oviposit, after which the mosquito was removed and the number of eggs in the test and control dishes was counted using an Olympus™ VT-II stereomicroscope. Once removed, mosquitoes were immediately placed in a -20°C freezer for 24 hours to kill them, stored individually in 5 ml Eppendorf Tubes™, and preserved with 70% ethanol.

2.5.1 Experiment1 – the effect of dung treatment and concentration on oviposition

To investigate the effect of dung infusion treatment and concentration on the proportion of eggs laid in test dishes when an alternative choice of deionised water is available, two types of assays were conducted: one in which two control dishes were offered (water vs water), which were considered the baseline reference group, and another where a treatment dish and a control dish (dung vs water) were offered (Okal et al., 2015; Eneh, Okal, et al., 2016). In brief, in the assay where two dishes of deionised water were offered (n = 19), both received 10 ml of deionised water, acting as the baseline reference group to which the assay where a control and treatment dish offered was compared. Here, one dish was arbitrarily assigned as the test dish, and the other as the control, though both contained untreated deionised water. Where a dung-infused treatment and a control dish were offered, the test dish received 10 ml of a dung infusion and the control dish received 10 ml of deionised water. This latter assay type was conducted for both dung types at concentrations of 0.125 (cow: n = 18; chicken: n = 16), 0.25 (cow: n = 15; chicken: n = 16), 0.5 (cow: n = 15; chicken: n = 18), and 1.0 g/100 ml (cow: n = 16; chicken: n = 15). The proportion of eggs laid in the test dish in the dual choice assay (dung vs water) was compared to the proportion of eggs laid in the test dish of the no-choice assay (water vs water) to account for stochastic variation in egg distribution integrating an empirically derived control data set, rather than assuming a theoretical 50:50 distribution (Okal et al., 2015; Eneh, Okal, et al., 2016). Additionally, the total number of eggs laid per female in the dual-choice assays (dung vs water) was compared to the number laid in the no-choice assays (water vs water) to investigate whether eggs were retained in the dual-choice assay.

2.5.2 Experiment 2 – the effect of dung treatment on egg retention

Egg retention in mosquitoes is the process by which a gravid female withholds laying a proportion of a mature egg batch (Meola and Lea, 1972) and in anophelines has been observed in response to a range of environmental cues (Dhar et al., 1996; Harris et al., 2013; Schoelitsz et al., 2020; Mwingira et al., 2020). To determine whether eggs were retained when only dung-infused oviposition water was presented of a single dung type, no choice assays were conducted where cow (*n* = 26) and chicken (*n* = 26) dung were assessed separately. In this case, both oviposition dishes received 10 ml of the same dung infusion at a concentration of 0.125 g/100 ml, as this was shown to elicit the greatest oviposition response. The total number of eggs laid across both dishes was compared to the total number of eggs laid in the baseline reference group.

2.5.3 Experiment 3 – relative response to cow or chicken dung containing oviposition sites

To determine if a greater proportion of eggs were laid in oviposition sites containing either cow or chicken dung, a dual choice assay was conducted where a choice of either cow or chicken dung infusion containing sites was presented (*n* = 26). One dish received 10 ml of cow dung infusion and the other the same volume of chicken dung infusion, both at a concentration of 0.125 g/100 ml as this was shown to elicit the greatest oviposition response. The proportion of eggs laid in the cow dung infusion containing dishes was compared to the proportion laid in those containing chicken dung infusion.

2.6 Indophenol blue method for the quantification of ammonia in cow and chicken manure

Ammonia comprises a major constituent compound produced from organic nitrogen in chicken dung (Witter, 1991; Kelleher et al., 2002), and has been suggested to modulate oviposition behaviour in *An. arabiensis* (Mutero et al., 2004). Therefore, here we aimed to examine the dissolved ammonia content of both cow and chicken dung. Three dung infusion solutions were prepared for cow and chicken dung at 0.125 g/100 ml, as described in section 2.2. Dissolved ammonia concentration was quantified as described previously (Tzollas et al., 2010) with minor modifications and all reagents were obtained from Sigma Aldrich©. 25 mL of sample extract was transferred to a 100 ml volumetric flask, then 1 ml of phenol solution (10%), 1 ml sodium nitroprusside solution (0.5% w/v) and 2.5 ml of an oxidising solution (160 g/l trisodium citrate, 8 g/l sodium hydroxide and 2% sodium hypochlorite). The samples were sealed with quick-fit stoppers and kept in the dark at room temperature (25 ±2°C) for 2 h. Five 25 ml ammonia standards were prepared from ammonium chloride (1, 0.5, 0.25, 0.125 and 0.0625 mg/l), processed using the same method as the experimental samples, and used to prepare the calibration curve. All absorbance readings were measured at 640 nm on a Jenway 6305 spectrophotometer, and readings of each sample including the standards were repeated three times at 10 min intervals.

2.7 Statistical analysis

All statistical analyses were performed in R (Version: 3.6.2, RStudio version: 1.2.5033) (R Core Team, 2019). All behavioural data were analysed using generalised linear models (GLM) fitted with binomial errors for proportion data, Poisson errors for count data, and quasi-models in the case of overdispersion, as previously described (Okal et al., 2015). All fitted models were subject to analysis of deviance to reveal if independent variables and their interactions had a significant effect on the dependent variable. In the case of quasi-models, F–tests were used, and a χ^2–test was used for non-quasi models (Pekár and Brabec, 2016; Crawley, 2013). A linear regression model was used to derive the slope and intercept of the calibration curve for the quantification of ammonia. These were used to calculate the dissolved ammonia concentration of the cow and chicken dung infusions. A *t*-test was used to determine if there was a significant difference between the mean dissolved ammonia concentration between the two dung types.

3. Results

3.1 Experiment 1 – the effect of dung treatment and concentration on oviposition

When *An. arabiensis* were presented with an equal choice of two oviposition sites containing deionised water, the distribution of eggs was very similar across the two dishes, where a mean proportion of 0.492 (SE ± 0.114) eggs were laid in the arbitrarily assigned test dish, validating the experimental design. In the choice tests of dung infusion vs water, treatment type had a significant effect on the proportion of eggs laid in the test dish (χ^2 = 11.137, *df* = 2, *P* = 0.004), where the presence of dung infusion was associated with a lower proportion of eggs laid for both cow and chicken dung (Fig. 2). Likewise, dung concentration also significantly affected the proportion of eggs laid (χ^2 = 6.274, *df* = 1, *P* = 0.012), where increasing concentrations were associated with decreasing proportions of eggs laid in the test dish. However, the interaction between treatment and concentration was not significant (χ^2 = 1.902, *df* = 1, *P* = 0.439), indicating the effect of dung concentration on the proportion of eggs laid did not vary by dung type. These findings suggest that gravid *An. arabiensis* lay fewer eggs in cow or chicken dung-infused water when an alternative of deionised water is available. Higher dung concentrations lead to lower proportions of eggs laid, though, cow and chicken dung seem to both reduce the proportion of eggs laid to a similar degree over the tested concentrations.

Moreover, neither treatment type ($F_{(2,145)}$ = 0.765, *P* = 0.467) nor concentration ($F_{(1,144)}$ = 0.116, *P* = 0.734) had a significant effect on the mean number of eggs laid by an individual female across treatment and control oviposition dishes (Fig. 3). Thus, there is no evidence that eggs are retained by *An. arabiensis* when presented with a dung infused oviposition site when an alternative of deionised water is available, for both cow and chicken dung at all concentrations tested.

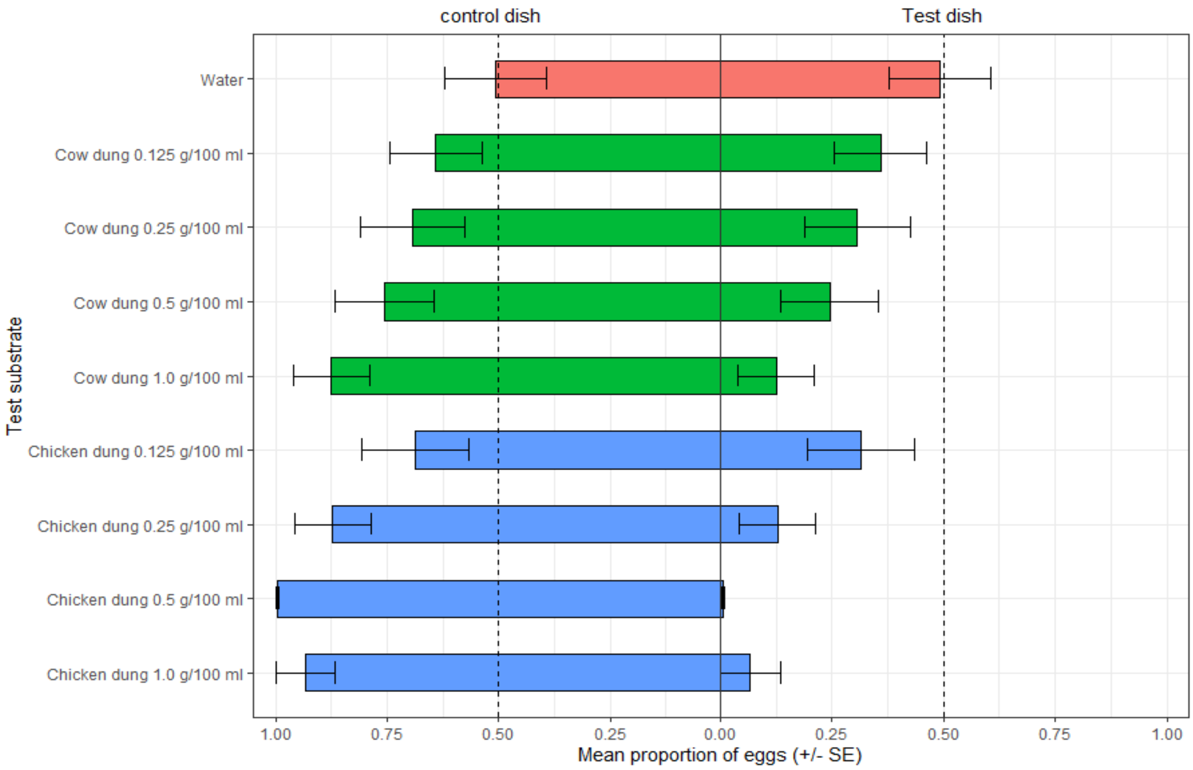

Fig. 2: Mean proportion of eggs laid in test and control dishes by gravid *An. arabiensis* during dual choice assays. Bars show mean proportion of eggs laid in test and control (deionised water) dishes; error bars display standard error of the mean. The water vs water group forms the baseline reference group against which dung infusions were tested in statistical analysis.

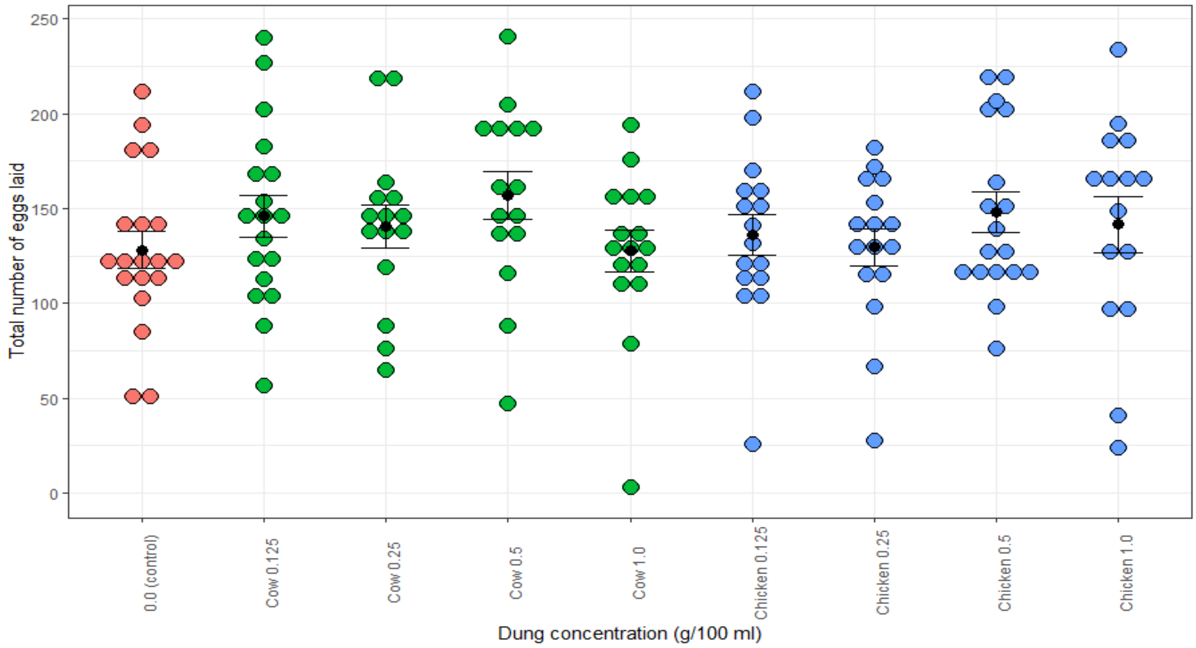

Fig. 3: Total number of eggs laid by gravid *An. arabiensis* when exposed to different treatments in dual choice oviposition assays (dung vs water) and the control (water vs water). Coloured points show actual counts and black dots show means with standard error bars.

3.2 Experiment 2 – the effect of dung treatment on egg retention

To examine whether gravid *An. arabiensis* may retain eggs when only dung infusion containing oviposition dishes are available, no choice oviposition assays were conducted with both cow and chicken dung. The total number of eggs laid in dung vs dung assays was compared with the total number of eggs laid in the water vs water assays. Treatment type was found to have no significant effect on the total number of eggs laid ($F_{(2,68)} = 1.122$, $P = 0.332$) (Fig. 4). When presented with only water, the mean number of eggs laid was 128 (SE ± 9.79), with cow dung infused water it was 102 (SE ± 13.9), and with chicken dung infused water it was 114 (SE ± 9.78). This suggests that gravid *An. arabiensis* do not retain eggs any more or less when presented with dung-infused water only than when only deionised water is available.

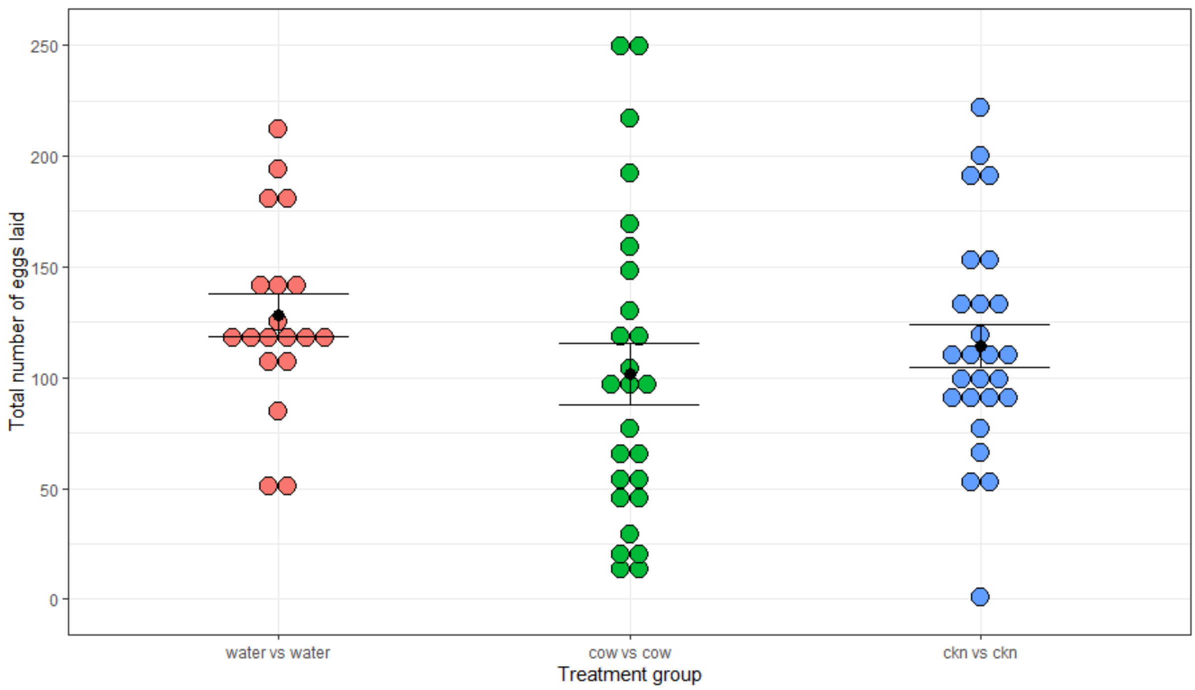

Fig. 4: Total number of eggs laid by gravid *An. arabiensis* when exposed to different treatments in no choice oviposition assays. Coloured points show actual counts and black dots show means with standard error bars.

3.3. Experiment 3 – relative response to cow or chicken dung containing oviposition sites

Dual choice assays measuring the proportion of eggs laid across a choice of either cow or chicken dung-infused water were conducted to examine whether gravid *An.* arabiensis exhibit a relative preference for either treatment. Treatment type had a significant effect on the mean proportion of eggs laid ($F_{(1,50)}$ = 6.502, P = 0.014), where a greater proportion was observed in the oviposition dishes treated with cow dung infusion (Fig. 5). The mean proportion of eggs laid in the cow dung infusion treated dishes was 0.664 (SE ± 0.0867), whereas for chicken dung the mean number of eggs laid was 0.336 (SE ± 0.0867). This suggests that when only cow or chicken dung-infused oviposition sites are available, *An. arabiensis* lay a greater proportion of eggs in the former.

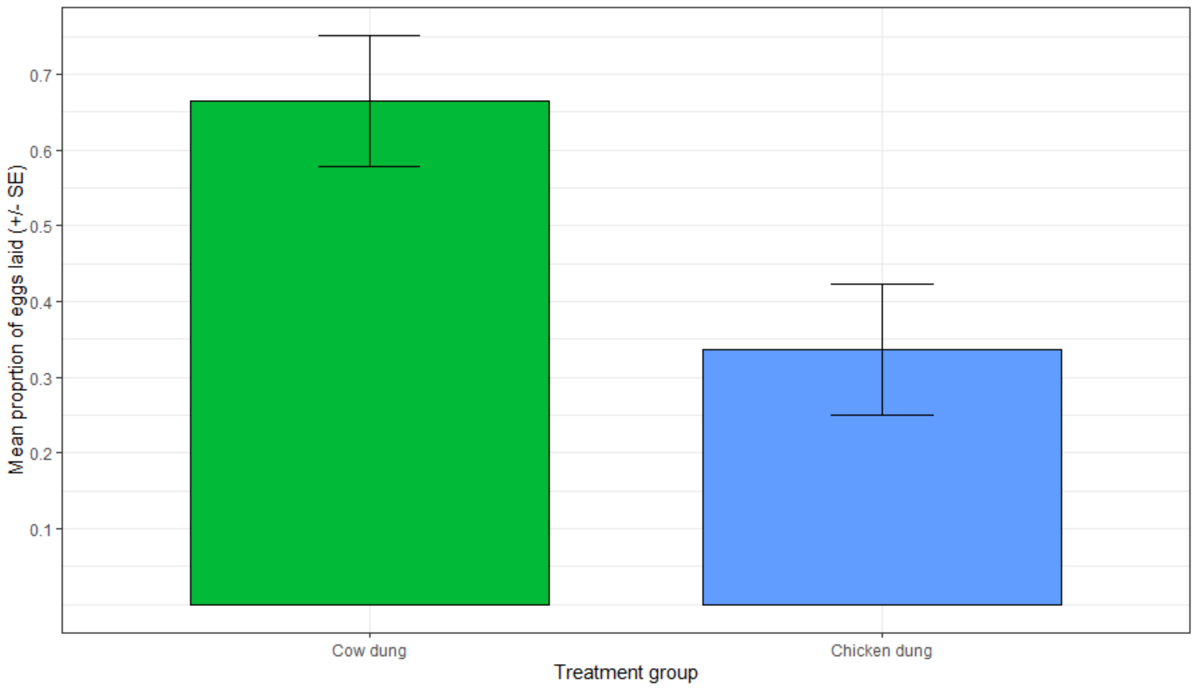

Fig. 5: Eggs laid by gravid *An. arabiensis* when presented two different treatments in dual choice assays. Bars show mean proportion of eggs laid in each treatment and error bars display standard error.

3.4 Indophenol blue method for the quantification of ammonia in cow and chicken manure

Chicken dung infusions at a concentration of 0.125 g/100 ml were found to have a significantly (t = 18.44, df = 8.045, P = <0.01) higher mean dissolved ammonia concentration than cow dung infusions at the same concentration. Chicken dung infusion had a mean dissolved ammonia concentration of 1.368 mg/l (± 0.065), which was over eight times greater than observed in cow dung (0.162 mg/l ± 0.003).

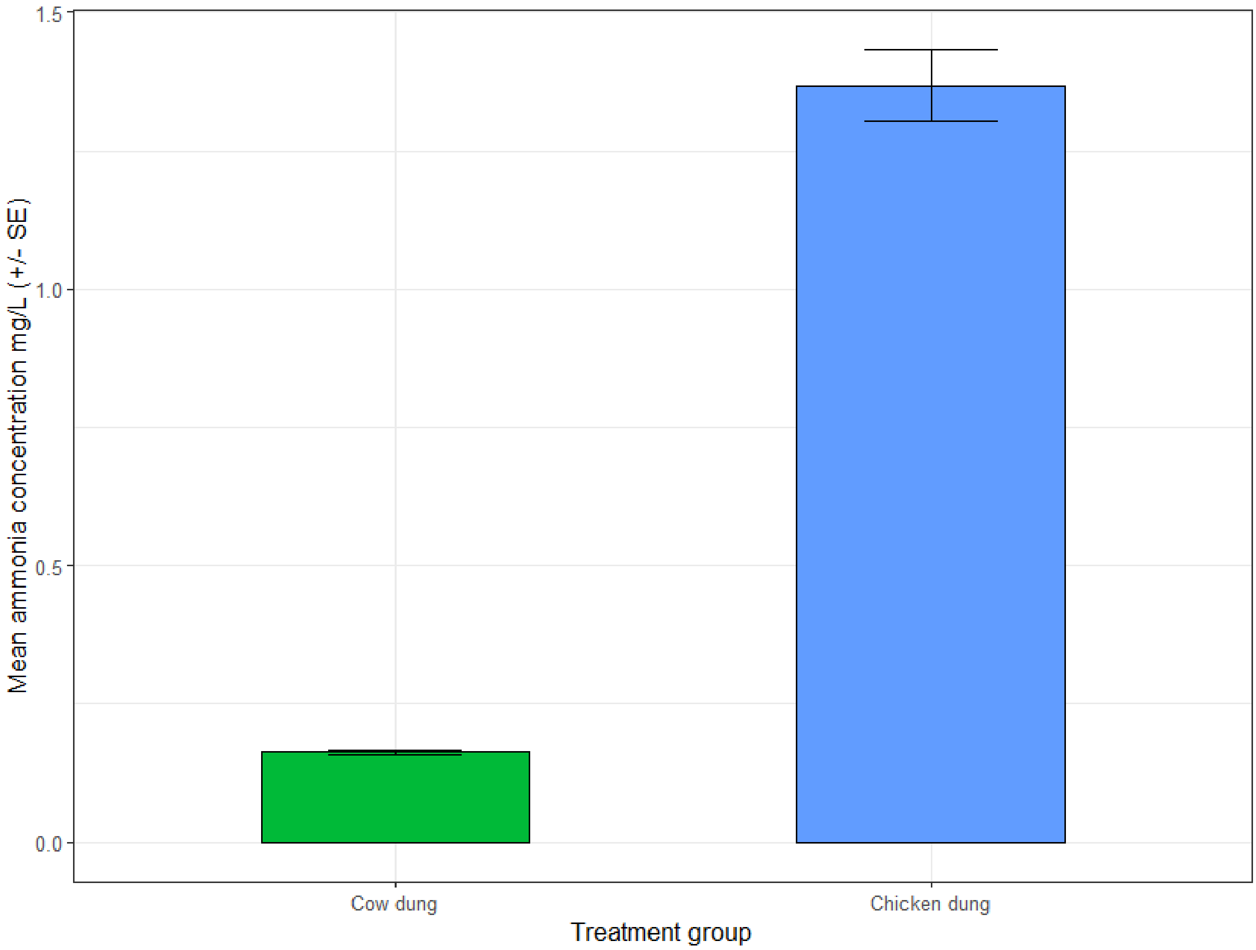

Fig. 6: Dissolved ammonia concentration of preparations of 0.125 g/100 ml of cow and chicken dung infusions in mg/l. Bars show mean dissolved ammonia concentration and error bars display standard error of the mean.

4. Discussion

The findings presented in this study demonstrate that cow and chicken dung in a potential egg-laying site affects the ultimate oviposition behaviour of *Anopheles arabiensis*. Overall, both these OFs reduced the proportion of eggs laid relative to a deionised water control, with higher concentrations further reducing the proportions of eggs laid in the dung infusions. Neither dung type produced any evidence of inducing egg retention across any of the treatment concentrations tested, regardless of whether or not deionised water was available. When the cow and chicken dung were presented together, a greater proportion of eggs were laid in cow dung infusions. These results, therefore, indicate that both cow and chicken dung may broadly act as oviposition deterrents (Dethier, Browne and Smith, 1960; Day, 2016) when alternative oviposition sites are available.

Considering both cow and chicken dung reduced oviposition, this may indicate they are associated with poor habitat suitability or even larval toxicity (Day, 2016), however, eggs were still laid in dishes containing infusions of either dung. Nevertheless, several other studies have demonstrated *Anopheles gambiae s.l.* avoid ovipositing in cow dung-containing water bodies, as observed here. In a laboratory trial, *Anopheles gambiae s.s.* laid significantly fewer eggs in water that contained cow dung infusion compared to water from Lake Victoria (Otienoburu et al., 2007). Other authors have evaluated cow dung as a potential agent in Anopheline larval source reduction. A study in Kenya found outdoor mesocosms treated with cow dung had significantly lower colonisation rates by *An. gambiae s.l.* than untreated control ponds, whilst the opposite was found for Culicine species (Mbuya, Kateyo and Lunyolo, 2014). This demonstrates that *An. gambiae s.l.* may lay fewer eggs in oviposition sites containing cow dung when more acceptable sites are available, broadly corroborating with this study. However, previous research in Botswana noted greater mosquito colonisation rates in cow dung-treated mesocosms, suggesting a possible oviposition attractant role of cow dung. Though, the Anophelines present were of an unidentified species which shared a high similarity with a taxonomically undescribed species (Buxton et al., 2020), possibly a cryptic member of the *An. funestsus* group (Ogola et al., 2019), and may therefore not be representative of such responses in *An. gambiae s.l*. Given this discontinuity, further work is required to elucidate the underlying factors for the observations of this study, that eggs were still laid in less acceptable oviposition sites, albeit a significantly lower proportion, when a more acceptable alternative was available.

The amount of dung used per unit volume of water, and hence its concentration, was demonstrated here as an important variable. Studies investigating the effect of cow dung on mosquito colonisation have utilised a range of dung concentrations: Buxton *et al.* (2020) ranged from 0.1 g/100 ml to 0.8 g/100 ml, whereas Otienoburu *et al.* (2007) used 10 g/100 ml, and Mbuya *et al.* (2014) used 20 g/100 ml. Also, the age of cow dung associated with each experiment differed substantially, ranging from 6 hrs after cow dung had been infused into water (Otienoburu *et al.*, 2007) to up to 21 days after dung inoculation (Buxton *et al*., 2020), while this variable was unspecified by Mbuya *et al.* (2014). The attractivity to *An. arabiensis* of oviposition sites containing cattle urine was reported to be affected by urine age, where fresh urine stimulated oviposition whilst aged urine inhibited it (Kweka et al., 2011). This suggests that the length of time in which cow excreta has since polluted a water body may be an important factor in its relative attractivity to a gravid mosquito. Lastly, variation in cattle diet has been associated with differences in the microbial community composition of cow dung (Van Vliet et al., 2007) and is therefore worth considering due to the importance of microbial fauna in oviposition site selection (Sumba et al., 2004). However, none of the aforementioned studies report the dietary composition of the host cows. In future studies, the effects of dung inoculation period, cattle diet, and microbial diversity of both dung and the aquatic habitat following inoculation should be considered.

Larval exposure to cow dung in *An. gambiae s.l.* hastens development rate and leads to larger adult size, which is strongly related to enhanced fitness, whilst having no effect on mortality (Hardy et al., 2022; Jeanrenaud, Brooke and Oliver, 2019). These positive effects on life history were also found to be greater at higher concentrations when examined between 0.25 and 1.0 g/100 ml. Therefore, considering oviposition site choice has, in part, evolved to maximise reproductive fitness by selecting for attraction to the most suitable habitats in terms of larval performance (Ignell and Hill, 2020; Yoshioka et al., 2012; Day, 2016; V. Mwingira et al., 2020), it would be expected that greater numbers of eggs are laid in sites containing cow dung. However, the present study demonstrated that for *An. arabiensis*, this may not be the case as cow dung infusion significantly reduced the number of eggs laid in an oviposition site in favour of an alternative site containing deionised water. This evidence suggests cow dung may present cues that do not strongly elicit oviposition in gravid mosquitoes and perhaps even inhibit it.

This study demonstrated that gravid *An. arabiensis* lay fewer eggs in oviposition sites containing chicken dung when an alternative of deionised water is available and increased dung concentrations result in reduced oviposition activity. However, unlike cow dung, chicken dung negatively affects *An. arabiensis* and *An. gambiae s.s.* larval fitness through reduced larval survival, development rate, and production of adults (Hardy et al., 2022). The avoidance of chicken dung containing oviposition sites thus fits with the oviposition preference-offspring-performance hypothesis (Ellis, 2008; Yoshioka et al., 2012) and aligns with the typical larval habitats of *An. arabiensis*, characterised by clean fresh water (Mwangangi et al., 2008). Ammonia is a major constituent and principal compound produced from organic nitrogen in chicken dung (Witter, 1991; Kelleher et al., 2002) and has been shown to act as an attractant in host-seeking *An. gambiae s.s.* (Braks, Meijerink and Takken, 2001; Meijerink and Braks, 2001). The application of ammonium sulphate-based fertilisers is known to liberate volatile ammonia (Powlson and Dawson, 2022) and enhance *An. arabiensis* colonisation (Mutero et al., 2004), suggesting ammonia may also act as an oviposition attractant. However, ammonia is toxic to *An. gambiae s.l.* larvae above certain concentrations, negatively impacting larval development at concentrations from 1.3 mg/l and above (Akpodiete and Tripet, 2021) so may elicit a hormetic (Mattson, 2008) oviposition response. In our experiments, even the lowest chicken dung infusion concentrations were above the threshold for eliciting deleterious effects.

Here we demonstrated that chicken dung infusions had significantly higher concentrations of dissolved ammonia, with the mean concentration being more than eight times higher than observed in cow dung. This may underly, at least in part, the finding of significantly fewer eggs laid in chicken dung treated oviposition sites, compared to those treated with cow dung when both were offered together. Furthermore, the ammonia concentration could be a contributing factor to the greater reduction in eggs laid in chicken dung-infused water, when compared to cow dung-infused water, when either was offered as an alternative to deionised water. Higher ammonia concentrations reduce *An. gambiae s.l.* larval fitness (Akpodiete and Tripet, 2021), therefore gravid *An. arabiensis* may avoid oviposition in sites with higher ammonia concentrations when sites with lower concentrations are available, in line with models which state oviposition site choice is mediated by optimal larval performance (Ellis, 2008; Yoshioka et al., 2012). However, chicken and cow dung-based organic fertilisers have been found to increase water ammonia content to a lesser degree than urea-based inorganic fertilisers (Das, Ayyappan and Jena, 2005), which are also known to enhance Anopheline larval densities (Victor and Reuben, 2000). Considering this, it is likely that multiple other unknown ovipositional cues are also involved with the reduction in egg laying observed. Furthermore, in *An. gambiae s.s.*, ammonia is known to activate attractivity to other host-seeking kairomones that are deterrents in isolation (Smallegange, 2005), so it may also modulate the effects of other oviposition cues and potentially enhance their oviposition inhibitory effects in *An. arabiensis*. Despite its role in host location, it is unclear what direct role ammonia plays in *Anopheles* oviposition behaviour and how it may affect the response to other volatiles associated with cow and chicken dung.

Due to the constraints of the methods used in the dual choice egg count bioassays conducted, it is not possible to deduce whether cow and chicken dung are repellent, acting as mid to long-range cues which orient gravid females away from the source, in gravid *An. arabiensis* (Kennedy, 1976;

Day, 2016). However, several volatiles known to display ovipositional inhibitory effects in *An. gambiae s.l.* have been identified from both cow and chicken dung (Summarised in Table 1). If present in the treatments, these may explain the lower proportion of eggs laid in both cow and chicken dung-treated water (Fig. 2). Future research in this area should pair behavioural bioassays with modern rigorous analytical approaches to chemical ecology, such as combined gas chromatography and single sensillum recording (Ignell, Ghaninia and Hill, 2022).

		Detected in	
Name	**Oviposition inhibitory action**	**Cow dung**	**Chicken dung**
3-methylindole (Skatole)	(Eneh, Okal, et al., 2016)	(Huang et al., 2007; Dehnhard, Bernal-Barragan and Claus, 1991)	(Huang et al., 2007; Nakai et al., 1999; Yasuhara, 1987)
4-methyl-phenol (Para-cresol)	(Eneh, Okal, et al., 2016)	(Huang et al., 2007)	(Huang et al., 2007)
Dimethyl disulphide	(Schoelitsz et al., 2020; Suh et al., 2016)	(Woodbury et al., 2016)	(Ranadheera et al., 2017)
Dimethyl trisulphide	(Schoelitsz et al., 2020; Suh et al., 2016)	(Woodbury et al., 2016)	(Ranadheera et al., 2017)
Indole	(Eneh, Okal, et al., 2016)	(Spiehs and Varel, 2009)	(Huang et al., 2007; Yasuhara, 1987)
Nonanal	(Eneh, Okal, et al., 2016)	(Huang et al., 2007)	(Huang et al., 2007; Cooperband et al., 2008)
Phenol	(Eneh, Okal, et al., 2016)	(Shabtay et al., 2009)	(Yasuhara, 1987)

Table 1: Known oviposition inhibitory compounds of *An. gambiae s.l.* and evidence of their presence in cow and chicken dung.

Dung-mediated oviposition attraction has been exhibited in other mosquito species, such as *Cx. pipiens*, *Cx. restuans* (Jackson et al., 2005), *Cx. quinquefasciatus* and *Cx. nigripalpus* (Allan, Bernier and Kline, 2005). Additionally, Dibal, Nok and Umar (2012) demonstrated mesocosms enriched with cow dung had greater larval abundance, compared to those enriched with a clay suspension, for *Culex nebulus, Aedes aegypti, Cx. quinquefasciatus,* and *Culex trigripase.* Similar observations have been reported for *Aedes albopictus* in controlled laboratory trials, showing the species had a strong oviposition preference for habitats polluted with cow dung and that the relative attraction of these sites increased with the number of days the cow dung was fermented (Rajapaksha and Jayatunga, 2021). However, the bacterial community present in the dung infusions in this study was not identified. Future studies directed towards identifying bacterial communities in cow and chicken dung and the semiochemicals they emit may provide useful insight into their modulation of *Anopheles* oviposition behaviour.

The results of this study demonstrate that both dung types may act as oviposition deterrents, as per the definition of Dethier *et al.* (1960), though it is uncertain how the application of cow or chicken dung in the field may affect *An. gambiae s.l*. They may influence the spatial distribution of *An. gambiae s.l.* in the rice agroecosystem by deterring gravid females and leading them to instead oviposit in dung-free habitats. However, this would assume that alternative habitats are both available and of superior quality to that of the dung-polluted rice field. As the present study revealed that egg retention was not displayed when only dung-containing oviposition sites were available, oviposition may still occur if the application of OF is distributed over a sufficiently large area or more acceptable sites are not available. Moreover, as higher dung concentrations were found to further reduce oviposition activity in both dung types, should a concentration gradient exist across a given environment where these OFs have been applied, then reduced oviposition may occur in areas of higher dung concentration relative to those with lower. Nevertheless, the oviposition behaviour of mosquitoes is highly complex and mediated by many interacting environmental factors and internal states (Bentley and Day, 1989; Day, 2016) and factors such as nutrient availability, predator presence, and proximity to hosts are important in the colonisation patterns of mosquitoes (Wondwosen et al., 2017; Pintar et al., 2018; Minakawa, Seda and Yan, 2002). However, oviposition repellents have been demonstrated to effectively reduce colonisation rates in other mosquitoes

(Benelli et al., 2014) and arthropod pests (Hough-Goldstein and Hahn, 1992; Wallingford et al., 2016) and so similar effects may be observed with the putative deterrents, cow and chicken dung.

The observations reported here present several potentially fruitful avenues for future research, primarily in elucidating the nuances of mosquito behaviour in response to cow and chicken dung, and the causative agents of such responses. Assays based on lab-reared mosquito strains may not be reflective of wild populations, posing a limitation to this study and its generalisability, as laboratory strains can be significantly genetically divergent from wild populations and their biological and behavioural responses may not be representative of the populations they were derived from (Gloria-Soria et al., 2019). Therefore, the transposability of these observations to a field-based setting must be examined in semi-field or field-based settings with wild mosquito populations. Given the assays presented here, it is not possible to elucidate whether the observed responses were due to volatile or gustatory cues, and future work should aim to answer this. Additionally, assays to determine if such cues qualify as repellents or deterrents along with those which explore the details of mosquito flight behaviour in response to dung will provide further insight. It may also be productive to examine the constituent compounds which make up potential volatile profiles of cow and chicken dung, and to which of these mosquitoes respond in isolation or blends. Lastly, ovary dissections were not performed here as there was no evidence of egg retention across any treatment groups, however, such post-experimental validation would be valuable in future studies, especially if there is indication of egg retention.

Overall, these findings provide evidence of chicken dung inhibiting *An. gambiae s.l.* oviposition, whilst previous studies have also demonstrated chicken dung's larvicidal qualities (Hardy et al., 2022). Furthermore, the application of chicken dung to aquatic environments has been demonstrated to enhance the density of several predatory invertebrate families, which effectively control *An. gambiae s.l.* even in the presence of alternative prey (Rapatsa and Moyo, 2015; Muiruri et al., 2013; Saha, Aditya and Saha, 2014). The promotion of terrestrial predators due to chicken dung application also reduces rice crop damage by several phytophagous pest species (Chen, 2007). Therefore, its use could satisfy the fertiliser demands of the growing crop, whilst also limiting the production of malaria vectors and controlling rice pests alike. The putative use of chicken dung as a combined fertiliser and partial oviposition inhibitor may inform the emphasis of promoting this practice in SRI, and to non-SRI practitioners equally, though field trials should consider the potential direct negative health impacts it may have on individuals working in rice paddies and its effects on non-target species.

5. Conclusion

This research demonstrates the effects of cow and chicken dung on the egg-laying behaviour of *An. arabiensis*, representing the first report in Anophelines on chicken dung. Both OFs were found to partially inhibit oviposition and with increased concentrations both elicited further reduced egg laying. When gravid mosquitoes were presented with a cow and a chicken dung treated oviposition site simultaneously, a higher proportion of eggs were laid in those containing cow dung. However, across all assays, there was no evidence of egg retention. These data suggest that these OFs may deter gravid *An. arabiensis* seeking an oviposition site, that higher concentrations of both likely enhance this effect, and that chicken dung may be relatively more unattractive than cow dung. The findings of this study indicate that the application of manure-based OFs, such as cow or chicken dung, may alter the spatial distribution of *An. arabiensis* mosquitoes by reducing the number of eggs oviposited by gravid females in dung polluted water. The promotion of OFs in SRI, and their use in rice agriculture more broadly, might therefore alter the malaria transmission dynamics of a given area and incidence in communities associated with rice cultivation. In this manner, chicken dung application may deter gravid mosquitoes from the applied area, reduce the larval fitness of mosquitoes breeding in the treated habitat, promote the biodiversity of predatory invertebrates which control mosquitoes and rice pests alike, and meet the fertiliser demands of a growing rice crop.

6. Declarations

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6.3 Availability of data and materials

All data is presented in the manuscript and is available upon reasonable request.

6.4 Authors contributions

Harrison Hardy: conceptualisation, methodology, formal analysis, investigation, writing – original draft, visualisation, project administration; **Frances Hawkes:** conceptualisation, methodology, writing – review & editing, supervision, funding acquisition, project administration; **Steven Harte**: methodology, formal analysis, investigation, writing – review & editing; **Richard Hopkins:** conceptualisation, writing – review & editing, supervision, funding acquisition; **Ladslaus Mnyone:** conceptualisation, writing – review & editing, supervision, funding acquisition.

6.5 Ethics approval and consent to participate

Not applicable.

6.6 Consent for publication

Not applicable.

6.7 Competing interests

The authors declare that they have no competing interests.

7. References

Akpodiete, N. O. and Tripet, F. (2021) Laboratory and microcosm experiments reveal contrasted adaptive responses to ammonia and water mineralisation in aquatic stages of the sibling species Anopheles gambiae (sensu stricto) and Anopheles coluzzii, *Parasites & Vectors*, 14(1), p. 17.

Albeny-Simões, D., Murrell, E. G., Elliot, S. L., Andrade, M. R., Lima, E., Juliano, S. A. and Vilela, E. F. (2014) Attracted to the enemy: Aedes aegypti prefers oviposition sites with predator-killed conspecifics, *Oecologia*, 175(2), pp. 481–492.

Allan, S. A., Bernier, U. R. and Kline, D. L. (2005) Evaluation of oviposition substrates and organic infusions on collection of Culex in Florida, *Journal of the American Mosquito Control Association*, BioOne, 21(3), pp. 268–273.

Asmare, Y., Hill, S. R., Hopkins, R. J., Tekie, H. and Ignell, R. (2017) The role of grass volatiles on oviposition site selection by Anopheles arabiensis and Anopheles coluzzii, *Malaria Journal*, 16(1), p. 65.

Benelli, G., Conti, B., Garreffa, R. and Nicoletti, M. (2014) Shedding light on bioactivity of botanical by-products: neem cake compounds deter oviposition of the arbovirus vector Aedes albopictus (Diptera: Culicidae) in the field, *Parasitology Research*, 113(3), pp. 933–940.

Bentley, M. D. and Day, J. F. (1989) Chemical Ecology and Behavioral Aspects of Mosquito Oviposition, *Annual Review of Entomology*, 34(1), pp. 401–421.

Blaustein, L., Blaustein, J. and Chase, J. (2005) Chemical detection of the predator Notonecta irrorata by ovipositing Culex mosquitoes, *Journal of Vector Ecology*, 30(2), pp. 299–301.

Braks, M. A. H., Meijerink, J. and Takken, W. (2001) The response of the malaria mosquito, *Anopheles gambiae* , to two components of human sweat, ammonia and L -lactic acid, in an olfactometer: *Sweat components as kairomone for* Anopheles gambiae, *Physiological Entomology*, 26(2), pp. 142–148.

Buxton, M., Cuthbert, R. N., Dalu, T., Nyamukondiwa, C. and Wasserman, R. J. (2020) Cattle-induced eutrophication favours disease-vector mosquitoes, *Science of The Total Environment*, 715, p. 136952.

Chan, K., Tusting, L. S., Bottomley, C., Saito, K., Djouaka, R. and Lines, J. (2022) Malaria transmission and prevalence in rice-growing versus non-rice-growing villages in Africa: a systematic review and meta-analysis, *The Lancet Planetary Health*, Elsevier, 6(3), pp. e257–e269.

Chen, Y. H. (2007) Biological control in rice: Testing the role of manure in enhancing predation, In *The 2007 ESA Annual Meeting, December 9-12, 2007*.

Clements, A. N. (1999) *The biology of mosquitoes. Volume 2: sensory reception and behaviour.*, CABI publishing.

Cooperband, M. F., McElfresh, J. S., Millar, J. G. and Cardé, R. T. (2008) Attraction of female Culex quinquefasciatus Say (Diptera: Culicidae) to odors from chicken feces, *Journal of Insect Physiology*, 54(7), pp. 1184–1192.

Crawley, M. J. (2013) *The R book*, Second edition, Chichester, West Sussex, UK, Wiley.

Das, P. C., Ayyappan, S. and Jena, J. (2005) Comparative changes in water quality and role of pond soil after application of different levels of organic and inorganic inputs, *Aquaculture Research*, 36(8), pp. 785–798.

Day, J. F. (2016) Mosquito Oviposition Behavior and Vector Control, *Insects*, 7(4), p. 65.

Dehnhard, M., Bernal-Barragan, H. and Claus, R. (1991) Rapid and accurate high-performance liquid chromatographic method for the determination of 3-methylindole (skatole) in faeces of various species, *Journal of Chromatography B: Biomedical Sciences and Applications*, 566(1), pp. 101–107.

Dethier, V. G., Browne, B. L. and Smith, C. N. (1960) The Designation of Chemicals in Terms of the Responses They Elicit from Insects, *Journal of Economic Entomology*, 53(1), pp. 134–136.

Detinova, T. S. (1962) *Age-grouping methods in Diptera of medical importance, with special reference to some vectors of malaria*, World Health Organization.

Dhar, R., Dawar, H., Garg, S., Basir, S. and Talwar, G. P. (1996) Effect of volatiles from neem and other natural products on gonotrophic cycle and oviposition of Anopheles stephensi and An. culicifacies (Diptera: Culicidae), *Journal of medical entomology*, Oxford University Press Oxford, UK, 33(2), pp. 195–201.

Dibal, D., Nok, I. and Umar, Y. (2012) Effect of color, cow dung and clay suspension on oviposition behavior of gravid female mosquitoes in Zaria, Kaduna state, Nigeria, *Detinova, T.S., Bertram, D.S. and World Health Organization, 1962. Age-grouping methods in Diptera of medical importance, with special reference to some vectors of malaria. World Health Organization.*, 2(6), pp. 223–226.

Eldridge, B. F. and Harbach, R. E. (1992) Conservation of the names Culex stigmatosoma and Culex thriambus, *J Am Mosq Control Assoc*, 8, pp. 104–105.

Ellis, A. M. (2008) Incorporating density dependence into the oviposition preference–offspring performance hypothesis, *Journal of Animal Ecology*, 77(2), pp. 247–256.

Eneh, L. K., Fillinger, U., Borg Karlson, A. K., Kuttuva Rajarao, G. and Lindh, J. (2019) *Anopheles arabiensis* oviposition site selection in response to habitat persistence and associated physicochemical parameters, bacteria and volatile profiles, *Medical and Veterinary Entomology*, 33(1), pp. 56–67.

Eneh, L. K., Okal, M. N., Borg-Karlson, A.-K., Fillinger, U. and Lindh, J. M. (2016) Gravid Anopheles gambiae sensu stricto avoid ovipositing in Bermuda grass hay infusion and it’s volatiles in two choice egg-count bioassays, *Malaria Journal*, 15(1), p. 276.

Eneh, L. K., Saijo, H., Borg-Karlson, A.-K., Lindh, J. M. and Rajarao, G. K. (2016) Cedrol, a malaria mosquito oviposition attractant is produced by fungi isolated from rhizomes of the grass Cyperus rotundus, *Malaria Journal*, 15(1), p. 478.

Gloria-Soria, A., Soghigian, J., Kellner, D. and Powell, J. R. (2019) Genetic diversity of laboratory strains and implications for research: The case of Aedes aegypti, Barker, C. M. (ed.), *PLOS Neglected Tropical Diseases*, 13(12), p. e0007930.

Hardy, H., Hopkins, R., Mnyone, L. and Hawkes, F. M. (2022) Manure and mosquitoes: life history traits of two malaria vector species enhanced by larval exposure to cow dung, whilst chicken dung has a strong negative effect, *Parasites & Vectors*, 15(1), p. 472.

Harris, C., Lwetoijera, D. W., Dongus, S., Matowo, N. S., Lorenz, L. M., Devine, G. J. and Majambere, S. (2013) Sterilising effects of pyriproxyfen on Anopheles arabiensis and its potential use in malaria control, *Parasites & Vectors*, 6(1), p. 144.

Hough-Goldstein, J. and Hahn, S. P. (1992) Antifeedant and Oviposition Deterrent Activity of an Aqueous Extract of Tanacetum vulgare L. on Two Cabbage Pests, *Environmental Entomology*, 21(4), pp. 837–844.

Huang, J., He, J., Zhang, J. and Yu, Z. (2007) Identification of volatile organic compounds in the manures of cow, hog and chicken by solid phase microextraction coupled with gas chromatography/mass spectrometry, *Se pu= Chinese Journal of Chromatography*, 25(3), pp. 425–429.

Ignell, R., Ghaninia, M. and Hill, S. R. (2022) Combined Gas Chromatography and Single Sensillum Recording for Mosquitoes, *Cold Spring Harbor Protocols*, Cold Spring Harbor Laboratory Press, (9), p. pdb-prot107869.

Ignell, R. and Hill, S. R. (2020) Malaria Mosquito Chemical Ecology, *Current Opinion in Insect Science*, 40, pp. 6–10.

Ijumba, J. N. and Lindsay, S. W. (2001) Impact of irrigation on malaria in Africa: Paddies paradox, *Medical and Veterinary Entomology*, 15(1), pp. 1–11.

Ijumba, J. N., Mosha, F. W. and Lindsay, S. W. (2002) Malaria transmission risk variations derived from different agricultural practices in an irrigated area of northern Tanzania, *Medical and Veterinary Entomology*, 16(1), pp. 28–38.

Ikeshoji, T. and Mulla, M. S. (1970) Oviposition Attractants for Four Species of Mosquitoes in Natural Breeding Waters, *Annals of the Entomological Society of America*, 63(5), pp. 1322–1327.

Jackson, B. T., Paulson, S. L., Youngman, R. R., Scheffel, S. L. and Hawkins, B. (2005) Oviposition preferences of Culex restuans and Culex pipiens (Diptera: Culicidae) for selected infusions in oviposition traps and gravid traps, *Journal of the American Mosquito Control Association*, BioOne, 21(4), pp. 360–365.

Jarju, L. B., Fillinger, U., Green, C., Louca, V., Majambere, S. and Lindsay, S. W. (2009) Agriculture and the promotion of insect pests: rice cultivation in river floodplains and malaria vectors in The Gambia, *Malaria Journal*, 8(1), p. 170.

Jeanrenaud, A. C. S. N., Brooke, B. D. and Oliver, S. V. (2019) The effects of larval organic fertiliser exposure on the larval development, adult longevity and insecticide tolerance of zoophilic members of the Anopheles gambiae complex (Diptera: Culicidae), Hülsen, T. (ed.), *PLOS ONE*, 14(4), p. e0215552.

Kabula, B., Kisinza, W., Tungu, P., Ndege, C., Batengana, B., Kollo, D., Malima, R., Kafuko, J., Mohamed, M. and Magesa, S. (2014) Co-occurrence and distribution of East (L1014S) and West (L1014F) African knock-down resistance in Anopheles gambiae sensu lato population of Tanzania, 00(00).

Kafy, H. T., Ismail, B. A., Mnzava, A. P., Lines, J., Abdin, M. S. E., Eltaher, J. S., Banaga, A. O., West, P., Bradley, J., Cook, J., Thomas, B., Subramaniam, K., Hemingway, J., Knox, T. B., Malik, E. M., Yukich, J. O., Donnelly, M. J. and Kleinschmidt, I. (2017) Impact of insecticide resistance in *Anopheles* *arabiensis* on malaria incidence and prevalence in Sudan and the costs of mitigation, *Proceedings of the National Academy of Sciences*, 114(52), pp. E11267–E11275.

Katambara, Z., Kahimba, F. C., Mahoo, H. F., Mbungu, W. B., Mhenga, F., Reuben, P., Maugo, M. and Nyarubamba, A. (2013) Adopting the system of rice intensification (SRI) in Tanzania: A review, *Agricultural Sciences*, 04(08), pp. 369–375.

Kawada, H., Dida, G. O., Ohashi, K., Komagata, O., Kasai, S., Tomita, T., Sonye, G., Maekawa, Y., Mwatele, C., Njenga, S. M., Mwandawiro, C., Minakawa, N. and Takagi, M. (2011) Multimodal Pyrethroid Resistance in Malaria Vectors, Anopheles gambiae s.s., Anopheles arabiensis, and Anopheles funestus s.s. in Western Kenya, Oliveira, P. L. (ed.), *PLoS ONE*, 6(8), p. e22574.

Kelleher, B. P., Leahy, J. J., Henihan, A. M., O’Dwyer, T. F., Sutton, D. and Leahy, M. J. (2002) Advances in poultry litter disposal technology – a review, *Bioresource Technology*, 83(1), pp. 27–36.

Kennedy, J. S. (1976) Behaviourally discriminating assays of attractants and repellents, *Pesticide Science*, Wiley Online Library, 7(6), pp. 632–632.

Killeen, G. F., Govella, N. J., Lwetoijera, D. W. and Okumu, F. O. (2016) Most outdoor malaria transmission by behaviourally-resistant Anopheles arabiensis is mediated by mosquitoes that have previously been inside houses, *Malaria journal*, Springer, 15, pp. 1–10.

Kitau, J., Oxborough, R. M., Tungu, P. K., Matowo, J., Malima, R. C., Magesa, S. M., Bruce, J., Mosha, F. W. and Rowland, M. W. (2012) Species shifts in the anopheles gambiae complex: Do LLINs successfully control anopheles arabiensis?, *PLoS ONE*, 7(3), pp. 1–7.

Kramer, W. L. and Mulla, M. S. (1979) Oviposition Attractants and Repellents of Mosquitoes: Oviposition Responses of Culex Mosquitoes to Organic Infusions, *Environmental Entomology*, 8(6), pp. 1111–1117.

Kweka, E. J., Owino, E. A., Mwang’onde, B. J., Mahande, A. M., Nyindo, M. and Mosha, F. (2011) The role of cow urine in the oviposition site preference of culicine and Anopheles mosquitoes, *Parasites & Vectors*, 4(1), p. 184.

Latif, M. A., Islam, M. R., Ali, M. Y. and Saleque, M. A. (2005) Validation of the system of rice intensification (SRI) in Bangladesh, *Field Crops Research*, 93(2–3), pp. 281–292.

Mattson, M. P. (2008) Hormesis defined, *Ageing Research Reviews*, 7(1), pp. 1–7.

Mbuya, N. P., Kateyo, E. and Lunyolo, F. (2014) Assessment of Anopheles Larval Source Reduction Using Cow Dung: Environmental Perspective on Pro-poor Tool for Malaria Vector Control, *International Journal of Innovation and Applied Studies*, 5(1), p. 30.

Meertens, H. C. C. (2001) The prospects for integrated nutrient management for sustainable rainfed lowland rice production in Sukumaland, Tanzania, *Nutrient Cycling in Agroecosystems*, 65(2), pp. 163–171.

Meijerink, J. and Braks, M. A. H. (2001) Olfactory receptors on the antennae of the malaria mosquito Anopheles gambiae are sensitive to ammonia and other sweat-borne components, *Journal of Insect Physiology*, p. 11.

Meola, R. and Lea, A. O. (1972) Humoral Inhibition of Egg Development in Mosquitoes, *Journal of Medical Entomology*, 9(1), pp. 99–103.

Messenger, L. A., Shililu, J., Irish, S. R., Anshebo, G. Y., Tesfaye, A. G., Ye-Ebiyo, Y., Chibsa, S., Dengela, D., Dissanayake, G., Kebede, E., and others (2017) Insecticide resistance in Anopheles arabiensis from Ethiopia (2012–2016): a nationwide study for insecticide resistance monitoring, *Malaria journal*, BioMed Central, 16(1), pp. 1–14.

Minakawa, N., Seda, P. and Yan, G. (2002) Influence of host and larval habitat distribution on the abundance of African malaria vectors in western Kenya., *The American Journal of Tropical Medicine and Hygiene*, 67(1), pp. 32–38.

Muiruri, S. K., Mwangangi, J. M., Carlson, J., Kabiru, E. W., Kokwaro, E., Githure, J., Mbogo, C. M. and Beier, J. C. (2013) Effect of predation on Anopheles larvae by five sympatric insect families in coastal Kenya, *J Vector Borne Dis*, 50(1), p. 45.

Muriu, S. M., Muturi, E. J., Shililu, J. I., Mbogo, C. M., Mwangangi, J. M., Jacob, B. G., Irungu, L. W., Mukabana, R. W., Githure, J. I. and Novak, R. J. (2008) Host choice and multiple blood feeding behaviour of malaria vectors and other anophelines in Mwea rice scheme, Kenya, *Malaria journal*, BioMed Central, 7(1), pp. 1–7.

Mutero, C. M., Ng'ang'a, P. N., Wekoyela, P., Githure, J. and Konradsen, F. (2004) Ammonium sulphate fertiliser increases larval populations of Anopheles arabiensis and culicine mosquitoes in rice fields, *Acta Tropica*, 89(2), pp. 187–192.

Muturi, E. J., Mwangangi, J. M., Beier, J. C., Blackshear, M., Wauna, J., Sang, R. and Mukabana, W. R. (2013) Ecology and behavior of Anopheles arabiensis in relation to agricultural practices in central Kenya, *Journal of the American Mosquito Control Association*, BioOne, 29(3), pp. 222–230.

Muturi, E. J., Shililu, J. I., Jacob, B. G., Mwangangi, J. M., Mbogo, C. M., Githure, J. I. and Novak, R. J. (2008) Diversity of Riceland Mosquitoes and Factors Affecting Their Occurrence and Distribution in Mwea, Kenya, *Journal of the American Mosquito Control Association*, 24(3), pp. 349–358.

Muturi, E. J., Shililu, J., Jacob, B., Gu, W., Githure, J. and Novak, R. (2006) Mosquito species diversity and abundance in relation to land use in a riceland agroecosystem in Mwea, Kenya, *Journal of Vector Ecology*, 31(1), pp. 129–137.

Mwangangi, J. M., Muturi, E. J., Shililu, J., Muriu, S. M., Jacob, B., Kabiru, E. W., Mbogo, C. M., Githure, J. and Novak, R. (2008) Contribution of different aquatic habitats to adult Anopheles arabiensis and Culex quinquefasciatus (Diptera: Culicidae) production in a rice agroecosystem in Mwea, Kenya, *Journal of Vector Ecology*, 33(1), pp. 129–138.

Mwangangi, J. M., Muturi, E. J., Shililu, J., Muriu, S. M., Jacob, B., Kabiru, E. W., Mbogo, C. M., Githure, J. and Novak, R. (2006) Survival of immature Anopheles arabiensis (Diptera: Culicidae) in aquatic habitats in Mwea rice irrigation scheme, central Kenya, *Malaria Journal*, 5(1), p. 114.

Mwangangi, J., Shililu, J., Muturi, E., Gu, W., Mbogo, C., Kabiru, E., Jacob, B., Githure, J. and Novak, R. (2006) Dynamics of immature stages of Anopheles arabiensis and other mosquito species (Diptera: Culicidae) in relation to rice cropping in a rice agro-ecosystem in Kenya, *Journal of Vector Ecology*, 31(2), pp. 245–251.

Mwingira, V., Mboera, L. E. G., Dicke, M. and Takken, W. (2020) Exploiting the chemical ecology of mosquito oviposition behavior in mosquito surveillance and control: a review, *Journal of Vector Ecology*, 45(2), pp. 155–179.

Mwingira, V. S., Spitzen, J., Mboera, L. E. G., Torres-Estrada, J. L. and Takken, W. (2019) The Influence of Larval Stage and Density on Oviposition Site-Selection Behavior of the Afrotropical Malaria Mosquito Anopheles coluzzii (Diptera: Culicidae), Reisen, W. (ed.), *Journal of Medical Entomology*, 57(3), pp. 657–666.

Mwingira, V. S., Spitzen, J., Mboera, L. E. G., Torres-Estrada, J. L. and Takken, W. (2020) The Influence of Larval Stage and Density on Oviposition Site-Selection Behavior of the Afrotropical Malaria Mosquito Anopheles coluzzii (Diptera: Culicidae), Reisen, W. (ed.), *Journal of Medical Entomology*, 57(3), pp. 657–666.

Nakai, Y., Niino, T., Ando, T. and Kodha, C. (1999) Microorganisms aerobically degrading skatole or indole in composting processes, *Nihon Chikusan Gakkaiho*, Japanese Society of Animal Science, 70(1), pp. 32–37.

Ogola, E. O., Chepkorir, E., Sang, R. and Tchouassi, D. P. (2019) A previously unreported potential malaria vector in a dry ecology of Kenya, *Parasites & Vectors*, 12(1), p. 80.

Okal, M. N., Francis, B., Herrera-Varela, M., Fillinger, U. and Lindsay, S. W. (2013) Water vapour is a pre-oviposition attractant for the malaria vector Anopheles gambiae sensu stricto, *Malaria Journal*, 12(1), p. 365.

Okal, M. N., Lindh, J. M., Torr, S. J., Masinde, E., Orindi, B., Lindsay, S. W. and Fillinger, U. (2015) Analysing the oviposition behaviour of malaria mosquitoes: design considerations for improving two-choice egg count experiments, *Malaria journal*, Springer, 14(1), pp. 1–17.

Oliver, S. V. and Brooke, B. D. (2018) The effect of commercial herbicide exposure on the life history and insecticide resistance phenotypes of the major malaria vector Anopheles arabiensis (Diptera: culicidae), *Acta Tropica*, 188, pp. 152–160.

Opoku, A. and Ansa-Asare, O. (2009) The occurrences and habitat characteristics of mosquitoes in Accra, Ghana, *West African Journal of Applied Ecology*, 11(1), [online] Available at: http://www.ajol.info/index.php/wajae/article/view/45730 (Accessed 9 November 2022).

Otienoburu, P. E., Bayoh, N., Gimnig, J., Huang, J., Walker, E. D., Otieno, M. F., Vulule, J. and Miller, J. R. (2007) Anopheles gambiae (Diptera: Culicidae) oviposition as influenced by type of water infused into black and red soils of western Kenya, *International Journal of Tropical Insect Science*, 27(01), p. 2.

Pekár, S. and Brabec, M. (2016) *Modern analysis of biological data: generalized linear models in R*, Masarykova univerzita.

Perugini, E., Guelbeogo, W. M., Calzetta, M., Manzi, S., Virgillito, C., Caputo, B., Pichler, V., Ranson, H., Sagnon, N., della Torre, A. and Pombi, M. (2020) Behavioural plasticity of Anopheles coluzzii and Anopheles arabiensis undermines LLIN community protective effect in a Sudanese-savannah village in Burkina Faso, *Parasites & Vectors*, 13(1), p. 277.

Pintar, M. R., Bohenek, J. R., Eveland, L. L. and Resetarits, W. J. (2018) Colonization across gradients of risk and reward: Nutrients and predators generate species-specific responses among aquatic insects, Seymour, C. (ed.), *Functional Ecology*, 32(6), pp. 1589–1598.

Powlson, D. S. and Dawson, C. J. (2022) Use of ammonium sulphate as a sulphur fertilizer: Implications for ammonia volatilization, *Soil Use and Management*, 38(1), pp. 622–634.

R Core Team (2019) *R: A Language and Environment for Statistical Computing*, Vienna, Austria, R Foundation for Statistical Computing, [online] Available at: https://www.R-project.org/ (Accessed 2 February 2021).

Rahman, M. M. (2013) Nutrient-Use and Carbon-Sequestration Efficiencies in Soils from Different Organic Wastes in Rice and Tomato Cultivation, *Communications in Soil Science and Plant Analysis*, 44(9), pp. 1457–1471.

Rajapaksha, R. D. T. and Jayatunga, D. P. W. (2021) Influence of Vertebrate Excreta on Attraction, Oviposition and Development of the Asian Tiger Mosquito, Aedes albopictus (Diptera: Culicidae), *Insects*, 12(4), p. 313.

Ranadheera, C. S., Mcconchie, R., Phan-Thien, K. and Bell, T. (2017) Strategies for eliminating chicken manure odour in horticultural applications, *World's Poultry Science Journal*, 73(2), pp. 365–378.

Rapatsa, M. M. and Moyo, N. A. G. (2015) An Investigation into the Physico-chemical Factors Affecting the Abundance and Diversity of Aquatic Insects in Organically Manured Aquadams and Their Utilization by *Oreochromis mossambicus* (Perciformes: Cichlidae), *Environmental Entomology*, 44(4), pp. 990–998.

Saha, N., Aditya, G. and Saha, G. K. (2014) Prey preferences of aquatic insects: potential implications for the regulation of wetland mosquitoes, *Medical and Veterinary Entomology*, 28(1), pp. 1–9.

Samuel, M., Brooke, B. D. and Oliver, S. V. (2020) Effects of inorganic fertilizer on larval development, adult longevity and insecticide susceptibility in the malaria vector *Anopheles arabiensis* (Diptera: Culicidae), *Pest Management Science*, 76(4), pp. 1560–1568.

Schoelitsz, B., Mwingira, V., Mboera, L. E. G., Beijleveld, H., Koenraadt, C. J. M., Spitzen, J., van Loon, J. J. A. and Takken, W. (2020) Chemical Mediation of Oviposition by Anopheles Mosquitoes: a Push-Pull System Driven by Volatiles Associated with Larval Stages, *Journal of Chemical Ecology*, 46(4), pp. 397–409.

Service, M. (1989) Rice, a challenge to health, *Parasitology Today*, Elsevier, 5(5), pp. 162–165.

Shabtay, A., Ravid, U., Brosh, A., Baybikov, R., Eitam, H. and Laor, Y. (2009) Dynamics of offensive gas-phase odorants in fresh and aged feces throughout the development of beef cattle, *Journal of Animal Science*, 87(5), pp. 1835–1848.

Sinka, M. E., Bangs, M. J., Manguin, S., Rubio-Palis, Y., Chareonviriyaphap, T., Coetzee, M., Mbogo, C. M., Hemingway, J., Patil, A. P., Temperley, W. H., Gething, P. W., Kabaria, C. W., Burkot, T. R., Harbach, R. E. and Hay, S. I. (2012) A global map of dominant malaria vectors, *Parasites & Vectors*, 5(1), p. 69.

Sinka, M. E., Manguin, S., , Maureen Coetzee, C. M. M., Hemingway, J., Patil, A. P., Temperley, W. H., Gething, P. W., Kabaria, C. W., Okara, R. M., Thomas Van Boeckel, H. C. J. G. and , Ralph E Harbach, S. I. Hay. (2010) The dominant Anopheles vectors of human malaria in Africa, Europe and the Middle East: occurrencedata, distribution maps and bionomic précis., *Parasites and Vectors*, 3, p. 117.

Sissoko, M. S., Dicko, A., Briët, O. J. T., Sissoko, M., Sagara, I., Keita, H. D., Sogoba, M., Rogier, C., Touré, Y. T. and Doumbo, O. K. (2004) Malaria incidence in relation to rice cultivation in the irrigated Sahel of Mali, *Acta Tropica*, 89(2), pp. 161–170.

Smallegange, R. C. (2005) Synergism between ammonia, lactic acid and carboxylic acids as kairomones in the host-seeking behaviour of the malaria mosquito Anopheles gambiae sensu stricto (Diptera: Culicidae), *Chemical Senses*, 30(2), pp. 145–152.

Spiehs, M. J. and Varel, V. H. (2009) Nutrient excretion and odorant production in manure from cattle fed corn wet distillers grains with solubles1,2, *Journal of Animal Science*, 87(9), pp. 2977–2984.

Stoop, W. A., Uphoff, N. and Kassam, A. (2002) A review of agricultural research issues raised by the system of rice intensification (SRI) from Madagascar: opportunities for improving farming systems for resource-poor farmers, *Agricultural Systems*, 71(3), pp. 249–274.

Suh, E., Choe, D.-H., Saveer, A. M. and Zwiebel, L. J. (2016) Suboptimal Larval Habitats Modulate Oviposition of the Malaria Vector Mosquito Anopheles coluzzii, Hansen, I. A. (ed.), *PLOS ONE*, 11(2), p. e0149800.

Sumba, L. A., Guda, T. O., Deng, A. L., Hassanali, A., Beier, J. C. and Knols, B. G. J. (2004) Mediation of oviposition site selection in the African malaria mosquito Anopheles gambiae (Diptera: Culicidae) by semiochemicals of microbial origin, *International Journal of Tropical Insect Science*, 24(3), pp. 260–265.

Takken, W. and Verhulst, N. O. (2013) Host Preferences of Blood-Feeding Mosquitoes, *Annual Review of Entomology*, 58(1), pp. 433–453.

Thakur, A. K. and Uphoff, N. T. (2017) How the System of Rice Intensification Can Contribute to Climate-Smart Agriculture, *Agronomy Journal*, 109(4), pp. 1163–1182.

Tirados, I., Costantini, C., Gibson, G. and Torr, S. J. (2006) Blood-feeding behaviour of the malarial mosquito Anopheles arabiensis: implications for vector control, *Medical and Veterinary Entomology*, 20(4), pp. 425–437.

Tzollas, N. M., Zachariadis, G. A., Anthemidis, A. N. and Stratis, J. A. (2010) A new approach to indophenol blue method for determination of ammonium in geothermal waters with high mineral content, *International Journal of Environmental Analytical Chemistry*, 90(2), pp. 115–126.

Van Vliet, P., Reijs, J., Bloem, J., Dijkstra, J. and De Goede, R. (2007) Effects of cow diet on the microbial community and organic matter and nitrogen content of feces, *Journal of Dairy Science*, Elsevier, 90(11), pp. 5146–5158.

Victor, T. and Reuben, R. (2000) Effects of organic and inorganic fertilisers on mosquito populations in rice fields of southern India, *Medical and veterinary entomology*, Wiley Online Library, 14(4), pp. 361–368.

Wallingford, A. K., Hesler, S. P., Cha, D. H. and Loeb, G. M. (2016) Behavioral response of spotted-wing drosophila, *Drosophila suzukii* Matsumura, to aversive odors and a potential oviposition deterrent in the field: *Drosophila suzukii* deterrents, *Pest Management Science*, 72(4), pp. 701–706.

Wang, Yan, Zhu, Y., Zhang, S. and Wang, Yongqiang (2018) What could promote farmers to replace chemical fertilizers with organic fertilizers?, *Journal of Cleaner Production*, Elsevier, 199, pp. 882–890.

Witter, E. (1991) Use of CaCl2 to decrease ammonia volatilization after application of fresh and anaerobic chicken slurry to soil, *Journal of soil science*, Wiley Online Library, 42(3), pp. 369–380.

Wondwosen, B., Birgersson, G., Seyoum, E., Tekie, H., Torto, B., Fillinger, U., Hill, S. R. and Ignell, R. (2016) Rice volatiles lure gravid malaria mosquitoes, Anopheles arabiensis, *Scientific Reports*, 6(1), p. 37930.

Wondwosen, B., Hill, S. R., Birgersson, G., Seyoum, E., Tekie, H. and Ignell, R. (2017) A(maize)ing attraction: gravid Anopheles arabiensis are attracted and oviposit in response to maize pollen odours, *Malaria Journal*, 16(1), p. 39.

Woodbury, B. L., Gilley, J. E., Parker, D. B., Marx, D. B. and Eigenberg, R. A. (2016) Emission of volatile organic compounds as affected by rate of application of cattle manure, *Transactions of the ASABE*, American Society of Agricultural and Biological Engineers, 59(3), pp. 885–895.

Yasuhara, A. (1987) Identification of volatile compounds in poultry manure by gas chromatography—mass spectrometry, *Journal of Chromatography A*, 387, pp. 371–378.

Yoshioka, M., Couret, J., Kim, F., McMillan, J., Burkot, T. R., Dotson, E. M., Kitron, U. and Vazquez-Prokopec, G. M. (2012) Diet and density dependent competition affect larval performance and oviposition site selection in the mosquito species Aedes albopictus (Diptera: Culicidae), *Parasites & Vectors*, 5(1), p. 225.