**Discussion**

In this study, we applied a Bayesian hierarchical framework to analyse two databases containing experiments used to determine mosquito lifespan: a published database of mark-release- recapture experiments and a compilation of mosquito dissection studies which we assembled via a literature search . By applying a single framework, we combined information across experiments which, individually, estimate lifespan with considerable uncertainty. Due to the assumptions required to analyse the field data, our estimates represent lower bounds on lifespan (LBL). Across both meta-analyses, the majority of estimated LBLs were less than 10 days, hinting that a minorityof mosquitoes live long enough to transmit disease. We determined that LBL varies across species and genera although most variance is explained by genus. The MRR analysis includes experiments conducted on each sex individually, and we estimate that, on average, females outlive males. Pre-release feeding with sugar also lengthens lifespan across all three genera, although this eﬀect is less marked than the sex diﬀerences. In contrast to some lab-based experiments (Yang et al., 2009; Brady et al., 2013), temperature was not determined to significantly impact lifespan. By fitting a range of survival models to the data in both meta-analyses, we assessed evidence for age-dependent mortality. Overall, the evidence for senescence was mixed: in the MRR experiments, in 8 of 33 species the data was better fit by models including a mortality hazard increasing with age, whereas the corresponding number for the dissection analysis was 2 out of 25 species,

MRR experiments are thought to under-estimate lifespan: lab experiments have shown that marking mosquitoes with dye can negatively impact survival (Verhulst, Loonen, and Takken, 2013; Dickens and Brant, 2014);

and MRR studies typically cannot diﬀerentiate between a mosquito dying and its dispersal from the study area meaning mortality is inflated. In this study, there was a positive correlation between lifespan estimates and trapping density, indicating better trapping coverage likely raises estimates towards their true value. We conducted an *in silico* Monte Carlo study to determine how accurately mosquito lifespan can be estimated in an ideal MRR experiment, where mosquitoes are not harmed by marking nor emigrate from the study area(see SOM). =This indicated that the the short study durations or small release sizes typical of the modal experiment results in high uncertainty in lifespan estimates (Fig. S12). This indicates pooling of data across experiments using a hierarchical model should lead to valuable increases in power. l.

Polovodova was the first to suggest that dissection data can be used to determine the number of gonotrophic cycles a female mosquito has undergone (Polovodova, 1948), termed their ``physiological age’’. The key assumptions in using dissection data to estimate mosquito age are : (i) physiological age can be accurately determined by ovariolar dissection (unlike MRR, this method can only be applied to females) and (ii) the relationship between physiological and chronological age is known. Dissection data likely understates physiological age because most ovarioles have fewer dilations than the number of gonotrophic cycles undergone (Fox and Brust, 1994; Hugo et al., 2008), an issue which is known to worsen with age (Fox and Brust, 1994), meaning a large number of ovarioles must be dissected to locate those representative few, which may be impractical (Gillies and Wilkes, 1965). The exchange rate between physiological age and chronological age is the duration of gonotrophic cycles and two methods are commonly used to estimate their duration: MRR studies (see, for example, Gillies and Wilkes, 1965), where marked mosquitoes are recaptured and dissected to determine the number of gonotrophic cycles occurring since release; and dissections of specimens from laboratory colonies (see, for example, Afrane et al., 2005). In our analysis, laboratory studies indicated a longer gonotrophic cycle of the two approaches (Fig. S9). The literature does not however favour one experimental method over another, and so to convert physiological age into calendar age we pooled data from both sources. It is possible, however, that aggregating data across both methods induces biases and an approach more entrenched in experimental knowledge would fare better. The dissection data yields estimates of the age structure of the population at snapshots in time, which can be used to determine mean lifespans (see SOM). This requires additional assumptions however and here we assume that the population is at equilibrium (recruitment matches mortality) and collected individuals are random samples from that population. If a population is shrinking, there are fewer young mosquitoes and the survival curve is flatter than at equilibrium, meaning we would overestimate lifespan. To balance those samples collected whilst the population density was declining with those taken during expansion, we aggregated capture data across collections occurring at different timepoints for each site. Field entomologists have argued that trapping methods do not yield unbiased samples from the population, although there is not consensus on whether this results in a relative paucity (Gillies and Wilkes, 1965) or inflation (Clements and Paterson, 1981) of actual nulliparous numbers. Though after excluding those data with a large deficit of nulliparous individuals, posterior predictive checks indicated that our models fit the data well across all age classes (see SOM) hinting the assumption of random sampling may be reasonable. Overall, the assumptions underpinning our analysis of dissection data suggest our estimates, like those from MRR, represent lower bounds on lifespan. The alternative dissection-based approach of Detinova (Detinova, 1962), based on dichotomous categorisation of female mosquito specimens as ‘parous’ or ‘unparous’ relies on fewer assumptions, and is also widely used. Further work examining parity rates in field specimens may be fruitful although, in principle, it oﬀers less information on the age structure of a population than Polovodova’s.

It is widely believed mosquitoes live artificially long under the benign conditions of the laboratory and their lifespans likely constitute an upper bound on wild populations. A large cage experiment of *Ae. aegypti* determined that females lived on average for nearly 32 days days (Styer et al., 2007), and a similar study with female *A. stephensi* determined their median lifespan was between 31 and 42 days (Dawes et al., 2009). These estimates are multiples of the modal estimates from either of our analyses. Without a gold standard method to measure mosquito lifespan in the field, it is diﬃcult to quantify the gap that exists between field and laboratory populations. The development of additional methods to estimate mosquito age such as ‘Near-Infrared Spectroscopy’ (Mayagaya et al., 2009; Sikulu et al., 2011; Lambert et al., 2018) if they are proven to work in the field, may be of considerable worth here.

In our analysis of the MRR data, we considered the effect of air temperature, sex and pre-release feeding status on lifespan. There is evidence mainly from laboratory studies that temperature modulates mosquito ecology and behaviour (Yang et al., 2009; Brady et al., 2013; Murdock et al., 2012; Beck-Johnson et al., 2013). The locations and times of year when the MRR studies were conducted encompassed a large range of average air temperatures, from approximately 10 oC to 35 oC yet we found no relationship between lifespan and temperature when pooling all series (Fig. S5) or for those individual species with the most data (Fig. S6). It is possible that by considering a raw average of air temperature across the month and ignoring rainfall (the historical data on rainfall is likely less reliable for a given location), this masked more complex interactions between temperature and lifespan. The observed laboratory relationship between lifespan and temperature, however, may not be as robust in the field if mosquitoes adjust their behaviour (such as, by seeking shade) in reaction to changes in temperature. As in laboratory studies (Styer et al., 2007; Dawes et al., 2009) our analysis indicates that females outlive male mosquitoes, although the magnitude of this difference is not as large in absolute or percentage terms. As ethical concerns are more often considered, it is now more common for MRR experiments to release males than females compared to historically (Fig. SM2). Since differences in lifespan exist between the sexes, it is possible that differences exist in other ecological parameters that can be determined by MRRs, highlighting the need for field methods that directly measure these characteristics of wild females. Our estimates of LBL indicate that mosquitoes that were sugar-fed prior to release lived on average 0.7 days longer than unfed individuals (Fig. S4). This difference may explain the recent successes of vector control methods reducing access of the insects to sugar or using toxic sugar baits (Müller, Kravchenko, and Schlein, 2008; Müller, Junnila, and Schlein, 2010; Müller et al., 2010a; Müller et al., 2010b; Beier et al., 2012).

It is reassuring that we estimated comparable overall lifespans from both the MRR (6.0 days) and dissection (5.5 days) analyses and that there was a positive (although insignificant) correlation between species-level estimates of lifespan from analyses of both experimental approaches. Across genera, the greatest discrepancy in estimates was for *Aedes*, where we estimated 8.1 days from the MRR studies compared to 3.5 days from the dissection analysis. This was followed by *Culex* (2.9 days from the MRRs versus 4.9 days from thedissection analysis) with the smallest discrepancy for *Anopheles* (6.8 versus 6.4 days). Some of the diﬀerences in estimates between the two approaches may be due to environmental and genetic diﬀerences between mosquitoes included in this analysis. However, we believe that part of the discrepancy can be explained by the methodological diﬀerences in approaches. We speculate that diﬀerences in dispersal rate can explain some of the discrepancy. Both *Anopheles* and *Culex* mosquitoes are generally thought to fly farther during their lifetimes than *Aedes* [Charles, do you have a reference here?], meaning that the estimates from MRR-based approaches will be most downwardly-biased for these genera. This is supported by our results since the dissection-based estimates (themselves not reliant on assumptions about dispersal) are similar or exceed the MRR estimates for *Anopheles* and *Culex* mosquitoes, but not for *Aedes*.

In our study, we determined that mosquito senescence could better explain patterns of mortality only in a minority of cases across both experimental approaches, contrasting with evidence from laboratory studies (Styer et al., 2007; Dawes et al., 2009) and field experiments (Harrington et al., 2008). To determine if experimental characteristics were responsible for our inability to detect senescence, we conducted a power analysis of MRR experiments (see SOM). This work indicated that

We conducted a power analysis of MRR experiments to determine whether typical experimental characteristics could detect senescence. Here we calculated the power of a maximum likelihood estimator of the ‘senescence parameter’ *β* of the Gompertz survival function (see Table SM3) for case study populations with three diﬀerent levels of senescence (Fig. S11A). This analysis indicated that power to detect senescence strongly depends on study length (Fig. S11B) but is insensitive to release size (Fig. S11C). Clements and Patterson (1981) conducted a meta-analysis of MRR and dissection-based field experiments and found evidence of an increasing risk of mortality hazard with age that is similar in magnitude to that of the ‘mild’ case considered above. For this case, detecting senescence with a power of 80% requires a study length of at least 18 days. Since the median study duration for experiments included in our analysis was 10 days (Table SM2) this could partly explain our failure to detect senescence at the species level. A number of experiments have found evidence of age-dependence in laboratory populations However, the artificially benign environment of the laboratory means mosquitoes live considerably longer than in the wild, where they may die because of exogenous

factors, before the eﬀects of physiological decline have had time to manifest. Field experiments have also found evidence for age-dependent mortality. Harrington et al. (2008) conducted a field experiment where mosquitoes reared under laboratory conditions were marked and released at diﬀerent ages. Analysis of the resultant MRR time-series indicated that mosquito mortality increases with age at release. It is possible, however, that this field experiment suﬀers from the same biases as laboratory-based approaches, because the released mosquitoes were often of ages considerably higher (up to 20 days) than typical estimates of wild mosquito lifespan.

In this work, we have used modern statistical methods to synthesise precious field data conducted by entomologists past and present, to produce lower bound estimates of mosquito lifespan. The importance of vector mortality for disease transmission has long been recognised, however, since even before 1957, when George Macdonald formulated the now famous Ross-Macdonald equation of R0 for malaria. Indeed, the recent declines in malaria prevalence in Sub-Saharan Africa were likely due to upscaling of interventions (insecticide-treated bednets and indoor residual spraying) that aim to reduce mosquito lifespan (Bhatt et al., 2015). Worryingly, resistance to pyrethroids, the only class of insecticide used in current insecticide-treated bednets and likely the only product to come to market in the near future, has been determined to be widespread and increasing in intensity across Sub-Saharan Africa (World Health Organization, 2018). This alarming trend highlights the need for continued MRR and dissection-based studies to monitor the eﬀectiveness of bednets and determine whether more expensive alternatives, such as nets incorporating piperonyl butoxide be deployed. It also emphasises the need for investment in new tools for real time monitoring of mosquito populations. In recent years, considerable funding has been allocated to molecular and genomic research into mosquitoes that strengthens existing interventions and suggest novel control strategies. Without commensurate funding allocated to applied vector ecology, our lack of knowledge in this area threatens our opportunity to capitalise on molecular advances and potentially hinders our ability to control of mosquito-borne disease.

The diﬀerent nature of the assumptions of each of the two methods means they oﬀer complimentary information on mosquito survival. We also note that Polovodova’s dissection-based studies require specialised expertise which will often be unavailable, whereas MRR methods can more readily be used. Furthermore, most if not all dissection methods that have been used previously are only applicable to female mosquitoes, whereas MRR can be applied to either sex and can additionally be used to determine other ecological parameters (for example, population size and dispersal). Although dissection data gives detailed of age-structure, we thus foresee a continued reliance on MRR experiments in field entomological experiments. Eﬀorts to use both approaches concurrently will be particularly useful and will allow quantification of the biases induced by the assumptions of each. Similarly, MRR experiments releasing large numbers of marked mosquitoes and recording spatiotemporally-disaggregated captures of wild and re-caught marked mosquitoes will continue be useful in estimating lifespan and dispersal.

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