Reviewer #1: Evans et al present an interesting preliminary study on the carry-over effects of urban larval environment on the transmission potential of Aedes albopictus.
The study is quite novel and interesting. The the study design and the statistical analysis seem adequate to give reliable estimate of the subject. The study suffers of the typical difficulties of dealing with mosquito field collection, but authors seem to have adequately account for that. However, I suggest authors to further stress the limitation of the study in the discussion. In my opinion the number of field collections is a bit on the short-hand for the estimation of site-level parameter within the proposed statistical methods. Nevertheless, I find the study reasonably solid and interesting as a whole. However, there are some issues in the manuscript that should be addressed or modified before being accepted for publication.

First, some general comments.

The authors should clearly state the number of females tested. In the text, the initial number of larvae and the overall number of mosquito is reported. However, I completely missed (if present) the number of emerged females which is the relevant quantity for growth rate and should comply with the assumption of the N0 parameter of the model.

> *In our study, the number of emerged females is equivalent to the number that survive to emergence, as represented by the egg-to-adult survival rate. We initially reported this rate in line 319 - 320 and have clarified that this is rate is for female mosquitoes. We list the sample sizes of infection assays in Table 4, as they varied per site due to low emergence rates in the fall replicate.*
>
> *Following Livdahl and Sugihara 1984 (https://www.jstor.org/stable/4535), we assume N_0 to represent the initial number of females before accounting for mortality during the larval stage. This enables the mortality rate to be included via the summed A_x/N_0 parameter. Setting N_0 equal to the number of emerged mosquitoes would imply a 100% larval survival rate, which was not the case in our study. Unfortunately, we cannot identify the sex of first-instar larvae, and must assume a ratio within the initial cohort. Because males develop more quickly than female mosquitoes, using the ratio of emerged mosquitoes could introduce bias if, for example, days following male emergence but prior to female emergence were thermal extremes, causing higher mortality within the female population. Fortunately, eggs used in the experiment were drawn from a laboratory colony which is known to have approximately a 50:50 male:female sex ratio. Therefore, we used this value in our calculations.*

Moreover, as they honestly reported (e.g. line 293), for some analysis the sample size was low (also considering the number of parameter estimated in the models i.e. 2 qualitative covariates (2 and 3 levels) and their interaction. I suggest authors include in the discussion section a paragraph addressing the difficulties of obtaining appropriate sample size and that in the conclusion section, the results on the carry-over effect on vectorial capacity should be taken as

a first indication that it is needed to take in account site-specific characteristic that could impact survival, development and carry-over effect.

This is indeed a big limitation of our study. As suggested, we have added additional narratives regarding sample size to the discussion (lines 484 - 494) and conclusion (lines 507 - 508):

"Our study was further limited by the difficulties in obtaining appropriate sample sizes. While semi-field experiments incorporate more realistic variation in environmental temperature than laboratory experiments, they require additional space and travel time in order to distribute replicates in a manner that meets assumptions of independence across sites. Given the size of our study area, nine was the maximum number of sites that it was possible to visit daily. Unexpectedly low emergence rates of mosquitoes in the fall further reduced our sample size of mosquitoes that could be used in infection assays. Despite this limitation, we did find significant differences in mosquito demographic rates across season and in infection and dissemination rates across land class, suggesting that site-specific characteristics can directly and indirectly impact vector-borne disease dynamics. Yet, due to the low replication across sites, these results must be interpreted conservatively."

Moreover, only dengue virus-2 was tested and maybe it should be explicitly indicated in the title rather than the more general "a mosquito-borne pathogen". This should be highlighted also in the discussion and conclusion section

Thank you for this suggestion. We have adjusted the title and added additional clarification in the discussion and conclusion section (lines 459-461, lines 506).

Second, some specific comments
Line 94. In-text authors used "miles", in the cited figure "kilometres", please unify. Also throughout the text authors use unit (e.g.: "feet" line 104) that are not unit of the International System of Units (SI). I would suggest to use SI units or at least include the equivalent unit in brackets to help the reader (e.g. line 104 ..at approximately 3 feet (0.9 m) …)

Thank you for this comment. We have standardized all units to SI throughout the manuscript (lines 101, 113,137, etc.).

Line 157/163/172, I suggest adding "Equation 1/2/3" near the equation

Good idea. We have added labels for equations (lines 195, 200, 214).

Line 158 Why did you assume N0 = 50% and instead of using the observed proportion? Please explain

This is a very good point which deserves additional clarification. As mentioned above, we could not identify the sex of the first-instar larvae used in the experiment. The observed proportion of emerged mosquitoes represents those that survived the larval environment to emergence, and, given our findings regarding the effects of the larval microclimate on larval survival, may not be representative of the initial cohort. In our study, males emerged 1 - 3 days earlier than females in the summer, and up to a week earlier than females in the fall. This additional time in the larval environment could have exposed female mosquitoes to stressful temperatures and lower resource concentrations than the male mosquitoes that emerged earlier, resulting in lower emergence rates. Therefore, we assumed a 50:50 male:female ratio of the initial cohort of first-instar larvae, as determined by our own monitoring of the laboratory colony used in the experiment.

Table 1 and line 183. Please clearly state what b(T) and c(T) are and give a short explanation on why your measure (the proportion of infectious mosquitoes per site) should be considered an acceptable estimate.

*The requested information has been added to lines 227 - 244**:***

"Conventionally, vector competence is the product of the proportion of mosquitoes that become infected after biting an infected human and the proportion of bites by infectious mosquitoes that infect humans. Our estimate, the proportion of infectious mosquitoes as measured by CPE assays, is the same as the product of the proportion of mosquitoes that become infected following an infectious blood meal and the proportion of infected mosquitoes that have DENV-2 virus particles in their saliva. With this formulation we are assuming that all infectious bites result in human infection, as we are not directly measuring dengue infection outcomes in humans (i.e. effects of human immunity on DENV infection)."

Also, Table 1 should include some summary statistics for each parameter, (e.g. the mean and the range or the mean and the 95% interval) to help the reader understand the variation in each parameter and also help understand and compare some estimate (e.g. egg-to-adult survival) to other settings.

We agree that this will help the reader better understand the parameters used in our models, we have added the mean and range of each parameter to Table 1.

Line 195 Please specify the distribution used. I supposed that being a generalized linear mixed model that it is not the Normal distribution.

Yes, this was wrongfully omitted in the original submission. We have rewritten the statistical analysis portion of the Methods section to include the distributions and link

functions of all statistical tests used in our analysis (lines 256 -261, 264, 281-282). Additionally, all of our code used to produce this analysis is hosted in a public repository on figshare.

Line 202 Could you please further address the reason why you switched to two-way ANOVA rather than analysing vectorial capacity by regression modelling?

We admit this was a point of confusion. Because the independent variables in this model were both factors (land class and season), a two-way ANOVA is identical to a regression model using two factorial independent variables, particularly as this model was used for hypothesis testing and not to generate regression coefficients. We have adjusted our language to refer to all analyses as regression models to reduce confusion (lines 270 - 273).

"Vectorial capacity was calculated at the site-level, and so did not require site to be included as a random effect. We therefore used a regression model to estimate the effect of land class, season, and their interaction on site-level vectorial capacity."

Line 210. I failed to understand over which period the mean temperature was calculated. I think the reader would appreciate if the authors include the period over which the mean was calculated. In the following sentence they stated "over the season", but this refer to the sampling period or all the summer and all the fall?

Thank you for comment and recognizing this confusing language. We have clarified this sentence to refer to each individual season (lines 279-280, 282).

Line 221 you included in the methods section that model validation (checking residuals) was carried out but then you did not include a short statement indicating that there was not any violation of model statistical assumptions. I assumed everything was reasonably fine, can you confirm?

This was an oversight on our part. We have added a statement confirming the results of model validation had no evidence that data failed to meet assumptions of normality (line 340-341).

Line 222 include R version and cite both R and lme4 accordingly

Thank you for noticing this. We have added the proper citations (line 299).

I appreciated that in the result section authors reported the mean and the standard error for all effects. However, I will suggest some improvements. First, the notation SE is not used consistently in the text (e.g. line 232 used SE, line 248 used, line 290 SE at the end).

We have changed our use of SE so that it is used consistently in the text (line 322, 336).

Second, when presenting percentage (%) as in line 240-241 I suggest authors to show 95% confidence intervals rather than SE because the latter could be misleading. As an example, I will expect in line 241 that that the 95% confidence interval of the fall egg-survival is 0.297 +/- 1.96*0.160 which results in negative values. Please compute and report appropriate confidence interval for proportion (you will find extensively example using lme4)

We have adjusted our reporting of proportions to use 95 % confidence intervals, rather than the mean and SE (line 311 - 312, 319 - 320). While doing this, we noticed that the mean relative humidity values had been incorrectly reported and were simply a copy of temperature values. We have replaced this in the main text (line 311-312) and Table 2.

Figure 3 is not clear. The points representing site-level means and vertical bands are not visible. Moreover, it is not clear if the points would be the mean of the 4 trays per site or the mean of the 3 sites within the selected landscape (rural/suburban/urban)

Thank you for pointing this out. We have added clarification to the figure legend to further explain the symbology (lines 713 - 716):

"The effect of larval temperature on predicted vectorial capacity at the site and seasonal level. Points represent site-level VC calculations for field based (circle) and grand mean (diamond) calculations, with colors representing the sites' land class. Boxes represent mean ± s.d. per calculation type (field based: dotted vs. grand mean: solid) and season (summer vs. fall). "

Reviewer #2:

1. It is necessary to review the whole article is in accordance with the regulations of the International System of Units, since there are some errors in the article, including the tables, where they do not mention what type of system are using. It must be specified in tables that are °C.

Thank you for noticing this omission. We have specified the units in Table 2.

2. In line 104 it is mentioned as "feet" measurement unit, but it must be changed to meters.
3. On line 94, "miles" is mentioned, it must be changed to kilometers.
4. Sometimes it uses "minutes" and other "min". standardize Lines 105, 120, 143, 150

We have standardized our use of SI throughout the manuscript (lines 100, 112,136, etc.).

5. The value of a quantity is written as a number followed by a space. This rule explicitly includes the percent sign (%) and the symbol for degrees of temperature (°C). Exceptions are the symbols for plane angular degrees, minutes, and seconds (°, ′, and ″), which are placed immediately after the number with no intervening space.

We appreciate your close eye, and have edited to manuscript to follow this format (lines 89, 96, 147-149, 159 - 162, 219, 311-312, etc.).

6. The article does not mention the safety conditions in the insectary for the management of mosquitoes infected with DENV-2. Is there a security protocol?

We did not mention our security protocol in the initial manuscript, and have added it on lines 172-191:

"All infection work was conducted in an arthropod containment level 2 (ACL-2) facility at the University of Georgia in the College of Veterinary Medicine. The physical space as well as experimental protocols have been reviewed and approved by the University of Georgia Office of Biosafety (2015-0038). Briefly, all DENV-2 exposed mosquitoes were counted initially and throughout the experiment, housed in secondary containment cages, and handled in a glove box and on ice when they were removed from secondary containment for forced salivations. All virus assays were also conducted in a biosafety cabinet in our biosafety level II (BSL-2) facility. Finally, we used designated and approved secondary containment to transport virus or infected tissues between our ACL-2 and BSL-2."

7. It is not clear whether the strain of the mosquitoes used was from the field or came from a colony previously established in the laboratory. Which generation was used, geographical origin. If it is a strain established in the laboratory, are there standardized procedures for reproduction, mainly for the feeds of females?

Thank you for mentioning this. It is an important detail that was initially looked over. We have added the requested information on lines 103 - 106:

"Ae. albopictus were from a laboratory colony obtained from the Centers for Disease Control (Atlanta, GA, USA) originating from Keyport, NJ, USA in 1995 (strain ATM-NJ95) [20] and maintained following standardized protocols."