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PROCEEDINGS B

Thermal thresholds heighten sensitivity of West Nile virus transmission to changing temperatures in coastal California

Nicholas K. Skaff, Qu Cheng, Rachel E. S. Clemesha, Philip A. Collender, Alexander Gershunov, Jennifer R. Head, Christopher M. Hoover, Dennis P. Lettenmaier, Jason R. Rohr, Robert E. Snyder and Justin V. Remais

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Review timeline

Original submission:	19 March 2020
1st revised submission:	8 May 2020
2nd revised submission:	7 July 2020
Final acceptance:	13 July 2020

Note: Reports are unedited and appear as submitted by the referee. The review history appears in chronological order.

Review History

RSPB-2020-0455.R0 (Original submission)

Review form: Reviewer 1 (Samuel Atkinson)

Recommendation
Accept with minor revision (please list in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
Excellent

General interest: Is the paper of sufficient general interest?
Good

Quality of the paper: Is the overall quality of the paper suitable?
Excellent

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
Yes

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible?
Yes

Is it clear?
Yes

Is it adequate?
No

Do you have any ethical concerns with this paper?
No

Comments to the Author
Please see uploaded pdf file. (See Appendix A)

Review form: Reviewer 2

Recommendation
Major revision is needed (please make suggestions in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
Good

General interest: Is the paper of sufficient general interest?
Good

Quality of the paper: Is the overall quality of the paper suitable?
Good

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
Yes

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
No

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Is it accessible?
Yes

Is it clear?
Yes

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
This study uses a long term West Nile virus dataset in Los Angeles, California to evaluate how temperature and other factors influence the probability of Culex infection with WNV and human cases. They find an interesting pattern where temperature in a narrow range was most associated with increased probability of Culex infection with WNV. The manuscript is well-written and I have a few overall comments as well as some specific comments below.

While looking at Figure 2, what is surprising is that at the highest end of the temperature range for the study region, you are not seeing an 'inhibitory' response in terms of Cx. infection probability. Did you check to see how Culex abundance varies along this temperature range? Although you are suggesting that the coastal region of LA is buffered by the water, perhaps this entire region is buffered relative to a place much further inland such as Phoenix, AZ? I would assume that in some places with WNV transmission, you would see more of a thermal optimum above which WNV transmission efficiency would decay.

Based on the methods I am seeing lots of different lag times for temp, precip, and soil moisture. It would be nice to see which of these lags actually correspond with the prior winter period given that some studies have shown that a mild winter can facilitate increased WNV transmission the following season.

While your study is focusing on how 'transcritical variation' influences WNV infection in Culex vectors, note that a recent study in Texas evaluated how weather variability (not just means), influences WNV infection in Culex (Poh et al. Science of the Total Environment 2019). I would think that more variation in temperature would also allow for more opportunity to enter a favorable temperature range. But perhaps for less time. It would be helpful if the authors can discuss this concept of variation in temperature measured as kurtosis or standard deviation as it compares to transcritical temperature.

Specific comments:

Ln. 195: You appear to be including Cx. quinq. abundance in the models but your methods does not explain how you are using these mosquito trap data. This would generally be best to consider the number of females per trap per day (unit flexible) as a standard metric. But in your case you already explain that you used data from CO_2 (with light?) and gravid traps so how did you consider the abundance data from these two traps? Generally gravid traps produce more Culex than light traps so it would be bad to ignore trap type unless you already found no statistical difference. Although you cite the supplemental materials which has more details for the methods, they still lack some detail on how mosquitoes were captured, processed, and tested. Ideally you can cite prior studies that give more details for different dimensions of the methods.

Ln. 279-280: You use the term 'Cx. infection probability' in the methods section so you can define this term in the methods instead of the results.

Ln. 285 (and Fig S1): When you say '# mosquitoes captured', is this the number of female Cx. quinq. captured per trap per day? This is related to the above comment in terms of a lack of detail in the methods section.

Ln. 286 (and Fig S1): How is the number of Cx. quinq. in a pool a predictor for the Cx. infection probability? I thought you used the number of individuals in a pool as an offset which then shouldn't be considered as an independent or fixed factor, right? Obviously the larger the pool size, the more likely to be positive, which is not very interesting to include in your results.

Ln. 370-371: Given this observation, it would be nice to have a supplemental figure showing human case locations for August and September in one map and then July and October in another map.

Ln, 374: If inland zones were too warm in Aug and Sept, why do you not see a decline in Cx. infection probabilities at those higher temps in Figure 2 (see comment above)?

Decision letter (RSPB-2020-0455.R0)

16-Apr-2020

Dear Professor Remais:

I am writing to inform you that your manuscript RSPB-2020-0455 entitled "Thermal thresholds heighten sensitivity of West Nile virus transmission to changing temperatures in coastal California" has, in its current form, been rejected for publication in Proceedings B.

This action has been taken on the advice of referees, who have recommended that substantial revisions are necessary, although both are positive overall. With this in mind we would be happy to consider a resubmission, provided the comments of the referees are fully addressed. However please note that this is not a provisional acceptance.

The resubmission will be treated as a new manuscript. However, we will approach the same reviewers if they are available and it is deemed appropriate to do so by the Editor. Please note that resubmissions must be submitted within six months of the date of this email. In exceptional circumstances, extensions may be possible if agreed with the Editorial Office. Manuscripts submitted after this date will be automatically rejected.

Please find below the comments made by the referees, not including confidential reports to the Editor, which I hope you will find useful. If you do choose to resubmit your manuscript, please upload the following:

1) A 'response to referees' document including details of how you have responded to the comments, and the adjustments you have made.
2) A clean copy of the manuscript and one with 'tracked changes' indicating your 'response to referees' comments document.
3) Line numbers in your main document.

To upload a resubmitted manuscript, log into http://mc.manuscriptcentral.com/prsb and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with

Decisions." Under "Actions," click on "Create a Resubmission." Please be sure to indicate in your cover letter that it is a resubmission, and supply the previous reference number.

Sincerely,
Professor Hans Heesterbeek
mailto: proceedingsb@royalsociety.org

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)
Please see uploaded pdf file.

Referee: 2

Comments to the Author(s)
This study uses a long term West Nile virus dataset in Los Angeles, California to evaluate how temperature and other factors influence the probability of Culex infection with WNV and human cases. They find an interesting pattern where temperature in a narrow range was most associated with increased probability of Culex infection with WNV. The manuscript is well-written and I have a few overall comments as well as some specific comments below.

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Specific comments:

Ln. 195: You appear to be including Cx. quinq. abundance in the models but your methods does not explain how you are using these mosquito trap data. This would generally be best to consider the number of females per trap per day (unit flexible) as a standard metric. But in your case you already explain that you used data from CO_2 (with light?) and gravid traps so how did you consider the abundance data from these two traps? Generally gravid traps produce more Culex than light traps so it would be bad to ignore trap type unless you already found no statistical difference. Although you cite the supplemental materials which has more details for the methods, they still lack some detail on how mosquitoes were captured, processed, and tested. Ideally you can cite prior studies that give more details for different dimensions of the methods.

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Author's Response to Decision Letter for (RSPB-2020-0455.R0)

See Appendix B.

RSPB-2020-1065.R0

Review form: Reviewer 3

Recommendation
Major revision is needed (please make suggestions in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
Acceptable

General interest: Is the paper of sufficient general interest?
Good

Quality of the paper: Is the overall quality of the paper suitable?
Acceptable

Is the length of the paper justified?
No

Should the paper be seen by a specialist statistical reviewer?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
No

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible?
Yes

Is it clear?
Yes

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
Please refer to the track changes in the attached document. Overall, this paper is very interesting and of high scientific merit. However, because of the obvious ambition in this work, as a reader with a strong background in ecological modeling, I often had frequent questions seeking additional information throughout. When referred to the supplemental materials (which was very frequent), I often found myself with not enough detailed information to repeat or understand the details. Personally, I love what was presented; the focal message was insightful, but I believe the authors would do much more service for the intended audience if this was submitted to a journal that emphasizes the modeling of infectious diseases that allowed additional page length.

Decision letter (RSPB-2020-1065.R0)

22-Jun-2020

Dear Professor Remais:

Your manuscript has now been peer reviewed and the reviews have been assessed by an Associate Editor. The reviewer's comments (not including confidential comments to the Editor) and the comments from the Associate Editor are included at the end of this email for your reference. As you will see, the reviewer has raised some issues with your manuscript and we would like to invite you to revise your manuscript to address them.

We do not allow multiple rounds of revision so we urge you to make every effort to fully address all of the comments at this stage. If deemed necessary by the Associate Editor, your manuscript will be sent back to one or more of the original reviewers for assessment. If the original reviewers are not available we may invite new reviewers. Please note that we cannot guarantee eventual acceptance of your manuscript at this stage.

To submit your revision please log into http://mc.manuscriptcentral.com/prsb and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions", click on "Create a Revision". Your manuscript number has been appended to denote a revision.

When submitting your revision please upload a file under "Response to Referees" in the "File Upload" section. This should document, point by point, how you have responded to the reviewers' and Editors' comments, and the adjustments you have made to the manuscript. We require a copy of the manuscript with revisions made since the previous version marked as 'tracked changes' to be included in the 'response to referees' document.

Your main manuscript should be submitted as a text file (doc, txt, rtf or tex), not a PDF. Your figures should be submitted as separate files and not included within the main manuscript file.

When revising your manuscript you should also ensure that it adheres to our editorial policies (https://royalsociety.org/journals/ethics-policies/). You should pay particular attention to the following:

Research ethics:
If your study contains research on humans please ensure that you detail in the methods section whether you obtained ethical approval from your local research ethics committee and gained informed consent to participate from each of the participants.

Use of animals and field studies:
If your study uses animals please include details in the methods section of any approval and licences given to carry out the study and include full details of how animal welfare standards were ensured. Field studies should be conducted in accordance with local legislation; please include details of the appropriate permission and licences that you obtained to carry out the field work.

Data accessibility and data citation:
It is a condition of publication that you make available the data and research materials supporting the results in the article. Datasets should be deposited in an appropriate publicly available repository and details of the associated accession number, link or DOI to the datasets must be included in the Data Accessibility section of the article (https://royalsociety.org/journals/ethics-policies/data-sharing-mining/). Reference(s) to datasets should also be included in the reference list of the article with DOIs (where available).

In order to ensure effective and robust dissemination and appropriate credit to authors the dataset(s) used should also be fully cited and listed in the references.

If you wish to submit your data to Dryad (http://datadryad.org/) and have not already done so you can submit your data via this link http://datadryad.org/submit?journalID=RSPB&manu=(Document not available), which will take you to your unique entry in the Dryad repository.

If you have already submitted your data to dryad you can make any necessary revisions to your dataset by following the above link.

For more information please see our open data policy http://royalsocietypublishing.org/data-sharing.

Electronic supplementary material:
All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI. Please try to submit all supplementary material as a single file.

Online supplementary material will also carry the title and description provided during submission, so please ensure these are accurate and informative. Note that the Royal Society will not edit or typeset supplementary material and it will be hosted as provided. Please ensure that the supplementary material includes the paper details (authors, title, journal name, article DOI). Your article DOI will be 10.1098/rspb.[paper ID in form xxxx.xxxx e.g. 10.1098/rspb.2016.0049].

Please submit a copy of your revised paper within three weeks. If we do not hear from you within this time your manuscript will be rejected. If you are unable to meet this deadline please let us know as soon as possible, as we may be able to grant a short extension.

Thank you for submitting your manuscript to Proceedings B; we look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Best wishes,
Professor Hans Heesterbeek
mailto: proceedingsb@royalsociety.org

Associate Editor
Comments to Author:
Thank you for addressing the reviewer concerns. I appreciate the time and effort that has gone into this. The manuscript has now been evaluated by an additional reviewer who has also noted several points, many of these are to help clarify technical points and thus make the information more accessible to a non-specialist reader, as well as highlight key messages of the manuscript.

Reviewer(s)' Comments to Author:
Referee: 3
Comments to the Author(s).
Please refer to the track changes in the attached document. Overall, this paper is very interesting and of high scientific merit. However, because of the obvious ambition in this work, as a reader with a strong background in ecological modeling, I often had frequent questions seeking additional information throughout. When referred to the supplemental materials (which was very frequent), I often found myself with not enough detailed information to repeat or understand the details. Personally, I love what was presented; the focal message was insightful, but I believe the authors would do much more service for the intended audience if this was submitted to a journal that emphasizes the modeling of infectious diseases that allowed additional page length.

Author's Response to Decision Letter for (RSPB-2020-1065.R0)

See Appendix C.

Decision letter (RSPB-2020-1065.R1)

13-Jul-2020

Dear Professor Remais

I am pleased to inform you that your manuscript entitled "Thermal thresholds heighten sensitivity of West Nile virus transmission to changing temperatures in coastal California" has been accepted for publication in Proceedings B.

You can expect to receive a proof of your article from our Production office in due course, please check your spam filter if you do not receive it. PLEASE NOTE: you will be given the exact page length of your paper which may be different from the estimation from Editorial and you may be asked to reduce your paper if it goes over the 10 page limit.

If you are likely to be away from e-mail contact please let us know. Due to rapid publication and an extremely tight schedule, if comments are not received, we may publish the paper as it stands.

If you have any queries regarding the production of your final article or the publication date please contact procb_proofs@royalsociety.org

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Electronic supplementary material:
All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI.

Thank you for your fine contribution. On behalf of the Editors of the Proceedings B, we look forward to your continued contributions to the Journal.

Sincerely,
Professor Hans Heesterbeek
Editor, Proceedings B
mailto: proceedingsb@royalsociety.org

Associate Editor:
Board Member
Comments to Author:
(There are no comments.)

Appendix A

Review: Thermal thresholds heighten sensitivity of West Nile virus transmission to changing temperatures in coastal California

Proceedings of the Royal Society B – Ecological Sciences

This is an important paper that examines a relatively unique question in relation to zoonotic infectious disease – a critical temperature transition zone. It is a well-articulated and well-written manuscript, using solid modeling approaches. However, I would like to see the Data Analysis section within Methods re-worked a bit. The last 2 paragraphs (out of 3) were some of the most technically dense descriptions I have ever read. While I am well versed with many of the tools mentioned, I was not familiar with all of them, which I suspect is similar to most of the potential readers of this paper. By the time I did a little work to figure out those unfamiliar methods, I was somewhat exhausted. I would suggest that these two paragraphs be expanded slightly to give a quick explanatory sentence attached to each tool mentioned that provides the reader with an "oh, that is why they are doing that" relief valve, so they don't have to work so hard to fully appreciate the work.

Finally, in terms of an overall comment, the paper does an excellent job of showing the possibility of a temperature transition zone that is related to WNV incidence. However, it never provides any biological reason why such a transition zone might actually have a cause-effect. What is it about the mosquito, the virus, the birds or humans that would be affected by temperatures crossing this transition zone? Ample research is cited covering lower and upper thresholds and optimum temperature effects and WNV, but the discussion in this paper should include at least some literature-based speculation as to what physical/chemical/biological mechanism might be at play when temperatures cross the transition zone. The potential "why" is the bottom line that the reader is looking for.

Overall, I found this paper to be excellent, and with a few minor modifications, I am confident that the Proceedings of the Royal Society B will publish this work.

A few specific comments:

In the abstract, the statement "Analyzing a massive … dataset .." made me pause. "Massive" is probably the wrong term to use because it is probably relative depending on experience. It might be best to quantify the size somehow (number of observations, data file size, etc.), or simply describe the dataset as "large". (line 52)

In the abstract, the statement "… temperatures … were more strongly associated with infection probability … ***because*** temperature variation …" (emphasis added – line 58). This implies an absolute cause and effect, which I don't believe has been shown. To remove the appearance of stating an absolute cause-effect, the sentence should be modified to something on the order of:

> Temperature variation in cooler marine-influenced coastal settings frequently traversed the narrow transitional temperature range during the most intense months of WNV transmission (August-September), which was strongly associated with increased infection probability in *Cx. quinquefasciatus.*

The methods section also needs a little clarification in 2 ways:
(1) Datasets with spatial resolution ranging from 4km to 10m were combined in the modeling, but there is no statement as to what final grid cell size was used for analysis, and how the georectification to a common grid size was accomplished. It makes a substantial difference if cells in each dataset were aggregated to 4km, or if they were all subdivided to 10m, or if an intermediate 30m was the final size used. The how and why needs to be explicitly stated.
(2) There is no statement as to if/how the datasets were separated into model development and model verification subsets. It is implied in the results section that there was a subset of data withheld from the training step (line 272), but it is not mentioned in the methods section. Ideally, the validation subset was based on stratified random sampling, stratified by both climatic region and by year, and where approximately 2/3 of the data were used in model development and 1/3 of the data were withheld for validation purposes.

Line 248: The statement "We identified ***the*** direct effects of average monthly mean temperature on monthly human WNV cases …" is not substantiated. The statement should be something on the order of "We identified significant relationships between average monthly temperature and monthly human WNV cases …"

Line 251: The sentence "An offset was used to account for differences in the human population…" needs clarification. Should this be "human population size", or is it some other demographic descriptor.

Line 309: insert the word "in" between "temperatures" and "the favorable range"

Line 337: Damn that autocorrect! Please do a search on Cx. and determine if the following letter should be capitalized or lowercase. Here is should be lower case.

Line 379: replace the word "determine" with the words "coincide with".

Appendix B

Response to reviewers
Manuscript RSPB-2020-0455

Thank you to the editor and both reviewers for the encouraging and helpful comments. We address each comment below with a reply in bold.

The Associate Editor comments:

Thank you for submitting your manuscript "Thermal thresholds heighten sensitivity of West Nile virus transmission to changing temperatures in coastal California" to Proceedings B. I have now received two reviews and evaluated to manuscript myself. We all agree that your manuscript is well-written and addresses an interesting topic. However, several points have been raised by the reviewers, which should all be addressed.

For example, as highlighted by reviewer 2, it would be interesting to know if Culex mosquito abundance varies along temperature ranges and if weather variability, not just average, influences outcomes.

We agree, and have fully addressed this point. See details below.

Reviewer 1 points out that need to expand and clarify parts of the description of the modeling methods and the importance of biologically justifying the results.

Yes, agreed. In the revision, we now fully address both of these issues. See details below.

Lastly, please make the human infection data used here more readily accessible, or soundly justify why it isn't possible in your response.

We have clarified data accessibility in the revision. All mosquito surveillance data used in the analysis are publicly accessible, and we point to the data source in the data accessibility statement. We are prohibited from making the human infection data directly available because they are classified as protected health information (PHI) that are available only to authorized California Department of Public Health (CDPH) staff. However, these data can be obtained for certain approved purposes by submitting a formal request to CDPH. We clarified this in the Data Availability statement (ln. 463-473).

> "The mosquito surveillance data are publicly accessible by data request to CalSurv [58]. Human case data are protected health information (PHI) with access restricted to authorized California Department of Public Health (CDPH) staff. Limited, deidentified human case data are available via California's Open Data Portal (https://data.ca.gov/). More complete human disease data can be obtained for approved purposes by submitting a formal request to the California Department of Public Health, Infectious Diseases Branch, Surveillance and Statistics Section."

Referee: 1

Review: Thermal thresholds heighten sensitivity of West Nile virus transmission to changing temperatures in coastal California -- Proceedings of the Royal Society B – Ecological Sciences

This is an important paper that examines a relatively unique question in relation to zoonotic infectious disease – a critical temperature transition zone. It is a well-articulated and well- written manuscript, using solid modeling approaches. However, I would like to see the Data Analysis section within Methods re-worked a bit. The last 2 paragraphs (out of 3) were some of the most technically dense descriptions I have ever read. While I am well versed with many of the tools mentioned, I was not familiar with all of them, which I suspect is similar to most of the potential readers of this paper. By the time I did a little work to figure out those unfamiliar methods, I was somewhat exhausted. I would suggest that these two *paragraphs be expanded slightly to give a quick explanatory sentence attached to each tool mentioned that provides the reader with an "oh, that is why they are doing that" relief valve, so they don't have to work so hard to fully appreciate the work.*

Thank you for letting us know that this section was a challenge! We've added three sentences describing the specific purpose of each method, one each accompanying the random forest (Ln 220-223,225-226), linear regression (Ln. 231-233), and mediation analysis details (Ln. 247-251). Note that we've now split the last two paragraphs you mentioned into 3 paragraphs (Ln. 220-265).

Finally, in terms of an overall comment, the paper does an excellent job of showing the possibility of a temperature transition zone that is related to WNV incidence. However, it never provides any biological reason why such a transition zone might actually have a cause-effect. What is it about the mosquito, the virus, the birds or humans that would be affected by temperatures crossing this transition zone? Ample research is cited covering lower and upper thresholds and optimum temperature effects and WNV, but the discussion in this paper should include at least some literature-based speculation as to what physical/chemical/biological mechanism might be at play when temperatures cross the transition zone. The potential "why" is the bottom line that the reader is looking for.

We agree, thanks for pointing this out. We were trying to avoid too much speculation, but we agree that we left the reader hanging a bit. We've added a paragraph to the discussion discussing potential mechanisms (Ln. 374-390).

> "The sigmoidal relationship between temperature and *Cx.* infection probability is likely the result of temperature sensitivity in several components of the WNV transmission cycle. In particular, the extrinsic incubation period (EIP), or the time between a mosquito's ingestion of an infectious blood meal and its ability to transmit the virus, may drive the upward phase of the curve[17,18,45]. EIP typically decreases non-linearly with increasing temperature[17,18,45] and has been shown to be longer in coastal Los Angeles compared to inland areas[45]. Additionally, high temperatures can constrain mosquito abundance by imposing limitations on multiple aspects of the mosquito life cycle, including reproduction, development time, longevity, fecundity and biting rate[20,46]. For example, increasing temperatures are linearly associated with reduced longevity and have threshold or parabolic relationships with egg production, blood feeding, and emergence rates of immature Culex[20]. Finally, the extreme diurnal temperature variability that was sometimes observed when monthly mean temperatures were very high may have disrupted transmission as temperatures oscillated into an extremely unfavorable range for a short period of time[30]. These phenomena probably contributed to the plateau in *Cx.* infection we observed at high temperatures[22,25]. Additional studies that disentangle the interactions between temperature's effects on different vector life history characteristics could more conclusively identify the mechanistic underpinning of the observed sigmoidal relationship."

Overall, I found this paper to be excellent, and with a few minor modifications, I am confident that the Proceedings of the Royal Society B will publish this work.

A few specific comments:
In the abstract, the statement "Analyzing a massive ... dataset .." made me pause. "Massive" is probably the wrong term to use because it is probably relative depending on experience. It might be best to quantify the size somehow (number of observations, data file size, etc.), or simply describe the dataset as "large". (line 52)

Our thanks – we changed this to 'large'.

In the abstract, the statement "... temperatures ... were more strongly associated with infection probability ... because temperature variation ..." (emphasis added – line 58). This implies an absolute *cause and effect, which I don't believe has been shown. To remove the appearance of stating an absolute cause-effect, the sentence should be modified to something on the order of:*

Temperature variation in cooler marine-influenced coastal settings frequently traversed the narrow transitional temperature range during the most intense months of WNV transmission (August-September), which was strongly associated with increased infection probability in Cx. quinquefasciatus.

We agree, and in the revision we removed the cause-effect language (Ln. 56-59):

> "Temperatures during the most intense months of WNV transmission (August-September) were more strongly associated with infection probability in *Cx.* quinquefasciatus pools in coastal LA where temperature variation more frequently traversed the narrow transitional temperature range compared to warmer inland locations."

The methods section also needs a little clarification in 2 ways:
(1) Datasets with spatial resolution ranging from 4km to 10m were combined in the modeling, but there is no statement as to what final grid cell size was used for analysis, and how the georectification to a common grid size was accomplished. It makes a substantial difference if cells in each dataset were aggregated to 4km, or if they were all subdivided to 10m, or if an intermediate 30m was the final size used. The how and why needs to be explicitly stated.

We agree. We did not need to downscale or upscale any of the gridded datasets, because the raster values were extracted from the coordinates of the mosquito surveillance site or in circular buffers surrounding each mosquito surveillance site. In other words, we weren't making any direct comparisons between the different gridded datasets that would've required resampling as you might need to do in a point process model or something similar. Unnecessary resampling would have sacrificed information in the higher resolution datasets (if they were upscaled), and introduced some amount of error in the lower resolution datasets (if they were downscaled). We clarified this in the supplemental methods.

> "We did not resample the gridded datasets because they were never directly compared in the analysis and any adjustments to the resolution would have introduced error or sacrificed relevant information."

(2) There is no statement as to if/how the datasets were separated into model development and model verification subsets. It is implied in the results section that there was a subset of data withheld from the training step (line 272), but it is not mentioned in the methods section. Ideally, the validation subset was based on stratified random sampling, stratified by both climatic region and by year, and where approximately 2/3 of the data were used in model development and 1/3 of the data were withheld for validation purposes.

We understand the request. We are at the very upper limit of the max page length for Proc B, so this information didn't make it into the main text, but we included these details in the supplemental materials (section 6). In short, out-of-bag performance estimates were generated on a random sample of 80% of the dataset, while model hyperparameters were selected with a sample of 20% of the dataset to avoid information leakage. Out-of-bag validation is the most common way to assess performance in a random forest, and is thought to be more robust than creating a discrete training and testing dataset. We also conducted a geographical and temporal validation by stratifying across months and years (temporal) and across surveillance sites (geographical). In the revision, we have clarified that further details are available in the SI-6: "see SI Appendix, Section SI-6 for detailed

information on random forest model development" (Ln. 218-219), and added some further information to the main text (Ln. 214-219).

> "Hyperparameter tuning was conducted on a random sample of 20% of the dataset, while out-of-bag validation and blocked cross validation (spatial and temporal) were conducted on the remaining 80%. These methods were used estimate overall model error (out-of-bag validation) and error on novel predictions in spatial (mosquito surveillance site) and temporal (yearly and monthly) domains respectively (see SI Appendix, Section SI-6 for detailed information on random forest model development)."

Line 248: The statement "We identified the direct effects of average monthly mean temperature on monthly human WNV cases ..." is not substantiated. The statement should be something on the order of "We identified significant relationships between average monthly temperature and monthly human WNV cases ..."

We apologize for the confusion on this. We needed some way to distinguish between the direct relationship between temperature and human WNV cases vs. indirect relationship operating through changes in *Cx.* infection. We used the typical language of mediation analysis/natural effect models, which classifies these as direct and indirect effects. In order to clarify that these relationships are not causal, we now refer to them as "direct relationship" and "indirect relationship". In the revision, we now state: "We identified the direct relationship between $\boldsymbol{T}$ and monthly human WNV cases, $\boldsymbol{H_i}$, and the indirect relationship between $\boldsymbol{T}$ and $\boldsymbol{H_i}$ operating through *Cx.* infection probability separately for each metropolitan LA climate zone" (Ln. 254-257).

We also put "total causal effects" in quotes to highlight that it is a specific term derived from the natural effect modeling literature and not our own language (Ln. 245).

Line 251: The sentence "An offset was used to account for differences in the human population..." needs clarification. Should this be "human population size", or is it some other demographic descriptor.

Yes, it should have been human population size. We have fixed this.

Line 309: insert the word "in" between "temperatures" and "the favorable range"

Thanks for catching that! Done.

Line 337: Damn that autocorrect! Please do a search on Cx. and determine if the following letter should be capitalized or lowercase. Here is should be lower case.

Fixed that instance and didn't find any others.

Line 379: replace the word "determine" with the words "coincide with".

Done

Response to reviewers
Manuscript RSPB-2020-0455

<u>Referee: 2</u>

Comments to the Author(s)
This study uses a long term West Nile virus dataset in Los Angeles, California to evaluate how temperature and other factors influence the probability of Culex infection with WNV and human cases. They find an interesting pattern where temperature in a narrow range was most associated with increased probability of Culex infection with WNV. The manuscript is well-written and I have a few overall comments as well as some specific comments below.

While looking at Figure 2, what is surprising is that at the highest end of the temperature range for the study region, you are not seeing an 'inhibitory' response in terms of Cx. infection probability. Did you check to see how Culex abundance varies along this temperature range? Although you are suggesting that the coastal region of LA is buffered by the water, perhaps this entire region is buffered relative to a place much further inland such as Phoenix, AZ? I would assume that in some places with WNV transmission, you would see more of a thermal optimum above which WNV transmission efficiency would decay.

We believe your point about the comparison between LA and AZ is spot on. Temperatures in LA are certainly more mild than many places just a few hundred miles to the east (Coachella Valley, Los Vegas etc), and we think this is an important reason we didn't see a strong inhibitory/downward response at high temps. When looking at Fig.2 panel b you can see that the smoothed line starts to show subtle hint of a downturn at the highest temperatures right before the data becomes scarce. Perhaps if temperatures in inland LA were a few degrees warmer we would see a more pronounced downturn. In the revision, we included some thoughts on this in the discussion, including why this makes it very hard to extrapolate this model to future warming scenarios (Ln. 420-424).

> "Mean temperatures in this study may not have sufficiently exceeded the optimal range for transmission in order for declines to be detected. This suggests that our findings, like the findings from many correlative empirical models, are limited in their applicability to "novel" future climates, especially in hot inland areas where warming is likely to raise temperatures beyond the observed range[49,50]."

Regarding the point about variation in abundance, what we saw across all the climate zones is that female *Cx. quinq.* abundance peaks in the spring, and declines though the summer. However, this is primarily a consequence of the availability of water for larval development, since precipitation is very rare during summers in LA and water sources like wetlands slowly dry throughout the summer. We controlled for variability in *Cx.* abundance in the RF model and added some information on the potential effects of temperature on abundance in the discussion (Ln. 379-390).

> "Additionally, high temperatures can constrain mosquito abundance by imposing limitations on multiple aspects of the mosquito life cycle, including reproduction, development time, longevity, fecundity and biting rate[20,46]. For example, increasing temperatures are linearly associated with reduced longevity and have threshold or parabolic relationships with egg production, blood feeding, and emergence rates of immature Culex[20]. Finally, the extreme diurnal temperature variability that was sometimes observed when monthly mean temperatures were very high may have disrupted transmission as temperatures oscillated into an extremely unfavorable range for a short period of time[30]. These phenomena probably contributed to the plateau in *Cx.* infection we observed at high temperatures[22,25]. Additional studies that

disentangle the interactions between temperature's effects on different vector life history characteristics could more conclusively identify the mechanistic underpinning of the observed sigmoidal relationship."

Based on the methods I am seeing lots of different lag times for temp, precip, and soil moisture. It would be nice to see which of these lags actually correspond with the prior winter period given that some studies have shown that a mild winter can facilitate increased WNV transmission the following season.

This is a little tricky because data were collected over the course of the spring, summer, and fall, so depending on when sampling took place a particular lag could include temperatures from the winter or spring, etc. The best way to look at this is through the larger seasonal/quarterly lags (these are lagging 3 month temp/precip averages). Seasonal lags of 2 or 3 would most often correspond with winter. However, temperature variables with these lags were not among the 10 most important predictors in the random forest model (see Fig. S1). We're not quite sure why that's the case, but it's potentially because LA winters are always pretty mild. We do suspect that WNV transmission in LA is sensitive to winter precipitation and we did find that precipitation with a 2 quarter lag (see Fig. S1) was the 8th most important predictor variable. We controlled for this by including precipitation at quite a few lags, but we have limited capacity to address precipitation in the main text given that we are at the upper edge of the page limit and we are really trying to focus on temperature. However, in the revision we have added text to the supplemental methods indicating that we are accounting for the possibility that winter climate conditions could influence the relationships we're examining.

"Large quarterly lags of temperature, precipitation and drought were included in order to account for the effects of climate conditions in the months prior to the WNV transmission season, including the preceding winter and fall."

While your study is focusing on how 'transcritical variation' influences WNV infection in Culex vectors, note that a recent study in Texas evaluated how weather variability (not just means), influences WNV infection in Culex (Poh et al. Science of the Total Environment 2019). I would think that more variation in temperature would also allow for more opportunity to enter a favorable temperature range. But perhaps for less time. It would be helpful if the authors can discuss this concept of variation in temperature measured as kurtosis or standard deviation as it compares to transcritical temperature.

We agree that temperature variation, including the diurnal range and sub-weekly heterogeneity, likely has important effects on WNV transmission patterns. We included several predictor variables in the random forest model that capture short-term temperature variability and found that diurnal variation (tmax-tmin) was most important. Figure S2 in supplemental section SI-2 illustrates how very high diurnal variability proceeding the mosquito trapping period was associated with decreases in the probability of detecting WNV in *Cx. quinq.* pools. Overall, our results indicate that while daily variability can be important, the effects are modest relative to broader monthly temperature patterns. At most we observed that diurnal temperature variation contributed to ~5% change in the probability of detecting WNV in collected pools, while average temperatures over a broader time scale were associated with a 40% change in this probability. That's ultimately why we focused on average temperatures, but we agree that further discussion of short-term variability could add some important context. In response to your suggestion, we clarified some existing sections (Ln. 116-122, 417-420) and added text to highlight the importance of weather variability (Ln. 384-390).

"Expanding on laboratory-derived vector-trait thermal response functions, machine learning approaches can accommodate vector and pathogen responses to weather variability at short time scales, such as diurnal temperature cycles and inter-daily temperature fluctuations. Such variation may regulate important physiological processes relevant to WNV transmission [28,29], in part because vector trait thermal responses are nonlinear and subject to Jensen's inequality — variation can lead to higher or lower transmission than would otherwise be expected under constant temperatures [25,30,31]."

"Though laboratory-derived estimates of WNV transmission depict a unimodal thermal response curve with declines in transmission at very high temperatures [22,24], our findings generally show a monotonic relationship with only the slight appearance of diminishing mosquito infection probabilities at the highest diurnal extremes."

"Finally, the extreme diurnal temperature variability that was sometimes observed when monthly mean temperatures were very high may have disrupted transmission as temperatures oscillated into an extremely unfavorable range for a short period of time[30]. These phenomena probably contributed to the plateau in *Cx.* infection we observed at high temperatures[22,25]."

Specific comments:

Ln. 195: You appear to be including Cx. quinq. abundance in the models but your methods does not explain how you are using these mosquito trap data. This would generally be best to consider the number of females per trap per day (unit flexible) as a standard metric. But in your case you already explain that you used data from CO2 (with light?) and gravid traps so how did you consider the abundance data from these two traps? Generally gravid traps produce more Culex than light traps so it would be bad to ignore trap type unless you already found no statistical difference. Although you cite the supplemental materials which has more details for the methods, they still lack some detail on how mosquitoes were captured, processed, and tested. Ideally you can cite prior studies that give more details for different dimensions of the methods.

You're correct, we did find that the CO2 (no light) and gravid traps captured different abundances of female *Cx. quinq.* (depending on location) and that pools from gravid traps had a higher probability of having detectable WNV. We controlled for these differences by including dummy variables in the random forest analysis for 'trap type' and 'vector control agency', which allowed for different probabilities of WNV infection in pools depending on which jurisdiction and trap type the pool came from. We also accounted for the differences in abundance among trap types by including predictor variables for both the number of female *Cx. quinq.* captured per trap night in addition to the number included in the tested pool (sometimes these numbers were different because not all captured females were tested). Based on the inclusion of these predictor variables, any interactions between trap type and abundance were accounted for in the model. Also, we did focus on the number of female *Cx. quinq.* captured per day per trap at each surveillance site (Ln. 157-162). We added some further clarification in the supplemental methods.

"To account for potential differences in trapping and processing methodology, we included dummy variables for each agency and for observations with an unreported agency (N=17,577 trap nights). A dummy predictor variable was also included for trap type to account for differences in the abundance of female *Cx. quinquefasciatus* captured and differences in the probability detecting WNV in pools among the two trap types (1, 2)."

"These data were subsetted to include only adult female *Cx. quinquefasciatus* surveillance records within metropolitan LA from 2006-2016 that were collected using a single CO2 (N=7,162 trap nights) or gravid (N= 29,308 trap nights) trap that was operated for one night without malfunctioning (totaling N=36,470 trap nights; see additional details in SI Appendix, Section SI-4). From this, we calculated the number of female *Cx. quinquefasciatus* captured per trap night at each surveillance location."

Ln. 279-280: You use the term 'Cx. infection probability' in the methods section so you can define this term in the methods instead of the results.

Thanks for pointing this out, we switched the order of the methods and results at some point and didn't adjust this. We have corrected this in the revision.

Ln. 285 (and Fig S1): When you say '# mosquitoes captured', is this the number of female Cx. quinq. captured per trap per day? This is related to the above comment in terms of a lack of detail in the methods section.

Good point, we clarified that it is female *Cx. quinq.* in several places and clarified that it is a per trap per day measure (Ln. 195-200).

"We developed a random forest model predicting the probability of WNV infection in adult female *Cx. quinquefasciatus* pools (hereafter '*Cx.* infection probability') based on the lagged climate and buffered land cover variables detailed above, as well as the total number of female *Cx. quinquefasciatus* captured per trap on the collection day, the number of female *Cx. quinquefasciatus* pooled and tested for WNV (which was occasionally less than the total number of *Cx. quinquefasciatus* captured)."

Ln. 286 (and Fig S1): How is the number of Cx. quinq. in a pool a predictor for the Cx. infection probability? I thought you used the number of individuals in a pool as an offset which then shouldn't be considered as an independent or fixed factor, right? Obviously the larger the pool size, the more likely to be positive, which is not very interesting to include in your results.

The random forest model we used doesn't allow for the specification of an offset or fixed vs. random effects as you might see in linear mixed models. We accounted for what you mention in your last sentence, that larger pool size is associated with greater likelihood of testing positive, by including pool size as a predictor in the model and by maximizing the mtry hyperparameter so that pool size is included as a predictor in each bootstrap iteration of the model. This controls for pool size, so when we look at the marginal effects for other predictors, the model is holding pool size constant (at the mean).

Ln. 370-371: Given this observation, it would be nice to have a supplemental figure showing human case locations for August and September in one map and then July and October in another map.

Human case locations during these two periods are not very insightful, because there are very few cases in the coastal zone during the July/October period, and then many more cases in across all zones in August/September. You really have to look over time to identify differences in the effects of temperature and the best support for this statement cited above comes from the mediation model coefficient estimates (Figure 4). However, we agree that it could be helpful to have a supplementary visual representation of these estimates. We added Fig. S5, which highlights the relationship between temperature changes and the probability of WNV infection separately during the July/October period

and the August-September period. Here, you can see that the effects of temperature are very similar during July and October, but the relationship is weaker in inland areas during August/September.

Ln, 374: If inland zones were too warm in Aug and Sept, why do you not see a decline in Cx. infection probabilities at those higher temps in Figure 2 (see comment above)?

In that section, what we meant to say is that temperatures in inland locations were above the inflection point in the curve in Fig 2a, not necessarily that there were so warm that *Cx.* lifespan or reproduction would be greatly affected. It's likely that temperatures were not hot enough to really start to see a decline in infection (though it did flatten out). In the revision, we include text in the discussion to clarify this (Ln. 417-424, 379-390):

> "Though laboratory-derived estimates of WNV transmission depict a unimodal thermal response curve with declines in transmission at very high temperatures [22,24], our findings generally show a monotonic relationship with only the slight appearance of diminishing mosquito infection probabilities at the highest diurnal extremes. Mean temperatures in this study may not have sufficiently exceeded the optimal range for transmission in order for declines to be detected. This suggests that our findings, like the findings from many correlative empirical models, are limited in their applicability to "novel" future climates, especially in hot inland areas where warming is likely to raise temperatures beyond the observed range[49,50]."

> "Additionally, high temperatures can constrain mosquito abundance by imposing limitations on multiple aspects of the mosquito life cycle, including reproduction, development time, longevity, fecundity and biting rate[20,46]. For example, increasing temperatures are linearly associated with reduced longevity and have threshold or parabolic relationships with egg production, blood feeding, and emergence rates of immature Culex[20]. Finally, the extreme diurnal temperature variability that was sometimes observed when monthly mean temperatures were very high may have disrupted transmission as temperatures oscillated into an extremely unfavorable range for a short period of time[30]. These phenomena probably contributed to the plateau in *Cx.* infection we observed at high temperatures[22,25]."

Appendix C

Response to reviewer #3
Manuscript RSPB-2020-1065

Thank you to the editor and reviewer #3 for the helpful comments. We have extracted major and minor suggestions from the tracked pdf submitted by reviewer #3 and reply to each comment in bold.

Associate Editor
Comments to Author:
Thank you for addressing the reviewer concerns. I appreciate the time and effort that has gone into this. The manuscript has now been evaluated by an additional reviewer who has also noted several points, many of these are to help clarify technical points and thus make the information more accessible to a non-specialist reader, as well as highlight key messages of the manuscript.
Our thanks to the additional reviewer for raising some helpful opportunities for clarification. We were able to address each comment and have improved the clarity and accessibility of the manuscript.

Referee: 3
Comments to the Author(s).
Please refer to the track changes in the attached document. Overall, this paper is very interesting and of high scientific merit. However, because of the obvious ambition in this work, as a reader with a strong background in ecological modeling, I often had frequent questions seeking additional information throughout. When referred to the supplemental materials (which was very frequent), I often found myself with not enough detailed information to repeat or understand the details. Personally, I love what was presented; the focal message was insightful, but I believe the authors would do much more service for the intended audience if this was submitted to a journal that emphasizes the modeling of infectious diseases that allowed additional page length.
We really appreciate your interest in this work. We have added additional details throughout (while bumping right up against the page limit). Along with our release of publicly available R-code and our detailed supplement document, we hope this revision fully clarifies our methods.

Major comments:

Ln. 163-164:
I have a number of questions here.
Most all of this information was available in the supplemental materials, though we appreciate that the reviewer's curiosity may mirror that of many other Proc B readers. We added information selectively as follows:

> *1.how many surveillence locations were there in the metro L.A. area? And were they consistently used each year or did they change locations or in operation.*
>
> **We added the total number of trapping sites to the main text (Ln. 165, "(N=928 sites)"). We also added supplemental text to clarify that sites were not operational every year and that locations of surveillance sites often differed between years (Supplementary info, section SI-4, page 9):**
>
> "Trap data were collected from January-December, although the trapping frequency differed between surveillance sites (N = 928 sites) and ranged from 1-441 observations

(mean=39.2) over the 11 year study period. The location and operational period of surveillance sites were determined based on the specific priorities of the vector control agencies and differed between years."

2. You mention Cx. tarsalis was rarely captured, but what about Cx. pipiens/restuans complex? Cx. tarsalis may not have been captured much in the highly urbanized areas, but they may have played larger roles in the enzootic cycle, possibly influencing or maintaining transmission of WNV during non-peak months, particularly in areas that are slightly more rural just outside L.A.?

Cx. quinquefasciatus is generally considered to be part of the _Cx. pipiens_ complex, and is typically found at lower latitudes in lieu of _Cx. pipiens_ (https://science.sciencemag.org/content/303/5663/1535). Though we only focus on Los Angeles in this manuscript, our larger CA-wide dataset reveals that the geographical transition between _Cx. pipiens_ and _Cx. quinquefasciatus_ occurs in the south-central Central Valley of California. Thus, Cx. pipiens are not typically found in the Los Angeles area. _Cx. restuans_ is most common east of the Continental Divide, particularly in the midwest and eastern US (http://vectorbio.rutgers.edu/outreach/species/rest.htm). None were collected in the LA surveillance records we have available. We added some text in the supplement (Section SI-4, page 9) to clarify that these vectors do not contribute to WNV transmission in LA.

"… several other nationally/globally important WNV vectors, *Cx. pipiens* and *Cx. restuans*, were not detected in our study area."

Your thoughts on potential enzootic transmission outside the study area are very interesting! However, our study area is generally bounded by either coastline or foothills/mountains. These dispersal barriers make it unlikely that _Cx. tarsalis_ outside the study area were seeding WNV transmission within the LA metro. It is possible that birds infected outside the study area dispersed into the study area, but ultimately this question is beyond the scope of what were able to assess based on the available data.

3. Not a criticism but more of a question out of curiousity: How were these data incorporated into the machine learning algorithm?

We incorporated the presence/absence of WNV in individual pools of captured _Cx. quinquefasciatus_ as the response variable in the random forest algorithm (Ln 198-209).

Since you have infection and abundance data, have the authors considered using vector index in areas of high transmission and MIR in areas of lower transmission?

We considered using MIR as the response variable, but not vector index because we wanted to focus exclusively on infection (while controlling for abundance). We used presence/absence instead of MIR because MIR estimates were unreliable when calculated based on data from a single trap night. A paragraph outlining this information is available in the supplemental methods (Section SI-4, page 9).

"WNV presence/absence in female Cx. quinquefasciatus pools was the outcome variable in the random forest model…Likewise, estimates of minimum infection rate (MIR), in which only one mosquito from each infected pool is assumed to be infected, contained significant outliers in cases where pools size was very small and offered no substantive advantage over presence/absence estimates since pool size was included as a predictor in the model."

Ln. 328: As a general comment, why is precipitation (and humidity) largely not discussed or addressed? Temperature may be the "more" important environmental predictor, but precipitation is consistently included as a top environmental parameter. If this is strucutred to be inferencing climatic changes over time, precipitation should certainly be aligned with temperature, as both have predicted changes with anticipated climate change.

You raise an important point, thank you. While precipitation is fairly important for WNV transmission in other parts of the county, rainfall during the summer months in Los Angeles is very rare. Precipitation during fall, winter or spring could influence transmission, but we investigated this and our results in Figure S1 suggest that the *most* important precipitation predictor variable is less than 5% as important as mean temperature. Thus, the influence of future changes in precipitation may be dwarfed by changes in temperature. What is more, mean precipitation in the region is generally not expected to change much (though rainfall intensity may increase) (https://www.energy.ca.gov/sites/default/files/2019-11/CCCA4-CEC-2018-005_ADA.pdf). We do agree that it would be valuable to briefly discuss why precipitation isn't a larger focus of the manuscript, and thus we added text to the limitations section of the discussion (Ln. 428-431):

"We also do not address future shifts in precipitation regimes, though changes in the region are expected to be small relative to current variability[48] and precipitation had relatively weak effects in our random forest model compared to temperature."

Ln. 386-390: This statement, and the title at large, seem to be based on statistically non-significant results. It is correct to claim they are associations, but even among the greatest sensitivity to tempreature, at defined by the coastal zone, the difference to central, and inland were not that different, and the 95 C.I. captured the range of all groups. This is how I have intrepreted these results and your discussion, thus far. The title and main message feel like they highlight a strong, distinctive link between climate and human cases across zones. However, the findings do not necessarily convey that.

We did in fact find statistically significant differences. Our mediation analysis found statistically significant differences (at the .05 level; represented by letters in Figure 4) between the mediation effects in the coastal and inland climate zones. As in many other circumstances (e.g., https://www.cscu.cornell.edu/news/statnews/stnews73.pdf), overlaps in our 95% confidence intervals should not be interpreted as precluding statistically significant differences between groups. We clarified in the main text that the coefficient estimates were significantly different (Ln. 347-350):

"Temperature increases mediated through Cx. infection probability contributed to significantly higher 1.66 (95% CI 1.33-2.07) and 1.58 (95% CI 1.22-2.04) fold increases in WNV incidence / 100,000 persons in coastal and central zones, respectively, than in the inland zone where no statistically significant effects were detected (1.16 [95% CI 0.89 - 1.51]; Fig. 4a)."

Minor comments:

Ln. 47: redundant – delete second use of temperature
We feel that the second usage of temperature is needed in the second clause for maximum clarity. We'd be concerned that readers might be confused about whether we're referring to "dynamics", "transmission", or "temperature".

Ln. 58-60: stay consistent with abbreviations; is it L.A. or LA? Make changes throughout
We now consistently use L.A. to refer to the Los Angeles metropolitan area throughout.

Ln. 91-92: please provide citation
We have combined the first and second sentences to emphasize that the four citations apply to both statements.

Ln. 105: I suggest that the authors describe WNV enzootic cycle a bit more – including dead-end hosts (not only humans, but equines, are also highly susceptible; insert 'human' between all and WNV
We clarified that humans are dead-end hosts and inserted 'human' at the requested location.

Ln. 110: Stay consistent – Culex or Cx.?
We now use Cx. after the first instance.

Ln. 122-123: Why not just state the relationship is nonlinear, and describe the nonlinear relationship? In mosquitoes and temperature, it's sigmoidal with respect to time and temperature; the way it's written seems like a convoluted way to state something simpler
The details on Jensen's inequality were added in response to a previous reviewer comment that asked us to clarify why nonlinearity could undermine the results of laboratory studies conducted at constant temperature. We hope the reviewer might agree that some readers may be interested in this technical point, and that it is thus worthwhile to include a few words here.

Ln. 128: Either insert 'the' or 'an' before
Yes, we added 'the'.

Ln. 131: Just write "We developed a spatiotemporal machine...for the L.A. metropolitan area."
We think it's important to emphasize that we have incorporated the improvements outlined in the previous paragraph.

Ln. 137, 152-154, 177: Are there really three climate zones in L.A.? (as defined by the Koppen-Geiger climate classifications)? Ecologists understand climate zones as permanent to semi-permanent regimes that span vast areas. It would be clearer to write microclimatic zones, as that is more relevant to both mosquito biology and the spatial scale that is being investigated.

-Per my comment above, I think I understand the "climate" portion of distinguishing into 3 zones – but I am conflicted by calling it that. These are not really designated as climate zones in the technical sense, but for your use, it seems appropriate. Maybe stick to calling them simply zones, rather than climate zones.
-Consider not calling it this, per my statements prior
You're correct that these are not Koppen-Geiger climate classifications. In order to avoid confusion with the tens or hundreds of climate classification systems recognized by ecologists/climatologists, we have clarified in the introduction that we identified these zones for the purposes of this study. The process for identifying these zones is available in the methods section. We feel that it is necessary to continue to clarify that these zones are determined based on climate conditions.

> "Ln. 136-138: We identify three distinct climate zones in metropolitan LA and investigate the effects of temperature on the spatiotemporal dynamics of human WNV incidence across these zones (Fig. 1)."

Ln. 171: Onset dates are highly variable when reported, largely because a vast majority of those with symptoms are mild and can not recall/waited to seek care. A statement addressing this and when expected exposure actually occurred should be provided.
We agree that this deserves mention. We have added text to the supplementary methods.

> "In general, onset dates can be unreliable because they depend on individual patients remembering when symptoms began, but for the purposes of our analysis we were able to attribute cases to months rather than specific days."

Ln. 178: The authors should also address that exposure to WNV may not have occurred at the residence, or census tract, that the individual lives, but this is the next best alternative.
We agree. This topic is addressed in the supplemental methods (Section SI-4, page 9):

> "The geographic location of human West Nile non-neuroinvasive and neuroinvasive disease cases was attributed to the census tract in which the individual resided. Though exposure to WNV may not have occurred in an individual's census tract of residence, it is likely to be a reasonable approximation of the location of exposure given that Culex vectors are most active at dawn and dusk when individuals are likely to be in closer proximity to their home."

Ln. 205: I think this is very important and should be a table in the main document, not supplemental materials.
Sadly, we are not able to include this large table in the main text given the strict space limits of Proc. B. While this information is not necessary to understand the main findings in the paper, it is available (along with our code) to those seeking to replicate our analysis.

Ln. 182-186: Have the authors ran univariate analysis of the higher resolution data? If this is a microclimatic, or fine-scale assessment, data available in <1km resolutions are more appropriate, and are less likely to lead to ecological inference issues (e.g. MAUP, fallacy) when linking associations, as opposed to lower resolution data, especially those exceeding 3,4 km. If these lower resolution variables were included, I wonder how valid any associations might be including these in the assessments.
We used the highest resolution data available across our study area for each of the spatial predictor variables included in the model. The two predictors that were derived from

datasets with >1km resolution, precipitation and drought status, likely do not vary systematically at fine-spatial scales, especially during the WNV transmission season when precipitation rarely occurs. Broader coastal to inland gradients in these variables were adequately captured in the lower resolution data.

Ln. 205: Also, I am confused with how total palustrine and riverine wetland areas were acquired. In S1-5, it is stated that these were estimated within radial buffers, but are later described as 1-m resolution data provided by NWI. This would not be estimations then, these are exact known measurements. Additionally, the spatial resolution is defined as vector - what does that mean? Why not just put 1-m resolution, since that is what the authors wrote the source provided was? This needs to be more clear, and highlights the importance for why this should be a main table.
Vectorized outlines of wetlands were delineated by the US FWS using ~1m resolution aerial imagery. For the purposes of this study, we quantified the wetland composition in the area around each surveillance location since wetlands tens or hundreds of meters way from a surveillance site can influence local mosquito populations. Therefore, we used radial buffers of various sizes to quantify the total area of wetlands around each site. We added some text to the supplement to clarify this (SI-5, page 10):

> "Palustrine and riverine wetland cover were acquired from the U.S. Fish and Wildlife Service National Wetlands Inventory (NWI), which created vectorized outlines of wetlands using at least 1:40,000 scale (1-meter ground resolution) aerial imagery captured in 2005 or 2006 (10)."

Ln. 209: Just to be clear: this is WNV presence/absence in a Cx. quinq pool, corect?
Yes, we clarify this as suggested. Ln 210 "…in *Cx. quinquefasciatus* pools…"

Ln. 252: Nit picky, but use / in both month combinations or -, but not each one
Yes, thank you. We now use / in both.

Ln. 287: AUC >0.90 are considered "high performing"; levels between 0.8 and 0.9 are considered "very good"
We now use "very good" instead of "high".

Ln. 300-301: This sentence prompted a question I wanted to ask earlier: when it was initially mentioned that lags were incorporated, what kind of lags? Were they daily, weekly, monthly, and for all variables, or just select ones? A vast majority of WNV modeling papers find a range of 2-4 week lags of temp and precip were major predictors for mosquito infection. Maybe consider changing one month to 4 week lag?
We use a variety of lags, including weekly lags, in the model, though the 1 month lag was by far the most important. These details are available in the supplementary methods (Section SI-5, page 10):

> "Daily temperature, and precipitation data were aggregated and lagged to generate several additional predictors, including average daily (1, 2, 3 day lags), average weekly (1-7, 8-14, 15-21 day lag), average monthly (1-30, 31-60, 61-90 day lags), and average quarterly (1-90, 91-180, 181-270 day lags) values during the period that preceded the date of collection at a mosquito surveillance trap."

Ln. 356-357: Could this be an artifact of human population density? If the authors were to plot human pop with their infection probability, there's a high chance this could follow a strong linear trend. Have the authors considered using, or at least discussed using, human infection rates, for the predicted outcome?

We accounted for differences in human population between the different zones by summing the total population of all the census blocks in each zone and using that as an offset in the mediation model. These details are available in the methods section of the main text:

> Ln 260-263: "An offset was used to account for differences in the human population size, Po, of each metropolitan LA climate zone. Climate zone population data were acquired by summing the US Census Bureau estimated 2010 population of all census blocks within each zone."

Ln. 424-427: I think this is the key message that needs to be highlighted/conveyed.

Yes, we agree that this is an important conclusion of the paper. This is reflected in the concluding sentence of the abstract and there is a paragraph discussing it in the discussion. Unfortunately, given the word limit, we are hard pressed to dedicate more space to this, and we hope that it is sufficiently emphasized in these locations.

Ln. 427-428: IPCC models need to be intorudced and referenced

We agree that it would be helpful to reference these models. We have added some further information to the main text:

> Ln 411-414: "For instance, global climate models from Climate Model Intercomparison Project version 5 (CMIP5) following Representative Concentration Pathway 4.5 anticipate a 1.26°C mid-century (2040-2069) increase in August – September mean temperature in LA's coastal zone[46]…"

Ln. 433: I should also add that RCP models also incorporate precipitation in their predictions; to provide these predictions is a stretch, considering the lack of discussion or inclusion of precipitation/humidity in the models

Yes, agreed. Please see our response regarding precipitation in the "Major Comments" section above.