THE ROYAL SOCIETY PUBLISHING

PROCEEDINGS B

The effect of resource limitation on the temperature dependence of mosquito population fitness

Paul J. Huxley, Kris A. Murray, Samraat Pawar and Lauren J. Cator

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Original submission:	13 October 2020
1st revised submission:	30 December 2020
2nd revised submission:	15 March 2021
3rd revised submission:	30 March 2021
Final acceptance:	31 March 2021

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

Review History

RSPB-2020-2548.R0 (Original submission)

Review form: Reviewer 1

Recommendation
Reject – article is not of sufficient interest (we will consider a transfer to another journal)

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

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

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
Title: Fine.
Abstract: Fine.
Introduction: Lines 42-44 are confusing. Rewrite for clarity.
Lines 85-91: Depending on the methods, I am not likely to be convinced that simply changing food density is sufficiently different from changing density, without doing both simultaneously. That is, while density has other effects (e.g. increased waste products, competition for breaking space, etc.), in most studies it seems likely the primary effect is through competition for food.
Methods. 3 reps? That seems VERY low for a simple 3x2 experiment. Lucky to have not spilled one. Only one water bath per temperature treatment (e.g. temperature is pseudoreplicated; I understand this is a necessity of some studies, but I would have liked to see some replication--what if one water bath had some problem--you would incorrectly infer a temperature effect when it was some other cause)?
Line 154-156: Assuming allometric relationships are the same across temperatures. If you normalize the survival (proxy for larval reserves) by size (wing lenghth), do any interesting patterns emerge?
That the food level (and water level?) was adjusted to # of larvae is good, but this does ignore the fact that nutrition is provided by more than just the fish food inputs (also not very realistic), but by the bacterial and fungal metabolism driven by the nutritional inputs. This process is also driven by temperature. This may help explain the temperature effect at higher food density, mechanistically.
Results: I think many of the results seem confirmatory to a variety of previous studies, on this species and other mosquitoes, including citations in this manuscript, as well as others.
I find the adult survival interesting data, but not sure how that showed by weighed with regards to fitness--again, see previous comment on allometry.
Discussion: A lot of reference to Mordecai et al, which is fine, but makes me wonder how much general appeal this work will have? The discussion is way too long.
Line 318: fecundity, not fertility, I think.
Line 324-332. I think this is an interesting element of the discussion. It would be nice to measure metabolic rate in these situations to understand the shape of that curve (it is linear between 22 and 32?) There is an article by Padmanabha et al. JIP 2012 that tackles these issues in this species. The authors should read this and other articles by Padmanabha on the subject.
Line 350: Fecundity was not measured, but estimated, right?
Line 370: Is the "however" because there was now a difference by food density?
Line 406-414: Your resource input is constant, but the supply to the larvae may not be, as they

may not be directly eating the ground fish food, but the microorganisms that are supplied by the fish good.

Review form: Reviewer 2

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?
Good

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

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Is it accessible?
No

Is it clear?
No

Is it adequate?
No

Do you have any ethical concerns with this paper?
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Comments to the Author
This study investigates the interaction of temperature and food resources on mosquito life history traits and population growth rate. Although these two environmental factors are rarely studied together, estimates of these life history parameters are often used to model mosquito populations and predict disease transmission, so the findings here are relevant to important problems affecting human health and likely to cited. I had no issues with the experimental design or analysis (aside from a single arithmetic error in the text), and the manuscript was generally well written. However, there were several places where I thought the text was confusing or could be revised to be better organized and more compelling. Additionally, I had one suggestion for two additional figure panels using the existing data and analysis. My suggested decision is minor

revisions, as all of my suggestions have to do with the presentation rather than the analysis.

Line by line comments:

Line 12: The effect of temperature on transmission is only partially through vector population dynamics – it also affects biting rate and pathogen traits (development rate and vector competence). Here in the abstract, adding "partially" should suffice. However, this comment makes me wonder if it's worth adding a sentence to the discussion about another future direction investigating the impact of temperature x resource interactions on these other non-mosquito-life-history traits that affect transmission as well.

Lines 36, 87: I'm personally a fan of Oxford commas, but it's not a hill worth dying on.

Line 44: The grammar and rhythm of this sentence was hard to parse on my first pass. I would revise to say: "a thermal maximum, and affect…."

Line 61, 64: I would cite the Mordecai et al. 2019 Eco Lett here instead of the Mordecai 2013 and 2017 papers, since it synthesizes these two papers along with several others from the lab group.

Line 88: "effects" should be "affects"

Line 132: I would specify "daily fecundity data" here, since you refer to daily and lifetime fecundity at various points, this will help the reader keep it all straight and the conversion later will make more sense.

Line 160: This second sentence in the fecundity section should explicitly say that this scaling relationship came from previously published data. At each step make it clear what is your data and what is the previously published data. (Switching from passive to active voice for the parts with your data, e.g., "we measured wing length" on line 161 will probably help.) I found this section very confusing initially, and didn't realize this was previously published data until I got to the next paragraph.

Line 165-6: This wasn't clear on my first read-through, I only understood it after reading the full manuscript. Perhaps add a sentence to clarify the biological meaning: "In other words, the effect of resource supply on fecundity occurs primarily through its effect on body size."

Line 174: I would explicitly state here that the Leslie model requires fecundity in daily units ("for the Leslie model" at the end of the sentence should suffice), so the logic is clear.

Line 183: I assume you calculated the standard errors of the mean from the raw data since they were available? Please be clear about this since this is not your data.

Line 240, 246, and 254: I would remove "rate of" from "rate of decrease" in these three places because the text is gives the raw difference, and rate to me in this context implies some sort of normalization like a percentage.

Line 244: "Adult lifespan" to be clear.

Figure 1: I would

Figure 2: Caption should explain what the boxplots are (I assume they are the observed data).
In general, the Discussion is really long and feels a bit unorganized. The individual sentences and paragraphs are all fine enough, but the whole is somehow less than the sum of the parts. I give some specific suggestions below for ways to condense and tweak the organization, but I suggest stepping back, rethinking your overall approach, and seeing if you can tighten it up and better emphasize what the most interesting and important points are. You don't have to talk in depth

about every single result. Currently it seems organized around methods and results sections, whereas it might work better to organize it around take home messages. For instance, the elasticity analysis might not need its own paragraph – if the main point of the elasticity analysis is that juvenile survival is the most important trait, include that information in the paragraph about juvenile survival.

Line 278-300: You might be able to combine these two paragraphs, and spend less time listing specific Mordecai et al. results.

Line 282: I think this should refer to panels a and b, not a and c. Additionally, given that the text here makes an argument about what these data say when expressed as rates, I think you should at least plot them as rates in the supplement. Otherwise, you're asking the reader to do too much work or simply take it on faith. Although actually, as I mention above, this paragraph can probably be condensed and moved somewhere else. It's more results than discussion, esp. since there are no citations of other work.

Line 286: I would replace "R0 predictions" with "trait responses" because the results you are citing are the latter not the former.

Line 295: I'm pretty sure this % is not correct – the Mordecai value is more than 2x as big so it should be over 100% bigger.

Line 320: "rate trajectories were negative" is not a very biological or intuitive way to describe this. Something like, "at low resource supply, the daily mortality rate started low and then increased over time, while at high resource supply the daily mortality rate started high and then decreased to very low levels." would be much easier for readers to understand. I also think this point would be even easier to get if you add two more panels for Figure 1 that show the daily mortality rates for each temperature x resource treatment calculated from the a and b coefficients.

Line 331-2: I don't understand what you're trying to say here.

Line 358-361: This feels like the most important and profound part of the discussion and it gets a bit lost in the middle of the Discussion here.

Line 362-90: These two paragraphs have some really good analysis (e.g., 384-6), but they also do a lot of repeating results and giving values from Table S2. The discussion is already pretty long, so this could be a good place to cut content that doesn't add much. You might even be able to merge them into a single paragraph since concluding sentence is essentially the same in both.

Line 391-4: Why is the main impact through size with few direct carry over effects? Is it because the energy and nutrients for reproduction come from the adult blood meal, and the extent to which blood meal sizes vary is due to body size differences?

Line 402: Relatedly, was does "adult food supply" mean? Size of blood meal? Quality of blood meal? Frequency of blood meal?

Line 405: There is no Figure 4, do you mean S1?

Decision letter (RSPB-2020-2548.R0)

01-Dec-2020

Dear Mr Huxley:

I am writing to inform you that your manuscript RSPB-2020-2548 entitled "The effect of resource limitation on the temperature-dependence of mosquito population fitness" 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. 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.
4) Data - please see our policies on data sharing to ensure that you are complying (https://royalsociety.org/journals/authors/author-guidelines/#data).

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,
Dr Locke Rowe
mailto: proceedingsb@royalsociety.org

Associate Editor
Board Member: 1
Comments to Author:
The manuscript has been assessed by two expert reviewers. While both are satisfied with the experimental approach, they differ in their appraisal of the novelty and broader appeal of this work. Reviewer 1 finds that the results are largely confirmatory of previous work, and discussed in such a way that speaks mainly to mosquito researchers. I agree, and recommend the authors focus the manuscript much more clearly on what is new about this work, and how the results would be of interest beyond mosquito (indeed, Aedes) biology, given the broader readership of Proceedings B. Otherwise, I think that this paper - in its current form - is better suited to a more specialised journal (e.g. Parasite and Vectors).

Reviewer 2 is much more positive in terms of the appeal of the study, but they do make a range of detailed suggestions on improving the clarity of the manuscript. I too found the manuscript challenging to read in places - the logical progression was not always clear in the introduction and discussion; and I agree with both reviewers that the discussion is too long and the writing could be streamlined. Moreover, with all the different traits measured, the manuscript would benefit from a schematic describing the experimental design, traits measured and how they

interact, which would help with following the methods and results. There is also a lot of detail in the methods which are presented in the supplementary material, which makes me wonder if the shorter format of Proceedings B is the best venue for this work.

I do not have any major comments to add beyond the detailed suggestions of the two reviewers and the summary points above. One last point: while not an expert on the Metabolic Theory of Ecology, from my reading of the paper, I was surprised to see the study pitched as considering metabolic explanations (l41, 45) yet metabolic rate is not itself measured (reviewer 1 also points out this would be 'nice to measure'). It would seem that this physiological trait is key for inferring why temperature and nutrition interact in these complex ways to determine population growth - currently treated as a black box. Perhaps there could be more in the discussion on obtaining these physiological measures alongside the life history traits, and whether any such data exist.

Reviewer(s)' Comments to Author:
Referee: 1
Comments to the Author(s)
Title: Fine.
Abstract: Fine.
Introduction: Lines 42-44 are confusing. Rewrite for clarity.
Lines 85-91: Depending on the methods, I am not likely to be convinced that simply changing food density is sufficiently different from changing density, without doing both simultaneously. That is, while density has other effects (e.g. increased waste products, competition for breaking space, etc.), in most studies it seems likely the primary effect is through competition for food.
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Referee: 2

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Figure 2: Caption should explain what the boxplots are (I assume they are the observed data).

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Line 402: Relatedly, was does “adult food supply” mean? Size of blood meal? Quality of blood meal? Frequency of blood meal?

Line 405: There is no Figure 4, do you mean S1?

Author's Response to Decision Letter for (RSPB-2020-2548.R0)

See Appendix A.

RSPB-2020-3217.R0

Review form: Reviewer 2

Recommendation
Accept with minor revision (please list 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?
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.
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 is my second time reviewing this study that investigates the interaction of temperature and food resources on Aedes aegypti life history traits and population growth rate. The authors have successfully incorporated all of my previous comments, and the manuscript is greatly improved (all sections flow nicely, the Discussion is a much better length and all of the content is interesting and feels pertinent). While I disagree with the other reviewer's previous assessment of this paper's importance and novelty, I do agree that the prior version of the manuscript did not adequately argue these points, and that it was my prior knowledge on the topic that determined my assessment (and provided my incentive to review this paper). I think that the question is novel and interesting from a general ecology/biology perspective and important because of its implications for modeling vector-borne diseases of human. While the Discussion section now makes the latter argument more effectively, and I have some suggestions (below) to improve the former. Additionally, I had one suggestion for additional data to report in the supplement. Finally, I have a handful of superficial comments to improve the text. My suggested decision is accept with minor revisions.

The novelty of the study and relevance to other systems:

To my knowledge, temperature and resource availability are rarely studied together for their interactive effects on population growth rates, especially in animals. Although I acknowledge that perhaps the other reviewer knows of papers that have factorially crossed these factors and quantitatively projected their effects of population growth rate in animals, the only three studies that I know of to have done this are in single-celled plankton. That said, I do think that the authors should add a sentence or two acknowledging this literature in the Discussion and argue that their results are relevant to the ecological literature beyond the more urgent applications to mosquitoes and vector-borne disease. The authors already cite reference 20, which found similar results (lower Topt under resource stress) in phytoplankton. This study using bacterial plankton communities in lakes had more mixed results that sometimes support that finding: https://www.int-res.com/articles/ame2007/49/a049p035.pdf. This other study, also in bacterial plankton, did not estimate Topt, but also found that temperature had a stronger effect on growth in resource limited environments: https://pubmed.ncbi.nlm.nih.gov/24185633/. The authors could read through the citations of those papers, and use Google Scholar to look at studies that cite them in order to look for other relevant examples. I also note a few places in the line by line comments below where you can generalize the argument.

Additional data to report in the supplement

When reading the new paragraph on comparing your trait estimates to prior studies used to parameterize R0 models (lines 281-290), I had a fleeting thought that this paragraph might be better done with larval mortality, since that was the most important trait in the model. But I quickly realized that most studies report larval mortality as a percentage reaching maturity rather than a daily mortality rate, so a direct comparison would be difficult. The daily mortality rate is arguably a better way to measure and report the data, but it would still be nice to be able to make a direct comparison with other studies. Therefore, I think it would advisable (and should be easy) to also report the percentage reaching maturity in the supplement.

Line by line comments:

Line 21: I would remove the word "exaggerated" – I found it confusing rather than helpful.

Line 37: I would add “interact to” before “affect the abundance”

Line 38-9: I would delete “interest is growing rapidly in the effects of” (this is a weak argument for importance) and add “can have large effects” before “on their population fitness”

Line 42: I would delete “Therefore” – it doesn’t match the logical flow

Line 44: “leading to interesting new insights” is also a weak argument in this vague form. I would delete “interesting” and add something like, “including XYZ” to give an example of one or more of these insights.

Line 46: I would replace “Yet” with “While”

Line 52: “this” -> “this prediction” (in general, you want to avoiding hanging this-es without nouns)

Line 56: I would delete “Ultimately” (it doesn’t match the logical flow) and change “this” -> “This resource limitation”

Line 64: I would delete “Additionally” for the start of a new paragraph

Line 67: I would delete “However” and change “too” -> “also”

Line 71: I would replace “may together” with “interact to”

Line 72: “through” -> “together through effects on”

Line 84: “disease vectors” could be “disease vectors and other arthropods” to generalize the scope

Line 89: The text refers to “this replication level” but the replication level is not given until the following paragraph, so I would move this sentence to after that is given or change the text to say “the replication level we chose” or something similar.

Line 180-1: A couple points here. 1) I would say “The trait data distributions for development time, lifespan, and wing length” for increased clarity (the juvenile mortality rate is also trait data, but not included here) and my personal preference for Oxford commas. 2) The phrasing “the trait data… were nonlinear, positive, and right-skewed” does not make sense. In my interpretation, positive and right-skewed refer to the data distributions for each treatment plotted as a histogram or density plot. However, “nonlinear” only applies in the context of “as a function of temperature,” no? So it doesn’t match the other descriptions or make sense as a description in this context.

Line 192: Table S3 shows that the authors did the model selection correctly, but just worded it in a confusing/wrong way here. Negatives (“did not improve”) are confusing generally, and this text actually says the opposite of what the authors did. I would say: “If removing a term worsened model fit (dAIC > -2), then it was retained. Otherwise it was removed.”

Line 229-31: I would switch the order here to match the paragraphs preceding it (larger decrease, followed by smaller decrease)

Line 260: The end of this first paragraph could be a place to put a sentence about the broader scope of results beyond mosquitoes.

Line 271-76: This paragraph is long series of hanging nounless this-es. I would change the sentences to start: “This is” -> “This pattern occurs” (271); “This is” -> “These effects” (273); “This is” -> “This result” (275); “This is a key finding” -> “This finding is key” (276)

Line 298: “this” -> “this hypothesis”

Line 300: If you change “natural mosquito populations” to “natural populations of mosquitoes and other arthropods” this could be another place to expand the scope.

Line 303: evaporation of breeding habitat is another threat that becomes worse over time and could be added here

Line 307: The end of this paragraph is another place to potentially expand the scope. You could point out that the outcome of climate change + resource limitation interactions on populations means different things for vectors and agricultural pests (higher pops = bad) than it does for species of conservation concern (higher pops = good). (Obviously with more nuance.)

Line 309: “this is” -> “this approach is appropriate”

Line 316: delete comma, delete period, delete “This is”

Line 319: delete “may have”

Line 328, 333: “we have not considered” reads better to me as “we did not consider”; same for “we have also not addressed” and “we also did not address”

Line 330: “This is” -> “This relationship occurs”

Line 342: delete both commas, replace “resource fluctuations” with “resource availability”

Line 356: End of final paragraph is another place to potentially expand scope and cite the plankton papers.

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?
Good

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

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?
No

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

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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
Summary of Huxley et al.
The authors conducted experiments with the mosquito Aedes aegypti at three different temperatures, where juveniles were exposed to either a high or low resource environment. They show that low resource levels lead to decreased population growth rate across the temperature range, and that the low resource treatment had a lower optimal temperature compared to the high resource treatment. They suggest that future projections of disease vectors and transmission should consider resource supply.

I think this is a well-conducted study with interesting results that should be of interest to a general readership, and I think that it can make a nice contribution to the literature. I do have four 'major' comments, which I detail below, that I hope can help improve the study.

Major comments

1. Juvenile survival results and Figure 1

When looking at the survival curves in Fig. 1a-b (and the corresponding estimates of the parameters in the survival curves in panels c and d) I'm wondering if there is an issue with dealing with competing rates. For example, in panel b where there were high resources, each of the three survival curves are almost completely flat by day 20, suggesting that juveniles will have extremely low mortality as time goes on. But isn't this actually because all of the juveniles have already emerged? Based on the development rate data shown in Figure 2, I'd think most individuals in the high resource treatment have already emerged by early on in the experiment, and mortality rate after the time when all individuals had either emerged or already died wouldn't be important.

Generally, it seems like juveniles could have three possible outcomes in this experiment: they stay in the tub for the duration of the experiment, they die, or they emerge. I'm wondering if an alternative figure would be more useful, where it depicts the fate of individuals over time, including death or emergence, in each of the six treatments. And does accounting for this affect the mortality rate estimates?

2. Adult longevity and its effects on population growth rate

Using their elasticity analysis, the authors found very minimal effects of adult survival and adult fecundity on population growth rate (r-max) (lines 239-250). However, I'm wondering if this is in part due to the fact that adults were starved, and therefore had shorter lifespans than may be observed in nature. For example, based on Fig. 2b, in this experiment the authors found mean adult lifespan was approximately 7 days and 11 days at 22C at low and high juvenile resources, respectively, and 2 days and 3 days at 32 degrees. In comparison, Aedes aegypti adult lifespan shown in Mordecai et al. 2017 (PLOS Neg. Trop. Diseases) looks to be approximately 26 days at 22C, and around 20 days at 32C. If adult lifespan is reduced here due to starvation, does this bias the elasticity analysis towards down-weighting lifespan's (and possibly fecundity's) effect on r-max, since a 10% perturbation around 3 days will be different than a 10% perturbation around 20 days?

3. Population growth rate at high resources increasing monotonically with temperature

The authors found that under high resources, population growth rate peaked at 32C (Fig. 3) and that it "increased monotonically with temperature" (line 237). On lines 259-260 in the first paragraph of the discussion, they concluded that "this lack of unimodality indicates that the temperature at which fitness peaks is higher than 32C when resources are abundant."

Unless the authors have additional information, I think this statement in the discussion should be removed. The experiment took place at 22C, 26C, and 32C. In my opinion, this six-degree gap between 26C and 32C leaves a very good chance that population growth rate actually peaks somewhere between 26C and 32C and then decreases again, but that it was not captured in the experiment. I apologize for not having a reference handy for this, but I believe a max growth rate for this species somewhere between 26C and 32C would be more in line with previous findings than a finding that max growth rate is above 32C. Either way, these alternative scenarios could be discussed in the Discussion section. I would also suggest removing the trend lines between points in Fig. 3, as it could give a potentially false sense to the reader that this is the pattern r-max will follow across temperature, even though it may actually be unimodal between points.

4. Implications for vector-borne disease and how we should model it

I liked the discussion, but I think a bit more could be added to strengthen it. For example, I like the idea of incorporating resource supply into mosquito projection models or vector-borne disease models. However, I was hoping for a bit more discussion for how this could potentially be done. If temperature is broadly predictable across time and space but resources aren't, how do we incorporate that?

Also, since the abstract and introduction introduce the idea of projecting disease transmission as one of the important reasons for understanding interactions between temperature and resources on mosquitos, I think some more could be said about possible effects of temperature and resources on other disease-relevant traits. Should we expect juvenile resource level to affect adult biting rate, a key trait for disease transmission rate? Should we expect it to affect vector competence? I realize the author's results don't explicitly speak to this, but I think some additional discussion about possibilities would be interesting, and would also highlight that changes in mosquito population growth rate won't necessarily correspond with the same changes in mosquito-borne disease transmission.

Minor comments

Abstract: suggest clarifying that resource levels are manipulated for juvenile but not adult mosquitos.

Line 278: I think "optima" should be "optimal" here.

Lines 284-285: Can the authors clarify in this sentence that this result is for the high resource supply treatment?

Figure 1. Could the authors use different colours in panels a and b to represent the different temperature treatments? It's hard to tell which confidence intervals are which.

Figure 2. Is there a strong reason for including both boxplots and GLM estimates here? If not, perhaps one or the other can be used to make the plots cleaner.

Decision letter (RSPB-2020-3217.R0)

15-Feb-2021

Dear Mr Huxley:

Your manuscript has now been peer reviewed and the reviews have been assessed by an Associate Editor. The reviewers' 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 reviewers and the Editors have raised some concerns 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 (https://royalsociety.org/journals/authors/author-guidelines/#data). 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,
Dr Locke Rowe
mailto: proceedingsb@royalsociety.org

Associate Editor Board Member
Comments to Author:
The revised manuscript has been reviewed by two independent experts (the second had reviewed the earlier submission). Both agree that this is an interesting study that will appeal to a broad readership, and they make a number of excellent suggestions to improve the manuscript further.

Reviewer 1 raises some pertinent suggestions concerning the interpretation of results (see major comments 1-3), and I agree that the authors should consider these points carefully and revise the manuscript accordingly.

While the revised manuscript presents a much stronger case for the novelty and relevance of the study beyond Aedes biology, both reviewers give good suggestions on how to improve this further - considering other potential consequences for disease transmission (Reviewer 1, point 4) or strengthening the point about the novelty of study in advancing fundamental ecology (Reviewer 2). As these points are not contradictory, I can see them both being incorporated without the manuscript becoming too long.

Related to these points, I would also request that the authors clarify how their work fits with other mosquito studies. At l.80-1, they state that 'studies that have considered temperature have not examined how the effects of temperature and resource supply together affect fitness through traits [36,37]'. But, from a quick read of ref. 36, the study does consider the interactive effects of temperature and diet (albeit also with density - thus a 4 x 4 x 4 design!) on development time and survival. This would seem also a relevant paper to cite in the discussion: how do the results compare? Moreover, they could mention related studies in Anopholes on temperature and food supply in the larval environment on adult body size and survival (Barreaux et al. Par & Vec 2018, 11:485), and vector competence (Barreaux et al. MWJ 2016, 7:8). (My apologies for not noticing these had not been cited in the previous version)

The reviewers also give a number of specific suggestions for improving clarity of writing. I agree with these all, and add a couple of small suggestions:
l.23-25: I found the phrase '_underestimate_ how resource supply _modulates_ the _temperature-dependency_' hard to follow (there are multiple relationships suggested here). I also wonder if the point is not so much whether it is underestimated, but more that the temperature-dependency of fitness is biased, as only traits measured under high/optimal resource supply are used in the projections.

l.40 'Biological rates ... such as mortality .. 'increases to an optimum before declining to zero' Be careful in wording here, do traits like metabolic rate, mortality rate actually approach zero at higher temperatures? And is it the case that mortality rate increases and declines, and not survival?

Reviewer(s)' Comments to Author:

Referee: 3
Comments to the Author(s).
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When looking at the survival curves in Fig. 1a-b (and the corresponding estimates of the parameters in the survival curves in panels c and d) I'm wondering if there is an issue with dealing with competing rates. For example, in panel b where there were high resources, each of the three survival curves are almost completely flat by day 20, suggesting that juveniles will have extremely low mortality as time goes on. But isn't this actually because all of the juveniles have already emerged? Based on the development rate data shown in Figure 2, I'd think most individuals in the high resource treatment have already emerged by early on in the experiment, and mortality rate after the time when all individuals had either emerged or already died wouldn't be important.

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be done. If temperature is broadly predictable across time and space but resources aren't, how do we incorporate that?

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Figure 1. Could the authors use different colours in panels a and b to represent the different temperature treatments? It's hard to tell which confidence intervals are which.

Figure 2. Is there a strong reason for including both boxplots and GLM estimates here? If not, perhaps one or the other can be used to make the plots cleaner.

Referee: 2

Comments to the Author(s).
This is my second time reviewing this study that investigates the interaction of temperature and food resources on Aedes aegypti life history traits and population growth rate. The authors have successfully incorporated all of my previous comments, and the manuscript is greatly improved (all sections flow nicely, the Discussion is a much better length and all of the content is interesting and feels pertinent). While I disagree with the other reviewer's previous assessment of this paper's importance and novelty, I do agree that the prior version of the manuscript did not adequately argue these points, and that it was my prior knowledge on the topic that determined my assessment (and provided my incentive to review this paper). I think that the question is novel and interesting from a general ecology/biology perspective and important because of its implications for modeling vector-borne diseases of human. While the Discussion section now makes the latter argument more effectively, and I have some suggestions (below) to improve the former. Additionally, I had one suggestion for additional data to report in the supplement. Finally, I have a handful of superficial comments to improve the text. My suggested decision is accept with minor revisions.

The novelty of the study and relevance to other systems:

To my knowledge, temperature and resource availability are rarely studied together for their interactive effects on population growth rates, especially in animals. Although I acknowledge that perhaps the other reviewer knows of papers that have factorially crossed these factors and quantitatively projected their effects of population growth rate in animals, the only three studies that I know of to have done this are in single-celled plankton. That said, I do think that the authors should add a sentence or two acknowledging this literature in the Discussion and argue that their results are relevant to the ecological literature beyond the more urgent applications to

mosquitoes and vector-borne disease. The authors already cite reference 20, which found similar results (lower Topt under resource stress) in phytoplankton. This study using bacterial plankton communities in lakes had more mixed results that sometimes support that finding: https://www.int-res.com/articles/ame2007/49/a049p035.pdf. This other study, also in bacterial plankton, did not estimate Topt, but also found that temperature had a stronger effect on growth in resource limited environments: https://pubmed.ncbi.nlm.nih.gov/24185633/. The authors could read through the citations of those papers, and use Google Scholar to look at studies that cite them in order to look for other relevant examples. I also note a few places in the line by line comments below where you can generalize the argument.

Additional data to report in the supplement

When reading the new paragraph on comparing your trait estimates to prior studies used to parameterize R0 models (lines 281-290), I had a fleeting thought that this paragraph might be better done with larval mortality, since that was the most important trait in the model. But I quickly realized that most studies report larval mortality as a percentage reaching maturity rather than a daily mortality rate, so a direct comparison would be difficult. The daily mortality rate is arguably a better way to measure and report the data, but it would still be nice to be able to make a direct comparison with other studies. Therefore, I think it would advisable (and should be easy) to also report the percentage reaching maturity in the supplement.

Line by line comments:

Line 21: I would remove the word "exaggerated" – I found it confusing rather than helpful.

Line 37: I would add "interact to" before "affect the abundance"

Line 38-9: I would delete "interest is growing rapidly in the effects of" (this is a weak argument for importance) and add "can have large effects" before "on their population fitness"

Line 42: I would delete "Therefore" – it doesn't match the logical flow

Line 44: "leading to interesting new insights" is also a weak argument in this vague form. I would delete "interesting" and add something like, "including XYZ" to give an example of one or more of these insights.

Line 46: I would replace "Yet" with "While"

Line 52: "this" -> "this prediction" (in general, you want to avoiding hanging this-es without nouns)

Line 56: I would delete "Ultimately" (it doesn't match the logical flow) and change "this" -> "This resource limitation"

Line 64: I would delete "Additionally" for the start of a new paragraph

Line 67: I would delete "However" and change "too" -> "also"

Line 71: I would replace "may together" with "interact to"

Line 72: "through" -> "together through effects on"

Line 84: "disease vectors" could be "disease vectors and other arthropods" to generalize the scope

Line 89: The text refers to "this replication level" but the replication level is not given until the following paragraph, so I would move this sentence to after that is given or change the text to say "the replication level we chose" or something similar.

Line 180-1: A couple points here. 1) I would say "The trait data distributions for development time, lifespan, and wing length" for increased clarity (the juvenile mortality rate is also trait data, but not included here) and my personal preference for Oxford commas. 2) The phrasing "the trait data… were nonlinear, positive, and right-skewed" does not make sense. In my interpretation, positive and right-skewed refer to the data distributions for each treatment plotted as a histogram or density plot. However, "nonlinear" only applies in the context of "as a function of temperature," no? So it doesn't match the other descriptions or make sense as a description in this context.

Line 192: Table S3 shows that the authors did the model selection correctly, but just worded it in a confusing/wrong way here. Negatives ("did not improve") are confusing generally, and this text actually says the opposite of what the authors did. I would say: "If removing a term worsened model fit (dAIC > -2), then it was retained. Otherwise it was removed."

Line 229-31: I would switch the order here to match the paragraphs preceding it (larger decrease, followed by smaller decrease)

Line 260: The end of this first paragraph could be a place to put a sentence about the broader scope of results beyond mosquitoes.

Line 271-76: This paragraph is long series of hanging nounless this-es. I would change the sentences to start: "This is" -> "This pattern occurs" (271); "This is" -> "These effects" (273); "This is" -> "This result" (275); "This is a key finding" -> "This finding is key" (276)

Line 298: "this" -> "this hypothesis"

Line 300: If you change "natural mosquito populations" to "natural populations of mosquitoes and other arthropods" this could be another place to expand the scope.

Line 303: evaporation of breeding habitat is another threat that becomes worse over time and could be added here

Line 307: The end of this paragraph is another place to potentially expand the scope. You could point out that the outcome of climate change + resource limitation interactions on populations means different things for vectors and agricultural pests (higher pops = bad) than it does for species of conservation concern (higher pops = good). (Obviously with more nuance.)

Line 309: "this is" -> "this approach is appropriate"

Line 316: delete comma, delete period, delete "This is"

Line 319: delete "may have"

Line 328, 333: "we have not considered" reads better to me as "we did not consider"; same for "we have also not addressed" and "we also did not address"

Line 330: "This is" -> "This relationship occurs"

Line 342: delete both commas, replace "resource fluctuations" with "resource availability"

Line 356: End of final paragraph is another place to potentially expand scope and cite the plankton papers.

Author's Response to Decision Letter for (RSPB-2020-3217.R0)

See Appendix B.

Decision letter (RSPB-2020-3217.R1)

23-Mar-2021

Dear Mr Huxley

I am pleased to inform you that your Review manuscript RSPB-2020-3217.R1 entitled "The effect of resource limitation on the temperature-dependence of mosquito population fitness" has been accepted for publication in Proceedings B.

The referee(s) do not recommend any further changes. Therefore, please proof-read your manuscript carefully and upload your final files for publication. Because the schedule for publication is very tight, it is a condition of publication that you submit the revised version of your manuscript within 7 days. If you do not think you will be able to meet this date please let me know immediately.

To upload your 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 Revision." Your manuscript number has been appended to denote a revision.

You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, upload a new version through your Author Centre.

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1) A text file of the manuscript (doc, txt, rtf or tex), including the references, tables (including captions) and figure captions. Please remove any tracked changes from the text before submission. PDF files are not an accepted format for the "Main Document".

2) A separate electronic file of each figure (tiff, EPS or print-quality PDF preferred). The format should be produced directly from original creation package, or original software format. Please note that PowerPoint files are not accepted.

3) Electronic supplementary material: this should be contained in a separate file from the main text and the file name should contain the author's name and journal name, e.g authorname_procb_ESM_figures.pdf
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 see: https://royalsociety.org/journals/authors/author-guidelines/

4) Data-Sharing and data citation
It is a condition of publication that data supporting your paper are made available. Data should be made available either in the electronic supplementary material or through an appropriate

repository. Details of how to access data should be included in your paper. Please see https://royalsociety.org/journals/ethics-policies/data-sharing-mining/ for more details.

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=RSPB-2020-3217.R1 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.

5) For more information on our Licence to Publish, Open Access, Cover images and Media summaries, please visit https://royalsociety.org/journals/authors/author-guidelines/.

Once again, thank you for submitting your manuscript to Proceedings B and I look forward to receiving your final version. If you have any questions at all, please do not hesitate to get in touch.

Sincerely,
Dr Locke Rowe
Editor, Proceedings B
mailto:proceedingsb@royalsociety.org

Associate Editor Board Member
Comments to Author:
The authors have revised the manuscript a second time after a second set of detailed suggestions from two reviewers, and have largely taken on board all of their suggestions in the main text - or explained in the response document why they have not, such as the point about elasticity analyses using starved lifespans. I have no further major comments, and believe that this study will be of interest to readers of Proceedings B as it provides important insights into how multiple stressors influence population growth of a disease vector, findings which are also generalisable to other ectothermic organisms. I have some minor suggestions below to improve phrasing further, and a reminder about data accessibility:

1. l.170-173: "Many biological rates (“functional traits”, such as metabolic, development, and fecundity [7]) of ectotherms increase approximately exponentially with temperature up to some optimum before declining to zero [16]."
- This confused me on first reading it was not obvious that 'metabolic' and 'development' were adjectives paired with 'rate'; maybe better as 'Many biological rates (including metabolic, development and fecundity rate [7]) ... ". It is not necessary to mention "functional traits" here. As per my previous comment, why not say 'declining to a baseline' than 'decline to zero' (the Dell et al. 2011 PNAS paper [ref 16] does not mention declining to zero, specifically).

2. While the authors have made some adjustments to Reviewer 3's comment 7 on 'monotonic' changes with temperature, there are other points in the paper where the functional form of relationships with temperature is discussed, which could be phrased more cautiously given that only 3 temperatures are tested. Specifically:
l.372: 'fitness was negative throughout and unimodal (declined steeply)' - could be 'fitness was negative for all three temperatures tested, and was much lower at 32C than 26C' ,
l.375: 'positive and monotonically increasing' - could say fitness was 'positive and increased moderately from 26C to 32C'
l.451 'increased nonlinearly with temperature... (Fig 1d) - should refer to 'Fig 2d' and could say instead 'increased with temperature, with a larger increase from 26C to 32C than between 22C and 26C'.

3. As per Reviewer 2, avoid the noun-less 'this'-es, paragraph at l.425, first 'This' could be "This reduction in survival is likely because ...", and then final sentence could start (if I have followed the logic correctly) "Such metabolic costs could explain why ... "

4. The link to the Dryad dataset does not work - in Dryad, it says 'No pages found" and when entered directly into a search engine a report "DOI not found" is generated.

Decision letter (RSPB-2020-3217.R2)

31-Mar-2021

Dear Mr Huxley

I am pleased to inform you that your manuscript entitled "The effect of resource limitation on the temperature-dependence of mosquito population fitness" 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

Data Accessibility section
Please remember to make any data sets live prior to publication, and update any links as needed when you receive a proof to check. It is good practice to also add data sets to your reference list.

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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,
Dr Locke Rowe
Editor, Proceedings B
mailto: proceedingsb@royalsociety.org

Associate Editor:
Board Member
Comments to Author:
The authors have incorporated the minor edits I suggested on the previous submission, and the Dryad dataset is now available (with a very comprehensive 'read me' file - well done!).

Appendix A

Responses to referees

Editor and Reviewers' comments are in **blue**, our responses in **black**.

Associate Editor's comments

1. The manuscript has been assessed by two expert reviewers. While both are satisfied with the experimental approach, they differ in their appraisal of the novelty and broader appeal of this work. Reviewer 1 finds that the results are largely confirmatory of previous work, and discussed in such a way that speaks mainly to mosquito researchers. I agree, and recommend the authors focus the manuscript much more clearly on what is new about this work, and how the results would be of interest beyond mosquito (indeed, Aedes) biology, given the broader readership of Proceedings B. Otherwise, I think that this paper - in its current form - is better suited to a more specialised journal (e.g. Parasite and Vectors).

We have revised the Introduction (lines 30-84) and the Discussion (251-356) extensively to better emphasize the novelty our study and put it in a broader context. We also have expanded the scope of our citations to underline how most publications is this area have ignored how resource limitation can profoundly affect the temperature-dependence of mosquito fitness. We now also more clearly emphasise that our findings underline the need for future research effort to be directed at better understanding how temperature and resource supply interact in the field, and how this interaction may influence other components of vector-borne disease transmission systems, such as pathogen growth and biting rates (lines 318-327).

2. Reviewer 2 is much more positive in terms of the appeal of the study, but they do make a range of detailed suggestions on improving the clarity of the manuscript. I too found the manuscript challenging to read in places - the logical progression was not always clear in the introduction and discussion; and I agree with both reviewers that the discussion is too long and the writing could be streamlined. Moreover, with all the different traits measured, the manuscript would benefit from a schematic describing the experimental design, traits measured and how they interact, which would help with following the methods and results. There is also a lot of detail in the methods which are presented in the supplementary material, which makes me wonder if the shorter format of Proceedings B is the best venue for this work.

We have substantially revised the Introduction and the Discussion to improve clarity, cohesion and precision. We have shortened the Introduction by 21% and Discussion by 31%, streamlined the Methods and provided a schematic of the experimental design and the traits measured (Fig. S1).

3. …while not an expert on the Metabolic Theory of Ecology, from my reading of the paper, I was surprised to see the study pitched as considering metabolic explanations (l41, 45) yet metabolic rate is not itself measured (reviewer 1 also points out this would be 'nice to measure'). It would seem that this physiological trait is key for inferring why temperature and nutrition interact in these complex ways to determine population growth - currently treated as a black box. Perhaps there could be more in the discussion on obtaining these physiological measures alongside the life history traits, and whether any such data exist.

Although we allude to Ecological metabolic theories (Metabolic Theory of Ecology (MTE) + Dynamic Energy Budget theory (DEB)) in the Introduction (lines 39-42 and 57-63), our

objective was not to explicitly link metabolic rate through other traits to fitness. Instead, we chose to take the practical approach of linking readily measurable life history traits (development, mortality, and fecundity rate) to fitness. This is the approach taken by the spate of current studies on the effects of temperature on disease vectors as well as more generally, holometabolous insects [1–4]. In part, we chose to take this practical approach because linking metabolic rate to fitness through life history traits of holometabolous insects (and arguably, ectotherms in general) is still a work in progress in both MTE and DEB [1,5]. While it is well-established that life history traits are closely related to operating temperature, body size and metabolic rate, the precise nature of these relationships varies across taxa [6]. For example, the mean activation energy value for some insect traits deviates from the value expected for this trait by MTE [7,8]. Such deviations from core MTE expectations could derive from how insect respiratory systems are structured [9]. Thus, while quantifying the relationships between size, metabolic rate and life history characteristics in insects could provide important insights into how they may respond to climatic warming, this line of investigation is beyond the scope of our study. We feel that the readers will appreciate the practicality of our approach, but if the reviewers and Editors deem it necessary, we are happy to clarify this issue in the Discussion of our paper. For example, as such, our results do also highlight the need to consider the role of resource supply in ontogenetic growth models under both MTE and DEB frameworks.

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Reviewer 1's Comments

4. Introduction: Lines 42-44 are confusing. Rewrite for clarity.

We have rewritten this paragraph to improve its clarity (lines 38-48).

5. Lines 85-91: Depending on the methods, I am not likely to be convinced that simply changing food density is sufficiently different from changing density, without doing both simultaneously. That is, while density has other effects (e.g. increased waste products, competition for breaking space, etc.), in most studies it seems likely the primary effect is through competition for food.

The reviewer raises a very important point. Indeed, larval competition is an important density-dependent mechanism that can affect population dynamics [10]. We have now revised the Introduction to acknowledge this and also make clear why we focus on resource supply *per se* (lines 71-82). Then, later, in the Discussion (lines 328-338) we revisit this issue to make clear that considering density dependence is a logical and potentially important avenue for future research.

6. Methods. 3 reps? That seems VERY low for a simple 3x2 experiment **[a]**. Lucky to have not spilled one. **[b]** Only one water bath per temperature treatment (e.g. temperature is pseudo replicated; I understand this is a necessity of some studies, but I would have liked to see some replication--what if one water bath had some problem--you would incorrectly infer a temperature effect when it was some other cause)?

[a] Prior to conducting the main experiment, we conducted a preliminary assay to determine the replication levels required to detect statistically significant effect sizes, and to calibrate our low-resource supply level (0.1 mg larva^{-1} day^{-1}). Analysis of these data showed that our level of replication was sufficient; we detected a significant effect of temperature on juvenile development time and adult lifespan at low-resource supply at 26 and 32°C (Table S4). As an

additional analysis, we compared the preliminary assay results with the corresponding treatment effects in the original manuscript. This analysis shows that there were no significant differences between these effects (Table S5). These analyses were not included in the original manuscript data, because, in the preliminary assay, we did not measure wing length, which is required to estimate fecundity and calculate r_{max}. Also, in the preliminary assay, we did not test the high-resource supply because it is consistent with the 'high food' regimes used in previous publications (e.g. [13,14])

Reviewer 1 suggested that main experiment lacked complexity, because it consisted of three temperature levels and two resource supply levels. However, disentangling the effects of temperature and resource supply across multiple traits is not straightforward, and required detailed planning and extensive daily maintenance, and overall make for an experiment that is novel and far from trivial in terms of logistical difficulty.

With respect to the second concern **[b]**, the water baths we used (Grant Instruments: JB Academy) display an error message, if they malfunction. In the pilot and the main experiment, we also checked the water temperatures twice daily with a glass immersion thermometer.

7. Line 154-156: Assuming allometric relationships are the same across temperatures. If you normalize the survival (proxy for larval reserves) by size (wing length), do any interesting patterns emerge?

We agree that this would be interesting, however, we show through our sensitivity analyses (Figs. 3, S3) that our r_{max} calculations are robust to variation in the scaling between wing length and fecundity. Please also refer to our response to Comment 3, which explains why we did not explicitly link metabolic rate through other traits. We are happy to carry out additional analyses, if the reviewer could elaborate on what they mean by "interesting patterns".

8. That the food level (and water level?) was adjusted to # of larvae is good, but this does ignore the fact that nutrition is provided by more than just the fish food inputs (also not very realistic), but by the bacterial and fungal metabolism driven by the nutritional inputs. This process is also driven by temperature. This may help explain the temperature effect at higher food density, mechanistically.

The Reviewer has raised a very interesting point**,** which we also as one of the authors (Pawar) now works extensively on the temperature dependence of microbial traits [15,16]. Accounting for microbial growth is outside the scope of our study, but we agree that it could contribute to larval nutrition. We now acknowledge this in the revised Discussion (lines 328-338), emphasizing that this is a potentially important avenue for future field research.

9. Results: I think many of the results seem confirmatory to a variety of previous studies, on this species and other mosquitoes, including citations in this manuscript, as well as others.

Our main finding is that juvenile resource regimes have far-reaching effects on the thermal response of population-level fitness, partly through carry-over effects on adult life history traits. While we do not report novel individual trait-level effects, we quantify the temperature dependence of fitness at two nutrient levels, and the contributions of juvenile and adult traits to it. This level of resolution of trait contributions to fitness has been called for [1,2,17], but datasets like ours remain largely absent. Our study underlines the importance of the effects of resource supply on the temperature-dependence of population-level fitness of an important disease vector. In doing so, our findings suggest many of the currently available projections

of how disease vectors and transmission risk may respond to climatic warming may need to be reconsidered. We have now rewritten the Introduction (especially, lines 38-48 and 71-84) and the Discussion (lines 251-267) to clarify the novelty our study and its findings.

10. I find the adult survival interesting data, but not sure how that showed by weighed with regards to fitness--again, see previous comment on allometry.

Please refer to our response to Comment 3.

11. Discussion: A lot of reference to Mordecai et al, which is fine, but makes me wonder how much general appeal this work will have?

Please refer to our response to Comment 9. In the Discussion, we also have expanded the focus of our citations to underline how most publications have ignored how resource limitation can profoundly affect the temperature-dependence of mosquito fitness. These findings are likely to apply to many organisms that are sensitive to temperature.

12. The discussion is way too long.

Please refer to our response to Comment 2.

13. Line 318: fecundity, not fertility, I think.

We have changed our wording of this throughout to make our usage more consistent.

14. Line 324-332. I think this is an interesting element of the discussion. It would be nice to measure metabolic rate in these situations to understand the shape of that curve (it is linear between 22 and 32?) There is an article by Padmanabha et al. JIP 2012 that tackles these issues in this species. The authors should read this and other articles by Padmanabha on the subject.

With regards to the comment about metabolic scaling, please refer to our response to Comment 3. We have now cited Padmanabha et al. [18] as an example of a study that focused on the temperature-dependency of individual traits. Our study differs because we quantify the mechanistic basis of temperature-dependent fitness.

15. Line 406-414: Your resource input is constant, but the supply to the larvae may not be, as they may not be directly eating the ground fish food, but the microorganisms that are supplied by the fish good.

Although we are interested in how the temperature-dependency of microbial growth affects food availability, examining this effect was beyond the scope of this study. We believe we did as much as possible to limit any confounding effect of microbial growth because we cleaned the tubs and provided clean water every day (lines 113-115). Also, please refer to our response to Comment 8.

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Reviewer 2's Comments to Author

16. Line 12: The effect of temperature on transmission is only partially through vector population dynamics – it also affects biting rate and pathogen traits (development rate and vector competence). Here in the abstract, adding "partially" should suffice. However, this comment makes me wonder if it's worth adding a sentence to the discussion about another future direction investigating the impact of temperature x resource interactions on these other non-mosquito-life-history traits that affect transmission as well.

We have addressed this comment by adding ‘partially’ on line 12. We have also added a short paragraph on lines 316-325 and sentence at the end of the Discussion, which notes how temperature × resource interactions could also have important effects on non-mosquito components of VBD systems.

17. Line 44: The grammar and rhythm of this sentence was hard to parse on my first pass. I would revise to say: “a thermal maximum, and affect….”

We have rewritten this paragraph to improve its clarity (lines 38-48).

18. Line 132: I would specify “daily fecundity data” here, since you refer to daily and lifetime fecundity at various points, this will help the reader keep it all straight and the conversion later will make more sense.

We have changed our wording of this throughout to make our usage more consistent.

19. Line 160: This second sentence in the fecundity section should explicitly say that this scaling relationship came from previously published data. At each step make it clear what is your data and what is the previously published data. (Switching from passive to active voice for the parts with your data, e.g., “we measured wing length” on line 161 will probably help.) I found this section very confusing initially, and didn’t realize this was previously published data until I got to the next paragraph.”

We have addressed this on lines 147-156.

20. Line 165-6: This wasn’t clear on my first read-through, I only understood it after reading the full manuscript. Perhaps add a sentence to clarify the biological meaning: “In other words, the effect of resource supply on fecundity occurs primarily through its effect on body size.

We have included this suggested sentence on lines 23-24 of the electronic supplementary material.

21. Line 174: I would explicitly state here that the Leslie model requires fecundity in daily units (“for the Leslie model” at the end of the sentence should suffice), so the logic is clear.

We have now explicitly stated this on lines 153-156.

22. Line 183: I assume you calculated the standard errors of the mean from the raw data since they were available? Please be clear about this since this is not your data.

We have now more clearly stated this on lines 160-164.

23. Line 240, 246, and 254: I would remove “rate of” from “rate of decrease” in these three places because the text is gives the raw difference, and rate to me in this context implies some sort of normalization like a percentage.

We have now removed ‘rate of’ in these places.

24. Figure 2: Caption should explain what the boxplots are (I assume they are the observed data).

We now describe what the boxplots are in Fig. 2’s caption (lines 567-570).

25. In general, the Discussion is really long and feels a bit unorganized. The individual sentences and paragraphs are all fine enough, but the whole is somehow less than the sum of the parts. I give some specific suggestions below for ways to condense and tweak the organization, but I suggest stepping back, rethinking your overall approach, and seeing if

you can tighten it up and better emphasize what the most interesting and important points are. You don't have to talk in depth about every single result. Currently it seems organized around methods and results sections, whereas it might work better to organize it around take home messages. For instance, the elasticity analysis might not need its own paragraph – if the main point of the elasticity analysis is that juvenile survival is the most important trait, include that information in the paragraph about juvenile survival.

We have rewritten the Discussion (lines 251-356) to address these very useful comments. Thank you.

26. Line 320: "rate trajectories were negative" is not a very biological or intuitive way to describe this. Something like, "at low resource supply, the daily mortality rate started low and then increased over time, while at high resource supply the daily mortality rate started high and then decreased to very low levels." would be much easier for readers to understand. I also think this point would be even easier to get if you add two more panels for Figure 1 that show the daily mortality rates for each temperature x resource treatment calculated from the a and b coefficients.

We have added the suggested additional plot to this panel (Fig. 2e) and used more intuitive language to describe this effect in the results (lines 204-205).

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Appendix B

Responses to referees

Editor and Reviewers' comments are in **blue**, our responses in **black**.

Associate Editor's comments

1. While the revised manuscript presents a much stronger case for the novelty and relevance of the study beyond Aedes biology, both reviewers give good suggestions on how to improve this further - considering other potential consequences for disease transmission (Reviewer 1, point 4) or strengthening the point about the novelty of study in advancing fundamental ecology (Reviewer 2). As these points are not contradictory, I can see them both being incorporated without the manuscript becoming too long.

We have added further information on the potential importance of considering the effects of temperature × resource interactions on other component of VBD systems to address **Reviewer 3's** suggestions (lines 461-467 and 472-483). We have added to the Discussion (lines 375-380 and 516-519) to address **Reviewer 2's** suggestions for strengthening the novelty of our study and have further highlighted its broader implications for ecology.

2. **Related to these points, I would also request that the authors clarify how their work fits with other mosquito studies. At l.80-1, they state that 'studies that have considered temperature have not examined how the effects of temperature and resource supply together affect fitness through traits [36,37]'. But, from a quick read of ref. 36, the study does consider the interactive effects of temperature and diet (albeit also with density - thus a 4 x 4 x 4 design!) on development time and survival**. This would seem also a relevant paper to cite in the discussion: how do the results compare? Moreover, they could mention related studies in Anopholes on temperature and food supply in the larval environment on adult body size and survival (Barreaux et al. Par & Vec 2018, 11:485), and vector competence (Barreaux et al. MWJ 2016, 7:8). (My apologies for not noticing these had not been cited in the previous version)

We have addressed the first part of this comment (**bold text**) on lines 161-163 by clarifying that [38, 39] did not investigate how the effects of temperature × resource supply on individual fitness traits can impact r_{max}. We have also now cited [38] in the Discussion for comparison of our low-resource temperature-dependent development rate. We have also cited the Barreaux et al. papers on line 464.

3. The reviewers also give a number of specific suggestions for improving clarity of writing. I agree with these all, and add a couple of small suggestions:
l.23-25: I found the phrase '_underestimate_ how resource supply _modulates_ the _temperature-dependency_' hard to follow (there are multiple relationships suggested here). I also wonder if the point is not so much whether it is underestimated, but more that the temperature-dependency of fitness is biased, as only traits measured under high/optimal resource supply are used in the projections.

We have rewritten this sentence as suggested to improve its clarity of meaning (lines 69-71).

4. l.40 'Biological rates ... such as mortality .. 'increases to an optimum before declining to zero' Be careful in wording here, do traits like metabolic rate, mortality rate actually approach zero at higher temperatures? And is it the case that mortality rate increases and declines, and not survival?

We have adjusted this sentence to improve its accuracy (lines 116-117).

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Reviewer 3's Comments

5. **Juvenile survival results and Figure 1**

> When looking at the survival curves in Fig. 1a-b (and the corresponding estimates of the parameters in the survival curves in panels c and d) I'm wondering if there is an issue with dealing with competing rates. For example, in panel b where there were high resources, each of the three survival curves are almost completely flat by day 20, suggesting that juveniles will have extremely low mortality as time goes on. But isn't this actually because all of the juveniles have already emerged? Based on the development rate data shown in Figure 2, I'd think most individuals in the high resource treatment have already emerged by early on in the experiment, and mortality rate after the time when all individuals had either emerged or already died wouldn't be important.
>
> Generally, it seems like juveniles could have three possible outcomes in this experiment: they stay in the tub for the duration of the experiment, they die, or they emerge. I'm wondering if an alternative figure would be more useful, where it depicts the fate of individuals over time, including death or emergence, in each of the six treatments. And does accounting for this affect the mortality rate estimates?

We have addressed these points by adjusting the survival curves in Fig. 1a and b so that they now match predicted development times. We have also included the predicted survival proportions in the caption, which addresses Comment 16 (**Reviewer 2**). While we appreciate **Reviewer 3's** suggestion for an alternative plot, we feel that the adjustments we have made are sufficient. We also feel it is important to point out that we carried out the survival analyses for inference and to show how resource limitation can significantly affect the temperature-dependence of juvenile survival. It is true that the mortality rate estimates would indeed change if they were predicted using development time estimates. However, this would not change our r_{max} estimates because we used the Kaplan-Meier survival estimates to populate the matrix projections models (lines 233-234).

6. **Adult longevity and its effects on population growth rate.**

> Using their elasticity analysis, the authors found very minimal effects of adult survival and adult fecundity on population growth rate (r-max) (lines 239-250). However, I'm wondering if this is in part due to the fact that adults were starved, and therefore had shorter lifespans than may be observed in nature. For example, based on Fig. 2b, in this experiment the authors found mean adult lifespan was approximately 7 days and 11 days at 22C at low and high juvenile resources, respectively, and 2 days and 3 days at 32 degrees. In comparison, Aedes aegypti adult lifespan shown in Mordecai et al. 2017 (PLOS Neg. Trop. Diseases) looks to be approximately 26 days at 22C, and around 20 days at 32C. If adult lifespan is reduced here due to starvation, does this bias the elasticity analysis towards down-weighting lifespan's (and possibly fecundity's) effect on r-max, since a 10% perturbation around 3 days will be different than a 10% perturbation around 20 days?

Our fitness estimates would not have qualitatively changed if we had used non-starved adults rather than starved adults. This is because elasticities quantify the relative change in fitness resulting from a relative change in underlying traits. This means that the relative contributions would be maintained even if non-starved adult lifespans had been used instead. Also, our finding is consistent with fitness studies on disease vectors and other holometabolous insects in showing that fitness is more sensitive to changes in juvenile traits than adult traits. This effect derives from how development time determines the rate of adult recruitment and the onset of reproduction, whereas survival determines the number of reproducing adults. We now have incorporated

additional references [61-63] into the Discussion (lines 399-400) to add further support our finding.

7. **Population growth rate at high resources increasing monotonically with temperature**

The authors found that under high resources, population growth rate peaked at 32C (Fig. 3) and that it "increased monotonically with temperature" (line 237). On lines 259-260 in the first paragraph of the discussion, they concluded that "this lack of unimodality indicates that the temperature at which fitness peaks is higher than 32C when resources are abundant."

Unless the authors have additional information, I think this statement in the discussion should be removed. The experiment took place at 22C, 26C, and 32C. In my opinion, this six-degree gap between 26C and 32C leaves a very good chance that population growth rate actually peaks somewhere between 26C and 32C and then decreases again, but that it was not captured in the experiment. I apologize for not having a reference handy for this, but I believe a max growth rate for this species somewhere between 26C and 32C would be more in line with previous findings than a finding that max growth rate is above 32C. Either way, these alternative scenarios could be discussed in the Discussion section. I would also suggest removing the trend lines between points in Fig. 3, as it could give a potentially false sense to the reader that this is the pattern r-max will follow across temperature, even though it may actually be unimodal between points.

We have removed the sentence from line 375: "this lack of unimodality indicates that the temperature at which fitness peaks is higher than 32C when resources are abundant." We have also removed the trend lines in Fig. 3.

8. **Implications for vector-borne disease and how we should model it**

I liked the discussion, but I think a bit more could be added to strengthen it. For example, I like the idea of incorporating resource supply into mosquito projection models or vector-borne disease models. **However, I was hoping for a bit more discussion for how this could potentially be done**. If temperature is broadly predictable across time and space but resources aren't, how do we incorporate that?

Also, since the abstract and introduction introduce the idea of projecting disease transmission as one of the important reasons for understanding interactions between temperature and resources on mosquitos, I think some more could be said about possible effects of temperature and resources on other disease-relevant traits. Should we expect juvenile resource level to affect adult biting rate, a key trait for disease transmission rate? Should we expect it to affect vector competence? I realize the author's results don't explicitly speak to this, but I think some additional discussion about possibilities would be interesting, and would also highlight that changes in mosquito population growth rate won't necessarily correspond with the same changes in mosquito-borne disease transmission.

We have added further information on the importance of considering temperature × resource interactions on other component of VBD systems to address **Reviewer 3's** suggestions (lines 461-467. On lines 472-484, we suggest potential ways for measuring effective fitness in the field and underline the importance of linking fitness to vector abundance dynamics and VBD transmission dynamics.

Minor comments

9. Abstract: suggest clarifying that resource levels are manipulated for juvenile but not adult mosquitos.

We have addressed this on lines 60 and 65 by inserting the words “juvenile” and “in the juvenile stages”, respectively.

10. Line 278: I think “optima” should be “optimal” here.

We have corrected this on line 403.

11. Lines 284-285: Can the authors clarify in this sentence that this result is for the high resource supply treatment?

We have inserted “high-resource supply into line 408.

12. Figure 1. Could the authors use different colours in panels a and b to represent the different temperature treatments? It’s hard to tell which confidence intervals are which.

We have addressed this by using colours to represent the temperature treatments in Fig. 1a and b.

13. Figure 2. Is there a strong reason for including both boxplots and GLM estimates here? If not, perhaps one or the other can be used to make the plots cleaner.

We have removed GLM estimates from Fig. 2.

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Reviewer 2

14. The novelty of the study and relevance to other systems:

To my knowledge, temperature and resource availability are rarely studied together for their interactive effects on population growth rates, especially in animals. Although I acknowledge that perhaps the other reviewer knows of papers that have factorially crossed these factors and quantitatively projected their effects of population growth rate in animals, the only three studies that I know of to have done this are in single-celled plankton. **That said, I do think that the authors should add a sentence or two acknowledging this literature in the Discussion and argue that their results are relevant to the ecological literature beyond the more urgent applications to mosquitoes and vector-borne disease. The authors already cite reference 20, which found similar results (lower Topt under resource stress) in phytoplankton**. This study using bacterial plankton communities in lakes had more mixed results that sometimes support that finding: https://www.int-res.com/articles/ame2007/49/a049p035.pdf. This other study, also in bacterial plankton, did not estimate Topt, but also found that temperature had a stronger effect on growth in resource limited environments: https://pubmed.ncbi.nlm.nih.gov/24185633/. The authors could read through the citations of those papers, and use Google Scholar to look at studies that cite them in order to look for other relevant examples. I also note a few places in the line by line comments below where you can generalize the argument.

Thank you, this was a very useful suggestion. We have now referenced some well-cited papers from the searches suggested here to broaden the relevance of our study. We also added lines 375-380 and 516-519.

15. Additional data to report in the supplement

When reading the new paragraph on comparing your trait estimates to prior studies used to parameterize R0 models (lines 281-290), I had a fleeting thought that this paragraph might be better done with larval mortality, since that was the most important trait in the model. But I quickly realized that most studies report larval mortality as a percentage reaching maturity rather than a daily mortality rate, so a direct comparison would be difficult. The daily mortality rate is arguably a better way to measure and report the data, but it would still be nice to be able to make a direct comparison with other studies. Therefore, I think it would advisable (and should be easy) to also report the percentage reaching maturity in the supplement.

This is another excellent suggestion. We have now added the survival proportions from each treatment to caption for Fig 1. On lines 382-387, we have also clarified that fitness was lower at low-resource supply due to the combined negative effect that it had on juvenile mortality (less reproducing adults) and development (lower rate of adult recruitment and a delayed onset of reproduction). We have also added a panel to the sensitivity analysis (Fig. S3) to show the combined relative contributions of juvenile and adult traits.

Line by line comments:

16. Line 21: I would remove the word "exaggerated" – I found it confusing rather than helpful.

We have removed this word from line 68.

17. Line 37: I would add "interact to" before "affect the abundance"

We have added these words to line 113.

18. Line 38-9: I would delete "interest is growing rapidly in the effects of" (this is a weak argument for importance) and add "can have large effects" before "on their population fitness"

We have made these changes on lines 115-116.

19. Line 42: I would delete "Therefore" – it doesn't match the logical flow

We have made this change on line 119.

20. Line 44: "leading to interesting new insights" is also a weak argument in this vague form. I would delete "interesting" and add something like, "including XYZ" to give an example of one or more of these insights.

We have made these changes on lines 121-122.

21. Line 46: I would replace "Yet" with "While"

We have made this change on line 124.

22. Line 52: "this" -> "this prediction" (in general, you want to avoiding hanging this-es without nouns)

We have made this change on line 130.

23. Line 56: I would delete "Ultimately" (it doesn't match the logical flow) and change "this" -> "This resource limitation"

We have made this change on line 134.

24. Line 64: I would delete “Additionally” for the start of a new paragraph

We have made this change on line 144.

25. Line 67: I would delete “However” and change “too” -> “also”

We have made this change on line 147.

26. Line 71: I would replace “may together” with “interact to”

We have made this change on line 152.

27. Line 72: “through” -> “together through effects on”

We have made this change on lines 161-163.

28. Line 84: “disease vectors” could be “disease vectors and other arthropods” to generalize the scope

We have made this change on line 165.

29. Line 89: The text refers to “this replication level” but the replication level is not given until the following paragraph, so I would move this sentence to after that is given or change the text to say “the replication level we chose” or something similar.

We have made this change on line 193.

30. Line 180-1: A couple points here. 1) I would say “The trait data distributions for development time, lifespan, and wing length” for increased clarity (the juvenile mortality rate is also trait data, but not included here) and my personal preference for Oxford commas. 2) The phrasing “the trait data… were nonlinear, positive, and right-skewed” does not make sense. In my interpretation, positive and right-skewed refer to the data distributions for each treatment plotted as a histogram or density plot. However, “nonlinear” only applies in the context of “as a function of temperature,” no? So it doesn’t match the other descriptions or make sense as a description in this context.

We have simplified this to improve its accuracy and clarity (lines 283-287).

31. Line 192: Table S3 shows that the authors did the model selection correctly, but just worded it in a confusing/wrong way here. Negatives (“did not improve”) are confusing generally, and this text actually says the opposite of what the authors did. I would say: “If removing a term worsened model fit (dAIC > -2), then it was retained. Otherwise it was removed.”

We have made these changes on lines 293-296.

32. Line 229-31: I would switch the order here to match the paragraphs preceding it (larger decrease, followed by smaller decrease)

We have made these changes on lines 338-339.

33. Line 260: The end of this first paragraph could be a place to put a sentence about the broader scope of results beyond mosquitoes.

We have added lines 375-380 to the Discussion to broaden the scope of our results.

34. Line 271-76: This paragraph is long series of hanging nounless this-es. I would change the sentences to start: “This is” -> “This pattern occurs” (271); “This is” -> “These effects” (273); “This is” -> “This result” (275); “This is a key finding” -> “This finding is key” (276)

We have made these changes on lines 394-400.

35. Line 298: “this” -> “this hypothesis”

We have made this change on line 433.

36. Line 300: If you change “natural mosquito populations” to “natural populations of mosquitoes and other arthropods” this could be another place to expand the scope.

We have made this change on lines 435-436.

37. Line 303: evaporation of breeding habitat is another threat that becomes worse over time and could be added here

We have made this change on lines 438-439.

38. Line 307: The end of this paragraph is another place to potentially expand the scope. You could point out that the outcome of climate change + resource limitation interactions on populations means different things for vectors and agricultural pests (higher pops = bad) than it does for species of conservation concern (higher pops = good). (Obviously with more nuance.)

We have made this change on lines 444-445.

39. Line 309: “this is” -> “this approach is appropriate”

We have made this change on lines 448.

40. Line 316: delete comma, delete period, delete “This is”

We have made this change on lines 454-456.

41. Line 319: delete “may have”

We have made this change on line 459.

42. Line 328, 333: “we have not considered” reads better to me as “we did not consider”; same for “we have also not addressed” and “we also did not address”

We have made these changes on lines 486 and 491.

43. Line 330: “This is” -> “This relationship occurs”

We have made this change on line 488.

44. Line 342: delete both commas, replace “resource fluctuations” with “resource availability”

We have made this change on line 502.

45. Line 356: End of final paragraph is another place to potentially expand scope and cite the plankton papers.

We have added lines 517-520 to the Discussion to broaden the scope of our results.