THE ROYAL SOCIETY PUBLISHING

PROCEEDINGS B

Experimental evidence of warming-induced disease emergence and its prediction by a trait-based mechanistic model

Devin Kirk, Pepijn Luijckx, Natalie Jones, Leila Krichel, Clara Pencer, Péter Molnár and Martin Krkošek

Article citation details
Proc. R. Soc. B **287**: 20201526.
http://dx.doi.org/10.1098/rspb.2020.1526

Review timeline

Original submission:	27 June 2020
Revised submission:	26 August 2020
Final acceptance:	16 September 2020

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

Review History

RSPB-2020-1526.R0 (Original submission)

Review form: Reviewer 1 (Alexander Strauss)

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?
Excellent

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

Is the length of the paper justified?
Yes

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

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

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

Is it accessible?
No

Is it clear?
N/A

Is it adequate?
N/A

Do you have any ethical concerns with this paper?
No

Comments to the Author
Kirk et al. experimentally show that gradual warming (from 10-13.5C) can lead to disease emergence in a Daphnia-microsporidian study system, and that this transition to an epidemic state was anticipated by a mechanistic trait-dependent model with temperature-dependent functions parameterized according to metabolic ecology theory. There is much to admire in this manuscript. The coupling of model and experiment is ambitious and compelling, the experiment is well-designed, and the results are novel as far as I can tell. The integration of metabolic theory into disease ecology is of general interest. The manuscript is very well written, and results are beautifully presented. With a few clarifications, I am confident that this paper will make a very nice contribution to the literature.
I identified three 'major' areas that would, in my mind, improve the manuscript, as well as several other minor suggestions.

Major:
1: Insufficient details for readers to understand the MTE foundations of the model.
Given that a major goal of this paper is to argue the benefits of MTE-temperature mechanistic disease models, I found the description of the models underwhelming. I don't see equations for the temperature-dependent functions in the main text or appendix (sorry if I missed them), nor information on how these functions were parameterized (which would strengthen the appendix, in my opinion). I understand that you "used these previously published MTE functions to represent the parameters in Eq. 1-4 that were temperature-dependent (Table 1, Fig. 1) [178]." However, a little more information should be presented so readers of this paper can see the equations that you used and understand more fully what you did. Otherwise, after an intro that explains why MTE functions are superior to phenomenological functions, all we see are the shapes in Fig. 1, which feels very phenomenological. Other than a verbal description, readers don't know how you got these shapes. I know that Proc B has stingy word limits, but I urge the others to find a way to include a little more information about the temperature-dependent functions in the model.

2: Underestimating host densities in the experiment
I am concerned that by visually estimating the number of large adults in the experiment (i.e., ignoring juveniles, and not conducting a thorough census), the authors are underestimating host density. Juveniles could vastly outnumber adults. This is not necessarily a major problem, but should be more clearly acknowledged, especially since host density is an important component of R0 (Fig. 2), and the introduction emphasizes the lack of population-level tests of MTE

temperature models for disease. Related, I would like to know a bit more about the potential role of juveniles for transmission. The authors write "it is difficult to detect O. colligate infections in juvenile D. magna" [221]. Is this a methodological issue, or are juveniles less likely to be infected? Any citations to explain more here? At the very least, all figures should be labeled to clarify that 'density' is actually 'density of large adults,' and that population level prevalence is probably much lower if you were to include juveniles. Perhaps one solution would be to state more explicitly that the model is only aimed at tracking large adults, rather than the entire host population. These limitations could also be brought up in the discussion.

3: Slightly overselling novelty
The introduction seems to slightly oversell the novelty of this modeling framework relative to some other relevant work. Do you consider Arrhenius function to be phenomenological? E.g., see the Shocket et al. papers that you cite; a similar Arrhenius form is also used in Hall et al. 2006 Ecology. I suspect that Arrhenius function is part of your MTE temperature functions, but not clear because I don't see your equations anywhere (see major 1). Contributions of this previous work could be clearer, as a counterexample to your claim that "equations used to relate trait performance to temperature are almost always described by data-intensive phenomenological functions." Also, depending on how you view the Arrhenius function, these papers seem like counterexamples to your claim that "most studies using MTE to predict infectious disease dynamics have focused on individual hosts rather than disease at the population level."

Minor:
Abstract:
43: Here and maybe elsewhere: Consider writing "gradual temporal warming," or something similar, as "gradual warming" could also imply a fine experimental gradient
49: Note that you don't actually show any of the "MTE sub-functions" in this paper with math… (see major 2)
Intro:
83: See major 3. I would expect a statement like this to end in "but see [citations]."
90: little data are available
98: Another place where 'but see' citations seem relevant.
105: "low disease state" seems vague… I think you mean $R0 < 1$
110: "The efficacy of trait-based models that use thermal performance curves arising from MTE sub-functions remains unknown" I'm not really sure what you mean here – How does point 3 differ from point 2 from earlier in the paragraph? The current study seems rather specific to be able to conclude a general 'efficacy' of this approach. The major strength of this paper (which is very cool!) seems to be demonstration that gradual warming can push disease into an epidemic state, and that the MTE trait mechanistic model explained the transition.
Methods:
137: [16,18] are references?
200: Can you state earlier in the methods that hosts were added to the experiment every three days? This was confusing on my first reading, until further on.
148: The harvesting term in the model is a bit confusing. It could be simplified by cancelling the (S+I) terms. It seems quite strange that harvesting rate is a constant per-capita processes, when in reality you removed the same number (not proportion) of hosts each sampling period. I understand that population density was more or less stable (but see major 2 above), but it still would make more sense to me to model 'harvest' the same way you model the additions to the experiment (phi_S). It might be clearer for readers to group phi_S and h together in the equations so that it looks like immigration and emigration.
164: Is S_eq defined? Sorry if I missed it.
184: Parasite intensity at death – is that w? Labeling could be a little more consistent.
Results:
270: Statistical analysis of increase in infection prevalence? I.e., would expect a significant interaction term in glm: Prevalence ~ day * treatment, link=logit
282: I'm a bit surprised to see a Mann-Whitney test reported… Why not a logistic regression (i.e., glm with logistic link?). It's not clear why you need a non-parametric test, since prevalence ought

to follow a binomial distribution. Sorry if I'm missing something obvious.
Discussion:
288: Didn't actually show the MTE functional forms, and details of the experiments to fit the forms are also lacking (major 1)
314: "employing the MTE" … what do you mean by that? Fitting temperature-dependent functions to biological rates and processes?
324: How do these suggested averages compare to the parameterized functions you used? Are they at all close? Without seeing your equations (major 1), it's hard to place this paragraph into context. I am by no means 'requiring' an additional analysis (I don't think it would fit in this paper), but this paragraph leads me to wonder how well a a-priori parameterization with 'suggested averages' would match your experiment.
359: Lack of information about juveniles seems important to mention here (major 2)
363: Perhaps it could be useful to estimate the degrees of temperature change per generation of host. Some species of long lived hosts may be experiencing rates of temperature change that are similar to your experiment.
398: Or larger gradients; And I suspect that density of these Daphnia populations would have responded to a larger (warmer) temperature gradient. Worth noting that the temperatures in the experiment were all quite cold.
Figures & Tables:
Overall, I really like the presentation. Figs 2-4 are very nicely designed and informative.
Table 1
Units on temperature-dependent parameters?
How did you estimate maximum per-capita recruitment? Not clear, and seems really high (1.33 day-1); I've usually measured little r around 0.2 – 0.4 for cladocerans.
Fig. 1
Can you highlight on this figure the range of temperatures considered in the current experiment?
Appendix:
43: Citation or figure for the degredation rate?
106: Where does size appear in the model? Is this an assumption about the data you use to parameterize the model? Another place where links to MTE could be clearer.
Table S1: This table would be clearer if you should us the range of model parameters. The quantities here seem to be observations that lead to model parameterization, not the parameters themselves, so it is difficult to gauge how they affect the model.

I hope that you find these comments and suggestions helpful.
Signed,
Alex Strauss

Review form: Reviewer 2

Recommendation
Accept as is

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

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

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

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
Kirk et al. developed a framework for predicting climate-induced disease emergence by combining models from the metabolic theory of ecology (MTE) with classical epidemiological models and tested whether the models can predict disease emergence in an experimentally warmed system of Daphnia and a microsporidian parasite. They also analyzed experimental and model-simulated data for ten early warning signals (EWS) of critical transitions of the disease-emergence bifurcation. I commend the authors for asking these questions. They are extremely important and, in my opinion, reach the Ecology Letters bar for scope and import of the question.

I reviewed this manuscript twice previously for other high profile journals. I very much liked the previous version and thought it was publishable then. The good thing is that the authors have very adequately addressed all previous concerns I raised. I do not have much to add. This is a fine piece of work integrating climate and seasonal change, a disease experiment, and MTE and SIR modeling. It is an extremely nice and important advance to the field. I support publishing this work and commend the authors for designing and writing a very interesting manuscript.

In particular, I appreciate the addition of the third and fourth paragraphs in the Discussion. In the third paragraph, the authors highlight that MTE was originally intended to offer a short cut, preventing having to attain all of the thermal performance curves for every trait important to transmission. Their study doesn't quite get us there but offers an important experimental proof of principle that MTE functions, when parameterized, can reasonably predict warming-induced disease dynamics. In the fourth paragraph, they provide guidance on how the discipline can move forward to fulfill MTE being this general short cut for predicting climate-dependent disease dynamics.

Minor comment
The Vasseur et al. paper is cited in the literature cited but not in the main text.

Decision letter (RSPB-2020-1526.R0)

22-Jul-2020

Dear Dr Kirk:

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 have raised some issues with your manuscript and we would like to invite you to revise your manuscript to address them.

In addition to the reviewers' comments, I also like the manuscript but I think you should provide additional explanation to justify the expression for R0. As the person who inventented the next-generation method, I'm always interested in how authors derive their R0. You provide no details but mention that it was derived as the dominant eigenvalue of the next-generation matrix. This seems odd because it is not immediately clear how this should be done in this system with pure environmental transmission. I can imagine doing this heuristically because R0 is basically a one-dimensional problem in cases where there is one state-at-infection, as in this case (see Diekmann, Heesterbeek & Roberts, J. R. Soc. Interface, 2010 for examples), and hence no matrix (or a 1x1-matrix if you wish). As I understand the model, each infected individual contributes spores to the environmental pool in two ways: 1) by direct shedding at rate lambda for an average of 1/(mu+alpha+h) time units, 2) by degrading completely after death, when in total omega spores as added to the pool. Spores stay viable for an average 1/gamma time units. Susceptible individuals come into contact with spores at a rate chi and per contact there is a probability sigma that the susceptible actually becomes infected. The first issue I have with the formula is that not every infected individual contributes to the pool after death. Only a fraction (mu+alpha)/(mu+alpha+h) die (1 minus this fraction leaves the system by harvesting). If h would be much larger than mu+alpha almost no infected would die and be able to shed omega spores upon degradation. In your formula, however, the shedding via the dead individuals would remain unchanged. You are correct that theta (decay rate) does not enter R0, as all dead individuals will decay eventually and this therefore only influences the speed of spore production, but not the total amount that enters R0. The second issue is with the idea of contacts. It is tempting to treat these as mass action. There is a difference, however, compared to direct interaction between S and I with some transmission rate beta. The infected in the interaction can meet and infect other susceptibles as long as it is infectious. A spore can only infect once. These issues, together with the fact that this is a one-dimensional R0 problem (and hence not a matrix problem) make me eager to see how you arrived at the expression in the manuscript. Please feel free to contact me directly if you want to discuss (j.a.p.heesterbeek@uu.nl).

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. Please see our Data Sharing Policies (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,
Professor Hans Heesterbeek
mailto: proceedingsb@royalsociety.org

Associate Editor
Board Member: 1
Comments to Author:
The authors should address the concerns of Reviewer 1. It is expected this will be straightforward.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)
Kirk et al. experimentally show that gradual warming (from 10-13.5C) can lead to disease emergence in a Daphnia-microsporidian study system, and that this transition to an epidemic state was anticipated by a mechanistic trait-dependent model with temperature-dependent functions parameterized according to metabolic ecology theory. There is much to admire in this manuscript. The coupling of model and experiment is ambitious and compelling, the experiment is well-designed, and the results are novel as far as I can tell. The integration of metabolic theory into disease ecology is of general interest. The manuscript is very well written, and results are beautifully presented. With a few clarifications, I am confident that this paper will make a very nice contribution to the literature.
I identified three 'major' areas that would, in my mind, improve the manuscript, as well as several other minor suggestions.

Major:
1: Insufficient details for readers to understand the MTE foundations of the model.
Given that a major goal of this paper is to argue the benefits of MTE-temperature mechanistic disease models, I found the description of the models underwhelming. I don't see equations for the temperature-dependent functions in the main text or appendix (sorry if I missed them), nor information on how these functions were parameterized (which would strengthen the appendix, in my opinion). I understand that you "used these previously published MTE functions to represent the parameters in Eq. 1-4 that were temperature-dependent (Table 1, Fig. 1) [178]." However, a little more information should be presented so readers of this paper can see the equations that you used and understand more fully what you did. Otherwise, after an intro that explains why MTE functions are superior to phenomenological functions, all we see are the shapes in Fig. 1, which feels very phenomenological. Other than a verbal description, readers don't know how you got these shapes. I know that Proc B has stingy word limits, but I urge the others to find a way to include a little more information about the temperature-dependent functions in the model.

2: Underestimating host densities in the experiment
I am concerned that by visually estimating the number of large adults in the experiment (i.e., ignoring juveniles, and not conducting a thorough census), the authors are underestimating host

density. Juveniles could vastly outnumber adults. This is not necessarily a major problem, but should be more clearly acknowledged, especially since host density is an important component of R0 (Fig. 2), and the introduction emphasizes the lack of population-level tests of MTE temperature models for disease. Related, I would like to know a bit more about the potential role of juveniles for transmission. The authors write "it is difficult to detect O. colligate infections in juvenile D. magna" [221]. Is this a methodological issue, or are juveniles less likely to be infected? Any citations to explain more here? At the very least, all figures should be labeled to clarify that 'density' is actually 'density of large adults,' and that population level prevalence is probably much lower if you were to include juveniles. Perhaps one solution would be to state more explicitly that the model is only aimed at tracking large adults, rather than the entire host population. These limitations could also be brought up in the discussion.

3: Slightly overselling novelty
The introduction seems to slightly oversell the novelty of this modeling framework relative to some other relevant work. Do you consider Arrhenius function to be phenomenological? E.g., see the Shocket et al. papers that you cite; a similar Arrhenius form is also used in Hall et al. 2006 Ecology. I suspect that Arrhenius function is part of your MTE temperature functions, but not clear because I don't see your equations anywhere (see major 1). Contributions of this previous work could be clearer, as a counterexample to your claim that "equations used to relate trait performance to temperature are almost always described by data-intensive phenomenological functions." Also, depending on how you view the Arrhenius function, these papers seem like counterexamples to your claim that "most studies using MTE to predict infectious disease dynamics have focused on individual hosts rather than disease at the population level."

Minor:
Abstract:
43: Here and maybe elsewhere: Consider writing "gradual temporal warming," or something similar, as "gradual warming" could also imply a fine experimental gradient
49: Note that you don't actually show any of the "MTE sub-functions" in this paper with math… (see major 2)
Intro:
83: See major 3. I would expect a statement like this to end in "but see [citations]."
90: little data are available
98: Another place where 'but see' citations seem relevant.
105: "low disease state" seems vague… I think you mean $R0 < 1$
110: "The efficacy of trait-based models that use thermal performance curves arising from MTE sub-functions remains unknown" I'm not really sure what you mean here – How does point 3 differ from point 2 from earlier in the paragraph? The current study seems rather specific to be able to conclude a general 'efficacy' of this approach. The major strength of this paper (which is very cool!) seems to be demonstration that gradual warming can push disease into an epidemic state, and that the MTE trait mechanistic model explained the transition.
Methods:
137: [16,18] are references?
200: Can you state earlier in the methods that hosts were added to the experiment every three days? This was confusing on my first reading, until further on.
148: The harvesting term in the model is a bit confusing. It could be simplified by cancelling the (S+I) terms. It seems quite strange that harvesting rate is a constant per-capita processes, when in reality you removed the same number (not proportion) of hosts each sampling period. I understand that population density was more or less stable (but see major 2 above), but it still would make more sense to me to model 'harvest' the same way you model the additions to the experiment (phi_S). It might be clearer for readers to group phi_S and h together in the equations so that it looks like immigration and emigration.
164: Is S_eq defined? Sorry if I missed it.
184: Parasite intensity at death – is that w? Labeling could be a little more consistent.

Results:
270: Statistical analysis of increase in infection prevalence? I.e., would expect a significant interaction term in glm: Prevalence ~ day * treatment, link=logit
282: I'm a bit surprised to see a Mann-Whitney test reported… Why not a logistic regression (i.e., glm with logistic link?). It's not clear why you need a non-parametric test, since prevalence ought to follow a binomial distribution. Sorry if I'm missing something obvious.
Discussion:
288: Didn't actually show the MTE functional forms, and details of the experiments to fit the forms are also lacking (major 1)
314: "employing the MTE" … what do you mean by that? Fitting temperature-dependent functions to biological rates and processes?
324: How do these suggested averages compare to the parameterized functions you used? Are they at all close? Without seeing your equations (major 1), it's hard to place this paragraph into context. I am by no means 'requiring' an additional analysis (I don't think it would fit in this paper), but this paragraph leads me to wonder how well a a-priori parameterization with 'suggested averages' would match your experiment.
359: Lack of information about juveniles seems important to mention here (major 2)
363: Perhaps it could be useful to estimate the degrees of temperature change per generation of host. Some species of long lived hosts may be experiencing rates of temperature change that are similar to your experiment.
398: Or larger gradients; And I suspect that density of these Daphnia populations would have responded to a larger (warmer) temperature gradient. Worth noting that the temperatures in the experiment were all quite cold.
Figures & Tables:
Overall, I really like the presentation. Figs 2-4 are very nicely designed and informative.
Table 1
Units on temperature-dependent parameters?
How did you estimate maximum per-capita recruitment? Not clear, and seems really high (1.33 day-1); I've usually measured little r around 0.2 – 0.4 for cladocerans.
Fig. 1
Can you highlight on this figure the range of temperatures considered in the current experiment?
Appendix:
43: Citation or figure for the degredation rate?
106: Where does size appear in the model? Is this an assumption about the data you use to parameterize the model? Another place where links to MTE could be clearer.
Table S1: This table would be clearer if you should us the range of model parameters. The quantities here seem to be observations that lead to model parameterization, not the parameters themselves, so it is difficult to gauge how they affect the model.

I hope that you find these comments and suggestions helpful.
Signed,
Alex Strauss

Referee: 2

Comments to the Author(s)
Kirk et al. developed a framework for predicting climate-induced disease emergence by combining models from the metabolic theory of ecology (MTE) with classical epidemiological models and tested whether the models can predict disease emergence in an experimentally warmed system of Daphnia and a microsporidian parasite. They also analyzed experimental and model-simulated data for ten early warning signals (EWS) of critical transitions of the disease-emergence bifurcation. I commend the authors for asking these questions. They are extremely important and, in my opinion, reach the Ecology Letters bar for scope and import of the question.

I reviewed this manuscript twice previously for other high profile journals. I very much liked the previous version and thought it was publishable then. The good thing is that the authors have

very adequately addressed all previous concerns I raised. I do not have much to add. This is a fine piece of work integrating climate and seasonal change, a disease experiment, and MTE and SIR modeling. It is an extremely nice and important advance to the field. I support publishing this work and commend the authors for designing and writing a very interesting manuscript.

In particular, I appreciate the addition of the third and fourth paragraphs in the Discussion. In the third paragraph, the authors highlight that MTE was originally intended to offer a short cut, preventing having to attain all of the thermal performance curves for every trait important to transmission. Their study doesn't quite get us there but offers an important experimental proof of principle that MTE functions, when parameterized, can reasonably predict warming-induced disease dynamics. In the fourth paragraph, they provide guidance on how the discipline can move forward to fulfill MTE being this general short cut for predicting climate-dependent disease dynamics.

Minor comment
The Vasseur et al. paper is cited in the literature cited but not in the main text.

Author's Response to Decision Letter for (RSPB-2020-1526.R0)

See Appendix A.

Decision letter (RSPB-2020-1526.R1)

16-Sep-2020

Dear Dr Kirk

I am pleased to inform you that your manuscript entitled "Experimental evidence of warming-induced disease emergence and its prediction by a trait-based mechanistic model" 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

Open Access
You are invited to opt for Open Access, making your freely available to all as soon as it is ready for publication under a CCBY licence. Our article processing charge for Open Access is £1700. Corresponding authors from member institutions (http://royalsocietypublishing.org/site/librarians/allmembers.xhtml) receive a 25% discount to these charges. For more information please visit http://royalsocietypublishing.org/open-access.

Your article has been estimated as being 10 pages long. Our Production Office will be able to confirm the exact length at proof stage.

Paper charges
An e-mail request for payment of any related charges will be sent out after proof stage (within approximately 2-6 weeks). The preferred payment method is by credit card; however, other payment options are available

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

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

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

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

Appendix A

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 have raised some issues with your manuscript and we would like to invite you to revise your manuscript to address them.

RESPONSE: Thank you very much for the thorough reviews, they have greatly improved our work. Below, we respond to each comment on a point-by-point basis.

In addition to the reviewers' comments, I also like the manuscript but I think you should provide additional explanation to justify the expression for R0. As the person who inventented the next-generation method, I'm always interested in how authors derive their R0. You provide no details but mention that it was derived as the dominant eigenvalue of the next-generation matrix. This seems odd because it is not immediately clear how this should be done in this system with pure environmental transmission. I can imagine doing this heuristically because R0 is basically a one-dimensional problem in cases where there is one state-at-infection, as in this case (see Diekmann, Heesterbeek & Roberts, J. R. Soc. Interface, 2010 for examples), and hence no matrix (or a 1x1-matrix if you wish). As I understand the model, each infected individual contributes spores to the environmental pool in two ways: 1) by direct shedding at rate lambda for an average of 1/(mu+ alpha+h) time units, 2) by degrading completely after death, when in total omega spores as added to the pool. Spores stay viable for an average 1/gamma time units. Susceptible individuals come into contact with spores at a rate chi and per contact there is a probability sigma that the susceptible actually becomes infected. The first issue I have with the formula is that not every infected individual contributes to the pool after death. Only a fraction (mu+alpha)/(mu+alpha+h) die (1 minus this fraction leaves the system by harvesting). If h would be much larger than mu+alpha almost no infected would die and be able to shed omega spores upon degradation. In your formula, however, the shedding via the dead individuals would remain unchanged. You are correct that theta (decay rate) does not enter R0, as all dead individuals will decay eventually and this therefore only influences the speed of spore production, but not the total amount that enters R0. The second issue is with the idea of contacts. It is tempting to treat these as mass action. There is a difference, however, compared to direct interaction between S and I with some transmission rate beta. The infected in the interaction can meet and infect other susceptibles as long as it is infectious. A spore can only infect once. These issues, together with the fact that this is a one-dimensional R0 problem (and hence not a matrix problem) make me eager to see how you arrived at the expression in the manuscript. Please feel free to contact me directly if you want to discuss (j.a.p.heesterbeek@uu.nl).

RESPONSE: Thank you for your comments on our R_0 formulation. You brought up several good points regarding this formulation, each of which we believe we can simply address. We cover them point-by-point below:

1. **<u>We did not use the next-generation matrix method to formulate R_0</u>**

The sentence labelling our method as using the next-generation method was mistakenly added in response to a reviewer's comment at a previous journal submission. After discussion, we realized that the method we used was not analogous to the next-generation

matrix method to calculate R_0. Instead, we formulated R_0 directly from the model equations, since it is a one-dimensional rather than structured multi-host system as you pointed out in your review. We apologize for our mistake and the resulting confusion on this. Lines 166-167 now read:

> **"The basic reproduction number (R_0) of the parasite is formulated from Eqs. 1-4, and is equal to:"**

2. <u>Not every infected individual will contribute to the spore pool after death</u>

We agree with your comment that not all infected individuals will remain in the population to release spores after death due to harvesting and that we had not accounted for this in our R_0 expression. To amend this, we have added a coefficient to $\omega(T)$ of $\frac{(\mu(T)+\alpha(T))}{(\mu(T)+\alpha(T)+h)}$ which gives the fraction of infected hosts that remain in the system until death, after which they settle to the bottom to decay and are no longer removed through harvesting. Eq. 5 now reads:

$$R_0(T) = \left(\frac{\lambda(T)}{\mu(T)+\alpha(T)+h} + \omega(T) * \frac{(\mu(T)+\alpha(T))}{(\mu(T)+\alpha(T)+h)}\right)\left(\frac{\chi(T) * \sigma(T) * S_{eq}}{\gamma}\right)$$

Adding the coefficient to $\omega(T)$ reduces R_0 slightly, but results in only very minor changes to the results. Our previous expression for R_0 predicted that R_0=1 at 11.71°C, whereas our new corrected expression predicts that R_0=1 at 11.97°C. The effect over the range of our experimental conditions (10°C – 13.5°C) is small because most spores are released by individuals shedding throughout their life and not after death. Since our experimental conditions increased by 0.5°C intervals, we still have the same prediction that R_0>1 once our experiment is warmed to 12°C. Fig. S1 below shows the differences in predictions of R_0 at our experimental host susceptible density in the previous expression (black line) and the new expression (blue line). While the general shape remains the same, there is a noticeable reduction in R_0 at the optimal temperature near 20°C, though this is a temperature range not tested in our experiment. We have adjusted Fig. 2 with these changes, which looks very similar to the previous version but with lower R_0 values around 20°C.

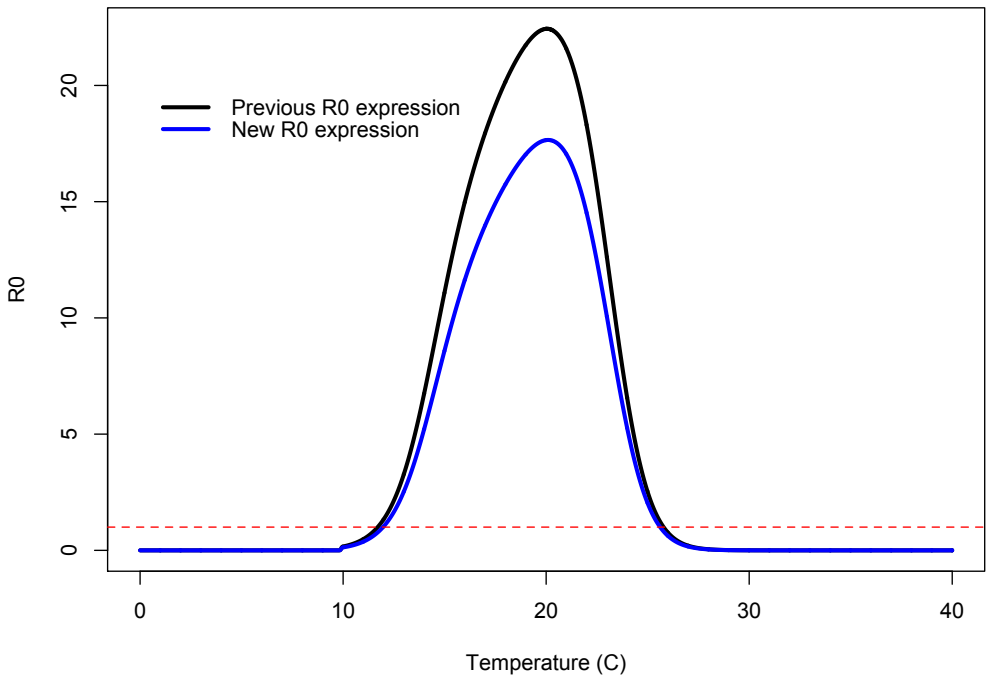

Fig. S1. Comparing R_0 expressions in relation to temperature. The temperature range of our experiment was 10.0°C – 13.5,

3. <u>Mass action and each spore can only infect once</u>

We fully agree that a spore can only infect once, which is different from a direct-transmission system in which an infected individual can continue to contact and infect susceptibles. However, this is not an issue with the R_0 expression, but rather that we did not sufficiently detail the R_0 formulation and that there were unstated assumptions and approximations that we have clarified as explained below.

In the manuscript equation 5 for R_0 and also in the revised R_0 expression in point (2) above, the first term in parentheses represents the total number of spores produced per infected individual, on average. The second term in parentheses represents the probability a spore infects a new host as opposed to being lost via medium removal, but there are unstated assumptions and approximations underlying its formulation. There are two key assumptions that we needed to make clear:

1) **Spores that are ingested but do not infect the host are expelled, re-enter the water column, and remain viable;**
2) **The rate of spore loss from the water column due to ingestion and infection is very small compared to spore loss via media removal; i.e., $\sigma\chi S << \gamma$.**

Regarding assumption 1, to our knowledge, there have been no studies in this host-parasite system that investigate the proportion of spores that remain viable after passing through the host gut. However, evidence from a similar system with this same host and a bacterial parasite, *Daphnia magna-Pasteuria ramosa*, shows that the parasite is not killed if it fails to infect the host (King et al. 2013, *Ecology and Evolution*). Moreover, based on our observations working with this system, we believe that at least a large proportion of spores must remain viable after passing through the host gut. This is because an average sized *Daphnia* filters ~1ml of medium per hour (Kirk et al. 2019), meaning that the dense populations we maintain under lab conditions (~200 hosts/2L at 20C) should filter through all of their medium in their mesocosm every 10 hours. If spores not causing infection were destroyed upon ingestion, this scenario would lead to very low levels of spores in the medium resulting in little or no infection in the population, which is not concordant with the high levels of infection prevalence we regularly observe in our stock populations (47% prevalence, ref: this study). Moreover, we know that new viable spores are created by spreading infection within the anterior of the *Daphnia* gut, and then have to pass through the remainder of the gut before entering the environment, implying that passage through the gut does not kill spores. Finally, microsporidian spores are generally durable, and have been shown to survive months of winter in other *Daphnia*-microsporidian systems (Ebert 2005). We have made this assumption explicit in our revision.

With the assumption that ingested spores that do not cause infection remain viable after passing through the gut, the probability that a spore causes an infection is

$$[\sigma\chi S/(\chi S+\gamma)]*\sum_{i=0}^{\infty}[(1-\sigma)\chi S/(\chi S+\gamma)]^{i}$$

where χ is the filtrate rate, σ is the probability an ingested spore causes infection, S is the abundance of susceptible hosts, and γ is the rate of medium exchange. This equation represents the sum of the probabilities that a spore infects a host upon its first ingestion ($i=1$), or it passes through the gut on its first ingestion and infects a host the second time it is ingested ($i=2$), or it passes through the host gut for the first two ingestions and infects a host on its third ingestion ($i=3$), and so on. Via the formula for the sum for a geometric power series the equation becomes

$$[\sigma\chi S/(\chi S+\gamma)]*[1-(1-\sigma)\chi S/(\chi S+\gamma)]^{-1}$$

which simplifies to

$$\sigma\chi S/(\sigma\chi S+\gamma)$$

This is to say that there are only two ultimate fates for a spore: either it is ingested and infects (at rate $\sigma\chi S$) or it is removed from the system via medium exchange (at rate γ). Additionally, spores could of course also die in the environment, but microsporidian spores are highly durable and we assume their death rate is negligible over the timescale of the experiment as we have noted in the previous version of the manuscript.

Under the approximation that $\sigma\chi S \ll \gamma$, we arrive at the expression for the second parenthesis for R_0: $\sigma\chi S/\gamma$, but as an approximation rather than being exactly correct.

This assumption that $\sigma\chi S \ll \gamma$ is strongly supported for our system, as we know that σ is very small (Kirk et al. 2019). For example, at 12°C, $\sigma\chi S$=0.000018 d^{-1}, while γ is constant across temperature and equals 0.0286 d^{-1}. This means that spore loss from medium exchange (γ) is nearly 1600x larger than spore loss from infection ($\sigma\chi S$), and the assumption that $\sigma\chi S \ll \gamma$ is valid. Because of this, if we look at the temperature range of the experiment (10°C -13.5°C), there is no discernible difference between predictions from our simpler R_0 expression that assumes spore loss only from medium removal (black line; Fig. S2) and a more complicated expression that explicitly accounts for removal of spores from infection (dashed blue line; Fig. S2).

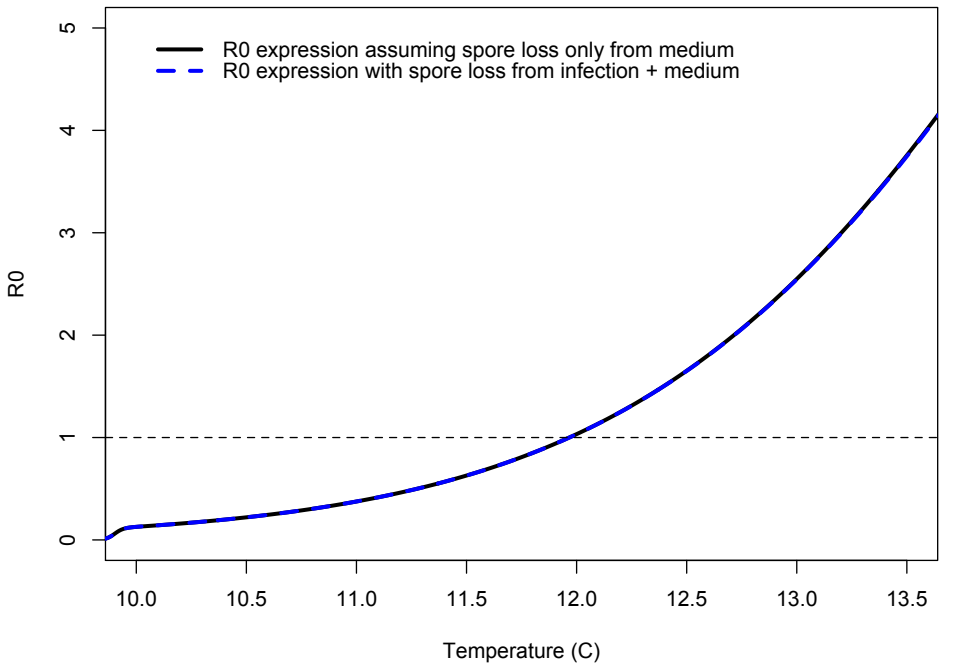

Fig. S2. Comparing R_0 expressions in relation to temperature, with (black line) and without (dashed blue line) the assumption that spore loss from infection is negligible compared to spore loss from medium removal. The temperature range of our experiment was 10.0°C – 13.5.

Lines 176-185 of the main text now read:

> **"Our R_0 formulation makes two key assumptions. First, we assume that spores that are ingested but do not infect the host are expelled, re-enter the water column, and remain viable. While there has not yet been an experimental test of this assumption in this system, evidence from a similar host – parasite system (*Daphnia magna – Pasteuria ramosa)* showed that the parasite was not killed if it failed to infect the host (King et al. 2013) and there are several reasons to believe the same**

holds for our system (Supporting Information). Second, we assume that the rate of spore loss from the water column due to ingestion and subsequent infection is very small compared to spore loss via media removal (i.e., $\sigma\chi S << \gamma$) and can therefore be ignored, which is supported in this system since σ is very small (Kirk et al. 2019). The Supporting Information contains additional details on the R_0 formulation."

We have also added an additional section labelled "R_0 formulation" to the Supporting Information that outlines much of the above response.

Referee: 1

Comments to the Author(s)
Kirk et al. experimentally show that gradual warming (from 10-13.5C) can lead to disease emergence in a Daphnia-microsporidian study system, and that this transition to an epidemic state was anticipated by a mechanistic trait-dependent model with temperature-dependent functions parameterized according to metabolic ecology theory. There is much to admire in this manuscript. The coupling of model and experiment is ambitious and compelling, the experiment is well-designed, and the results are novel as far as I can tell. The integration of metabolic theory into disease ecology is of general interest. The manuscript is very well written, and results are beautifully presented. With a few clarifications, I am confident that this paper will make a very nice contribution to the literature.
I identified three 'major' areas that would, in my mind, improve the manuscript, as well as several other minor suggestions.

RESPONSE: Thank you very much for your review; your suggestions have greatly improved our work.

Major:
1: Insufficient details for readers to understand the MTE foundations of the model.
Given that a major goal of this paper is to argue the benefits of MTE-temperature mechanistic disease models, I found the description of the models underwhelming. I don't see equations for the temperature-dependent functions in the main text or appendix (sorry if I missed them), nor information on how these functions were parameterized (which would strengthen the appendix, in my opinion). I understand that you "used these previously published MTE functions to represent the parameters in Eq. 1-4 that were temperature-dependent (Table 1, Fig. 1) [178]." However, a little more information should be presented so readers of this paper can see the equations that you used and understand more fully what you did. Otherwise, after an intro that explains why MTE functions are superior to phenomenological functions, all we see are the shapes in Fig. 1, which feels very phenomenological. Other than a verbal description, readers don't know how you got these shapes. I know that Proc B has stingy word limits, but I urge the others to find a way to include a little more information about the temperature-dependent functions in the model.

RESPONSE: Thank you, this is a very good point. To address this, we've now added a new equation (Eq. 6) that shows the Sharpe-Schoolfield expression that underlies the MTE

functions, and have added text to the methods that describes the different parameters that underly the Sharpe-Schoolfield model. Lines 186-201 read:

> **"The temperature-dependent parameters of the model were described using thermal relationships arising from the MTE (Table 1). Specifically, we used the Sharpe-Schoolfield equation (Schoolfield et al. 1981; Eq. 6) and its variants (Molnár et al. 2013, Kirk et al. 2018) to capture (a) the Boltzmann-Arrhenius relationship that describes the thermal dependence of a process' rate within intermediate temperature ranges based on the process' activation energy and Boltzmann's constant (Brown et al. 2004), and (b) how reaction rates are altered at high or low temperatures (T) when biological processes are impeded.**
>
> $$x(T) = x_0 \, e^{\frac{-E_x}{k}\left(\frac{1}{T}-\frac{1}{T_0}\right)} \left[1 + e^{\frac{E_{Lx}}{k}\left(\frac{1}{T}-\frac{1}{T_{Lx}}\right)} + e^{\frac{E_{Hx}}{k}\left(-\frac{1}{T}+\frac{1}{T_{Hx}}\right)}\right]^z \qquad \textbf{(Eq. 6)}$$
>
> **The Sharpe-Schoolfield equation can be described by Eq. 6, where x_0 is the rate of a given process x at the reference temperature T_0, E_x is the activation energy, k is Boltzmann's constant (k = 8.62 x 10^{-5} eV K^{-1}), and E_{Lx} and E_{Hx} are the inactivation energies at the low (T_{Lx}) and high (T_{Hx}) temperature thresholds, respectively. All temperatures are recorded in degrees Kelvin. For unimodal curves where x decreases past the temperature thresholds z is equal to -1, whereas z is equal to +1 if x increases past the temperature thresholds (e.g., if x is mortality rate; Molnár et al. 2013, Kirk et al. 2018). Some processes x may only have low or high temperature thresholds rather than both."**

Additionally, while space constraints mean that we do not have room to describe entire experiments and model fitting details of previous studies, we agree that it is important to make clear that Eq. 6 is the general Sharpe-Schoolfield model, but that the different MTE functions shown in Fig. 1 take slightly different forms from each other based on how they were previously estimated in Kirk et al. 2018, 2019. We describe which form each parameter takes, while referring readers to the previous studies for further details on experiments and model fitting. This information is now on lines 202-221:

> **"Previous work has shown that these metabolic models can accurately capture the thermal dependencies of many host and parasite traits in the *Daphnia* – *Ordospora* system at the scale of individual hosts (Kirk et al. 2018, 2019), and we used these previously published MTE functions to represent the parameters in Eq. 1-4 that were temperature-dependent (Table 1, Fig. 1). In other words, the temperature-dependence of our model was parameterized using experiments that were completely independent from the one reported here. While we refer readers to the previous studies for details on parameter experiments and model fitting, briefly: contact rate (χ) was modeled using a Sharpe-Schoolfield function with only an upper-temperature threshold (Fig. 1b; Kirk et al. 2019); within-host infection intensity was modeled using a Sharpe-Schoolfield function with upper and lower temperature thresholds and a negative activation energy, causing infection intensity to decrease across the intermediate temperature range (Fig. 1d; Kirk et al. 2018). Natural mortality rate (μ; Fig. 1a) and probability of infection (σ; Fig. 1c) were each**

composite functions of other terms (e.g., aging rate, infection rate) that were represented by Sharpe-Schoolfield functions (Kirk et al. 2018, 2019; see Supporting Information for more details). Additionally, we note that contact rate (χ) increases with, and the probability of infection (σ) decreases with, *Daphnia* size (Kirk et al. 2019). We therefore assumed that large, female adult *Daphnia* size was constant in both treatments at 2700μm and found that our model predictions were not strongly impacted by this and other parameter assumptions (Fig. S2). The model parameters shedding rate (λ), parasite-induced mortality (α), and parasite intensity at death (ω) were each modeled as proportional to within-host infection intensity."

2: Underestimating host densities in the experiment
I am concerned that by visually estimating the number of large adults in the experiment (i.e., ignoring juveniles, and not conducting a thorough census), the authors are underestimating host density. Juveniles could vastly outnumber adults. This is not necessarily a major problem, but should be more clearly acknowledged, especially since host density is an important component of R0 (Fig. 2), and the introduction emphasizes the lack of population-level tests of MTE temperature models for disease. Related, I would like to know a bit more about the potential role of juveniles for transmission. The authors write "it is difficult to detect O. colligate infections in juvenile D. magna" [221]. Is this a methodological issue, or are juveniles less likely to be infected? Any citations to explain more here? At the very least, all figures should be labeled to clarify that 'density' is actually 'density of large adults,' and that population level prevalence is probably much lower if you were to include juveniles. Perhaps one solution would be to state more explicitly that the model is only aimed at tracking large adults, rather than the entire host population. These limitations could also be brought up in the discussion.

RESPONSE: Thanks, this issue is due to us not clearly communicating that, as you noted at the end of your comment above, our model is aimed at tracking large adults, rather than the entire host population. First, we have edited the manuscript in places we refer to host density to make clear that we are referring to adult host density. We have also changed this in Table 1 and Figure 2. Next, we added a sentence at the start of the *Model* section directly before the Equations are shown (lines 147-150) that reads:

"We note that our model is aimed at tracking disease dynamics across large, female adult *Daphnia* in each population, as we did not inspect juvenile or male *Daphnia* for infections (see Experimental Methods for more detail and justification)."

We have also made clear in the Figure captions that:

"Prevalence and size of population are measured in terms of large, adult female *Daphnia*."

We have added text to the Experimental Methods section on lines 254-267 to better explain our rationale for modeling and experimentally investigating infections in large, female *Daphnia*:

"On day three and subsequently every three days for the duration of the 120-day experiment, we randomly collected twelve large females from each experimental population. In the model and experiment, we focused on large adult females (rather than males or juveniles) for three reasons. First, large adult females should be the primary contributors to the force of infection in the population, as they have significantly higher parasite loads compared to males (Zukowski et al. 2020) and have much higher parasite loads compared to juveniles (i.e. infection has less time to develop in juveniles) (Kirk et al. 2018). Second, *Daphnia* populations are often female biased (as asexual reproduction is predominant reproductive mode), further reducing the role males play in disease transmission. And third, it is methodologically fraught to quantify *O. colligata* abundance in juvenile *D. magna* due to their small body size that renders dissections unreliable and makes it difficult to characterize the typically low parasite abundances in juveniles, resulting in increased false negatives in juveniles. Our measures of prevalence and host density should thus be considered as the prevalence of infection in adult female *Daphnia* and the density of adult female *Daphnia*, respectively."

We have also added text about this to the Discussion paragraph on our model caveats. Lines 425-431 now read:

"Additionally, our model neglects the contribution to transmission from juveniles in the population and, focuses on large, adult female *Daphnia* only, both because the latter are the predominant contributors to the force of infection and because infections in juveniles are hard to detect. If infections were able to be detected reliably in juveniles, a more complicated stage- or age-structured model that explicitly tracks infections across juveniles and adults may provide more information on the overall disease dynamics in the population, but this is unlikely to alter model predictions for R_0 due to the small contribution of juveniles to the transmission dynamics."

3: Slightly overselling novelty

The introduction seems to slightly oversell the novelty of this modeling framework relative to some other relevant work. Do you consider Arrhenius function to be phenomenological? E.g., see the Shocket et al. papers that you cite; a similar Arrhenius form is also used in Hall et al. 2006 Ecology. I suspect that Arrhenius function is part of your MTE temperature functions, but not clear because I don't see your equations anywhere (see major 1). Contributions of this previous work could be clearer, as a counterexample to your claim that "equations used to relate trait performance to temperature are almost always described by data-intensive phenomenological functions." Also, depending on how you view the Arrhenius function, these papers seem like counterexamples to your claim that "most studies using MTE to predict infectious disease dynamics have focused on individual hosts rather than disease at the population level."

RESPONSE: Thanks, this is a very good point and we are admirers of the *Daphnia*-temperature work out of Spencer Hall's lab. We agree that the Arrhenius forms the basis for many MTE functions, including the rising temperature region of the unimodal Sharpe-

Schoolfield functions we employ in this work and should therefore be used as counterexamples to two of our claims here.

We have edited the sentence on lines 79-85 to now read:

> **"This method has predicted temperature's effects on several host – parasite systems (Mordecai et al. 2013, 2017, Shapiro et al. 2017, Shocket et al. 2018, Gehman et al. 2018, Huber et al. 2018, Tesla et al. 2018), but the equations used to relate trait performance to temperature are often described by fitting system-specific phenomenological functions, such as quadratic or Briere functions, to data-rich experiments (but see Shocket et al. 2018 and Hall et al. 2006 which use monotonic Arrhenius functions to model temperature-dependence in *Daphnia* – fungal parasite populations)."**

And have followed up on lines 95-97 once the MTE is introduced to make clear that Arrhenius function is linked to biological rates and the MTE:

> **"MTE functions that have their bases in biological reaction rates, such as the Arrhenius or Sharpe-Schoolfield functions, have been shown to capture disease-related traits…"**

We also agree that those two papers that employ Arrhenius functions can serve as counterexamples to our claim that most MTE disease studies focus on the individual-level, and have added text on lines 99-102 that read:

> **"However, to date, most studies using the MTE to predict infectious disease dynamics have focused on individual hosts rather than disease at the population level (but see Shocket et al. 2018 and Hall et al. 2006)."**

Minor:
Abstract:
43: Here and maybe elsewhere: Consider writing "gradual temporal warming," or something similar, as "gradual warming" could also imply a fine experimental gradient

RESPONSE: Thank you for this suggestion, we have edited the text throughout to read "gradual temporal warming" rather than "gradual warming".

49: Note that you don't actually show any of the "MTE sub-functions" in this paper with math… (see major 2)

RESPONSE: Thank you, we have now added a new equation (Eq. 6) that shows the form of the Sharpe-Schoolfield function.

Intro:
83: See major 3. I would expect a statement like this to end in "but see [citations]."

RESPONSE: We've edited this statement to read:

> **"This method has predicted temperature's effects on several host – parasite systems (Mordecai et al. 2013, 2017, Shapiro et al. 2017, Shocket et al. 2018, Gehman et al. 2018, Huber et al. 2018, Tesla et al. 2018), but the equations used to relate trait performance to temperature are often described by fitting system-specific phenomenological functions, such as quadratic or Briere functions, to data-rich experiments (but see Shocket et al. 2018 and Hall et al. 2006 which use monotonic Arrhenius functions to model temperature-dependence in *Daphnia* – fungal parasite populations)."**

90: little data are available

RESPONSE: We've now made this change.

98: Another place where 'but see' citations seem relevant.

RESPONSE: We've edited this statement to read:

> **"However, to date, most studies using the MTE to predict infectious disease dynamics have focused on individual hosts rather than disease at the population level (but see Shocket et al. 2018 and Hall et al. 2006)."**

105: "low disease state" seems vague… I think you mean R0 < 1

RESPONSE: Thank you, we have now changed lines 107-110 to read:

> **"First, while predicted by theory, we still lack experimental evidence that a slowly warming system can be pushed from a disease-free or low-disease state (where $R_0<1$) into an epidemic state (where $R_0>1$)."**

110: "The efficacy of trait-based models that use thermal performance curves arising from MTE sub-functions remains unknown" I'm not really sure what you mean here – How does point 3 differ from point 2 from earlier in the paragraph? The current study seems rather specific to be able to conclude a general 'efficacy' of this approach. The major strength of this paper (which is very cool!) seems to be demonstration that gradual warming can push disease into an epidemic state, and that the MTE trait mechanistic model explained the transition.

RESPONSE: We agree that this last sentence is a bit confusing and mostly redundant, and have now removed it.

Methods:
137: [16,18] are references?

RESPONSE: Yes, thank you, this was a formatting mistake left over from a prior submission. We have changed these to match the written in-text citation of the rest of the manuscript.

200: Can you state earlier in the methods that hosts were added to the experiment every three days? This was confusing on my first reading, until further on.

RESPONSE: We have edited the final paragraph of the introduction to clarify this. Lines 120-123 now read:

> **"Experimentally, we drove populations of the host – parasite system *Daphnia magna – Ordospora colligata* (a microsporidian parasite) with constant, low immigration of infected individuals through slowly warming conditions (10C – 13.5C over 120 days) and compared the course of the resultant epidemics to constant-temperature controls."**

148: The harvesting term in the model is a bit confusing. It could be simplified by cancelling the (S+I) terms. It seems quite strange that harvesting rate is a constant per-capita processes, when in reality you removed the same number (not proportion) of hosts each sampling period. I understand that population density was more or less stable (but see major 2 above), but it still would make more sense to me to model 'harvest' the same way you model the additions to the experiment (phi_S). It might be clearer for readers to group phi_S and h together in the equations so that it looks like immigration and emigration.

RESPONSE: We agree with the reviewer that there are different possible ways to express the harvesting term, and that it makes sense to cancel the (S+I) terms. However, the harvesting term does have a key difference from the terms for additions into the experiment (phi_S and phi_I). For adding in individuals, the relative number of susceptibles and infecteds that are added in is constant over time, since they are coming from stock populations. In contrast, while we are harvesting a constant number of individuals, the relative number of infected and susceptibles that are harvested changes over time depending on the true number of infected and susceptibles in the population. Therefore, harvesting should be written as a per-capita process here.

We have taken the reviewers suggestion to cancel the (S+I) terms to simplify the equations, and harvesting is now written as *h*S* and *h*I* for susceptibles and infeceds, respectively.

164: Is S_eq defined? Sorry if I missed it.

RESPONSE: Thanks for catching this, we have now defined it when it first appears in text.

184: Parasite intensity at death – is that w? Labeling could be a little more consistent.

RESPONSE: Thank you, we have now added the symbol labelling here and have also added it throughout the methods for the other parameters to be clearer.

Results:
270: Statistical analysis of increase in infection prevalence? I.e., would expect a significant interaction term in glm: Prevalence ~ day * treatment, link=logit

RESPONSE: Thank you for this suggestion. We have now run a mixed effects model to account for the fact that observations within each population are not independent. Additionally, we scaled the "day" variable to promote model convergence.

Our analysis now has the formula: glmer(prevalence ~ treatment * scaled(day) + (1|population,) family=binomial("logit").

As expected, treatment, day, and the interaction between them are all significant.

We have added text to the methods on lines 293-297 to read:

> **"To test for increases in infection prevalence over time, we used a similar generalized linear model with random effects where the response was prevalence, the fixed predictors were treatment, day scaled to be centered on zero, and an interaction between these two predictors, and the random effect was the replicate population."**

and in the results on lines 327-331 to read:

> **"This trend of increased disease prevalence continued as temperature increased in warming populations, whereas the control populations that remained at 10C never experienced a large increase in disease incidence (i.e., there was a significant interaction between treatment and day in the *glmer* model: p = 0.002; Fig. 3a)."**

282: I'm a bit surprised to see a Mann-Whitney test reported… Why not a logistic regression (i.e., glm with logistic link?). It's not clear why you need a non-parametric test, since prevalence ought to follow a binomial distribution. Sorry if I'm missing something obvious.

RESPONSE: Thank you for this suggestion, you are right that this is a much more appropriate test than the Mann-Whitney we had previously used. We have re-run the analysis with a glmer for final prevalence ~ treatment using family=binomial("logit") and replicate population as a random effect, which estimates a strong statistical difference in final prevalence between warming and constant treatments (p=2.46e-6). We have revised the methods on lines 290-293 to read:

> **"We used a generalized linear model with random effects (glmer function from the R package *lme4*; Bates et al. 2015) with family=binomial and link=logit to test for significant differences in the final number of infected individuals in the warming versus constant treatments where the random effect was replicate population."**

and in the results on lines 338-341 to read:

"We found that the warming population prevalence (mean ± SE = 0.229 ± 0.043) had significantly higher disease prevalence ($p < 0.0001$; Fig. 4) at the conclusion of the experiment relative to the constant 10C populations (mean ± SE = 0.031 ± 0.010)."

Discussion:
288: Didn't actually show the MTE functional forms, and details of the experiments to fit the forms are also lacking (major 1)

RESPONSE: Thank you, we've now added the MTE functional form as described in our response to major 1 above. While space limitations mean it is not possible to repeat details here of parameter estimation experiments from previous studies, we've added text to the methods to explain how functional forms differ between the different parameters and that these were informed from previously published experiments. Lines 202-221 now read:

> **"Previous work has shown that these metabolic models can accurately capture the thermal dependencies of many host and parasite traits in the *Daphnia – Ordospora* system at the scale of individual hosts (Kirk et al. 2018, 2019), and we used these previously published MTE functions to represent the parameters in Eq. 1-4 that were temperature-dependent (Table 1, Fig. 1). In other words, the temperature-dependence of our model was parameterized using experiments that were completely independent from the one reported here. While we refer readers to the previous studies for details on parameter experiments and model fitting, briefly: contact rate (χ) was modeled using a Sharpe-Schoolfield function with only an upper-temperature threshold (Fig. 1b; Kirk et al. 2019); within-host infection intensity was modeled using a Sharpe-Schoolfield function with upper and lower temperature thresholds and a negative activation energy, causing infection intensity to decrease across the intermediate temperature range (Fig. 1d; Kirk et al. 2018). Natural mortality rate (μ; Fig. 1a) and probability of infection (σ; Fig. 1c) were each composite functions of other terms (e.g., aging rate, infection rate) that were represented by Sharpe-Schoolfield functions (Kirk et al. 2018, 2019; see Supporting Information for more details). Additionally, contact rate (χ) increases with, and the probability of infection (σ) decreases with, *Daphnia* size (Kirk et al. 2019). We therefore assumed that large, female adult *Daphnia* size was constant in both treatments at 2700μm and found that our model predictions were not strongly impacted by this and other parameter assumptions (Fig. S2). The model parameters shedding rate (λ), parasite-induced mortality (α), and parasite intensity at death (ω) were each modeled as proportional to within-host infection intensity."**

314: "employing the MTE" … what do you mean by that? Fitting temperature-dependent functions to biological rates and processes?

RESPONSE: Thanks, we agree this was vague. We have amended this sentence on lines 371-373 to read:

"Indeed, if experiments need to be conducted for each host – parasite system to fit temperature-dependent MTE functions to data, its purported advantage (using little to no data) would be negated."

324: How do these suggested averages compare to the parameterized functions you used? Are they at all close? Without seeing your equations (major 1), it's hard to place this paragraph into context. I am by no means 'requiring' an additional analysis (I don't think it would fit in this paper), but this paragraph leads me to wonder how well a a-priori parameterization with 'suggested averages' would match your experiment.

RESPONSE: Thanks, we originally didn't want to delve too deeply into this point here as our prior work which was focused on estimating these parameter values (rather than using them to predict spread as we did here) has discussed this more, but it is certainly a worthwhile point to bring up in this section briefly. We've now edited the text on lines 379-393 to read:

"Moving forward, leveraging the MTE in a trait-based mechanistic model for data-poor systems will require parameter values to be input into the MTE functions *a priori*. A potential starting point could be to use parameter values near the broad averages found in other systems (e.g. activation energies in the 0.60 – 0.70 eV range; Gillooly et al. 2001, Brown et al. 2004), but this approach is unlikely to have performed well at predicting R_0 in this study, as several traits in this system differ significantly in their activation energies and temperature thresholds from one another (Kirk et al. 2018, 2019). This highlights that until further meta-analyses, such as the ones that have shown how activation energy varies among free-living species with co-variates such as taxon, trait function, or habitat (Dell et al. 2011), are performed for disease systems, accurately parameterizing the MTE functions *a priori* will be difficult. However, as we learn more about the generalities of activation energies, inactivation energies, and temperature thresholds, we should eventually be able to make predictions about how these MTE parameters may deviate from means based on the characteristics of the system or the trait in question (Molnár et al. 2017). This would allow for the possibility of parameterizing models for data-poor disease systems."

359: Lack of information about juveniles seems important to mention here (major 2)

RESPONSE: We agree. We've now added text to lines 425-431 that reads:

"Additionally, our model neglects the contribution to transmission from juveniles in the population and, focuses on large, adult female *Daphnia* only, both because the latter are the predominant contributors to the force of infection and because infections in juveniles are hard to detect. If infections were able to be detected reliably in juveniles, a more complicated stage- or age-structured model that explicitly tracks infections across juveniles and adults may provide more information on the overall disease dynamics in the population, but this is unlikely to

alter model predictions for R_0 due to the small contribution of juveniles to the transmission dynamics."

363: Perhaps it could be useful to estimate the degrees of temperature change per generation of host. Some species of long lived hosts may be experiencing rates of temperature change that are similar to your experiment.

RESPONSE: Thanks for this point. While outside the scope of our goals in this study, we agree that this could be helpful in some systems and have now added a sentence at the end of this section on lines 437-440 that reads:

"When considering temperature fluctuations and the speed of temperature change in a system, it may also be worthwhile to account for how long-lived the particular host species is, as different systems can experience temperature change over a range of temporal scales."

398: Or larger gradients; And I suspect that density of these Daphnia populations would have responded to a larger (warmer) temperature gradient. Worth noting that the temperatures in the experiment were all quite cold.

RESPONSE: We've edited this section to both note that it was a relatively small and cold temperature range, and that these Daphnia populations could have responded to a larger temperature gradient. Lines 465-472 now read:

"We did not see any evidence of a temperature effect on adult female *Daphnia* abundances over our relatively small temperature range (10C-13.5C; Fig. S1), suggesting that differences in population size were not the driver of higher disease prevalence in the warming treatments versus in the constant 10C treatments. Because of this, we did not allow population densities to vary with temperature in our model. However, densities will be influenced by temperature in many other systems, as well as potentially in this one if a wider temperature range were considered, and these changes can again be captured by the MTE, as demonstrated in phytoplankton (Bernhardt et al. 2018b)."

Figures & Tables:
Overall, I really like the presentation. Figs 2-4 are very nicely designed and informative.

RESPONSE: Thank you.

Table 1
Units on temperature-dependent parameters?

RESPONSE: Thanks, we've now added this to Table 1.

How did you estimate maximum per-capita recruitment? Not clear, and seems really high (1.33 day-1); I've usually measured little r around 0.2 – 0.4 for cladocerans.

RESPONSE: Thanks, we should have made this more clear that we did not explicitly estimate this value, but instead chose this value to keep adult female *Daphnia* population abundances approximately constant at the carrying capacity of 170 individuals. It also may be interpreted slightly different from a typically estimated little r, as it is for recruitment to the adult population (i.e., there can be many juveniles in the population that can then be recruited to the adult population). That being said, if we had used lower values such as 0.4, it would not have significantly affected our predictions as population size would remain constant at ~167 adult individuals in the population, rather than the ~169 individuals we have when we run the model with recruitment = 1.33 to keep the population closer to the average population size we empirically estimated. Importantly, these choices merely set the population size at equilibrium, and are inconsequential to the analysis focused on disease invasion so long as the demographic parameters for *Daphnia* chosen give a host abundance equilibrium that matches the experimental mean. We have clarified this in the methods section on lines 305-311, which now reads:

> **"The mean population size across all populations throughout the experiment was 169.5, so we set the density-dependent constraint on adult recruitment (K) to be 170 in our model, and set maximum per-capita recruitment to the adult class (ψ) at a relatively high value of 1.33 to keep population-size constant over time at ~169 individuals. We note that here the choice of K and ψ are inconsequential to the analysis focused on disease invasion so long as the chosen parameters generate a host abundance equilibrium that matches the experimental mean."**

Fig. 1
Can you highlight on this figure the range of temperatures considered in the current experiment?

RESPONSE: Yes, thank you for this idea. We've now shaded the temperatures considered in the current experiment and updated the figure caption.

Appendix:
43: Citation or figure for the degredation rate?

RESPONSE: We've added a citation to the unpublished data on this, as well as provided summary statistics for the degradation rate and some additional detail on how it was estimated. Lines 39-49 of the Supporting Information now read:

> **"Infected corpse degradation rate (θ), which does not affect R_0 but affects the timing of how quickly spores are released into the environment, was set to 0.1 d^{-1}. This is the average degradation rate in an independent experiment that we conducted, where we visually assessed the time point at which the *Daphnia* gut was completely degraded (mean degradation rate = 0.108 d^{-1}, standard deviation = 0.0659; Kirk et al. unpublished data). We note that this value (0.108 d^{-1}) was averaged across nine experimental temperatures (5°C - 32°C) and that the experiment used *Daphnia* of varying sizes, and while degradation did increase with temperature, we did not use MTE to model it, as the parameter does not appear in**

the R_0 equation (Eq. 5) and therefore does not affect the critical transition temperature to an epidemic."

106: Where does size appear in the model? Is this an assumption about the data you use to parameterize the model? Another place where links to MTE could be clearer.

RESPONSE: Thanks, we should have made this clear in the main text. We've now added text to lines 216-219 in the methods that reads:

> **"Additionally, contact rate (χ) increases with, and the probability of infection (σ) decreases with, *Daphnia* size (Kirk et al. 2019). We therefore assumed that large, female adult *Daphnia* size was constant in both treatments at 2700μm and found that our model predictions were not strongly impacted by this and other parameter assumptions (Fig. S2)."**

Table S1: This table would be clearer if you should us the range of model parameters. The quantities here seem to be observations that lead to model parameterization, not the parameters themselves, so it is difficult to gauge how they affect the model.

RESPONSE: Sorry for the confusion – this table is the range of model parameters that you're requesting. We've edited the caption on the table to make clear that these are the *parameter values* used in simulations to test model sensitivity to assumptions.

I hope that you find these comments and suggestions helpful.
Signed,
Alex Strauss

RESPONSE: Thank you very much for the thorough comments and suggestions, they've greatly improved the manuscript!

Referee: 2

Comments to the Author(s)
Kirk et al. developed a framework for predicting climate-induced disease emergence by combining models from the metabolic theory of ecology (MTE) with classical epidemiological models and tested whether the models can predict disease emergence in an experimentally warmed system of Daphnia and a microsporidian parasite. They also analyzed experimental and model-simulated data for ten early warning signals (EWS) of critical transitions of the disease-emergence bifurcation. I commend the authors for asking these questions. They are extremely important and, in my opinion, reach the Ecology Letters bar for scope and import of the question.

I reviewed this manuscript twice previously for other high profile journals. I very much liked the previous version and thought it was publishable then. The good thing is that the authors have very adequately addressed all previous concerns I raised. I do not have much to add. This is a fine piece of work integrating climate and seasonal change, a disease experiment, and MTE and

SIR modeling. It is an extremely nice and important advance to the field. I support publishing this work and commend the authors for designing and writing a very interesting manuscript.

In particular, I appreciate the addition of the third and fourth paragraphs in the Discussion. In the third paragraph, the authors highlight that MTE was originally intended to offer a short cut, preventing having to attain all of the thermal performance curves for every trait important to transmission. Their study doesn't quite get us there but offers an important experimental proof of principle that MTE functions, when parameterized, can reasonably predict warming-induced disease dynamics. In the fourth paragraph, they provide guidance on how the discipline can move forward to fulfill MTE being this general short cut for predicting climate-dependent disease dynamics.

RESPONSE: Thank you very much for reviewing the manuscript (three times), and for noticing the additional changes we have made based on previous reviews at other submissions. All of the previous and current reviews have greatly improved our manuscript.

Minor comment
The Vasseur et al. paper is cited in the literature cited but not in the main text.

RESPONSE: Thank you, we have removed this reference from the literature cited.