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The biophysical basis of thermal tolerance in fish eggs

Benjamin T. Martin, Peter N. Dudley, Neosha S. Kashef, David M. Stafford, William J. Reeder, Daniele Tonina, Annelise M. Del Rio, J. Scott Foott and Eric Danner

Article citation details
Proc. R. Soc. B **287**: 20201550.
http://dx.doi.org/10.1098/rspb.2020.1550

Review timeline

Original submission:	30 June 2020
Revised submission:	18 September 2020
Final acceptance:	28 September 2020

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

Review History

RSPB-2020-1550.R0 (Original submission)

Review form: Reviewer 1

Recommendation
Accept with minor revision (please list in comments)

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

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

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

Is the length of the paper justified?
Yes

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

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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?
N/A

Do you have any ethical concerns with this paper?
No

Comments to the Author
General:

This is an elegant study with good theoretical basis supported by detailed experimental studies. The model and experiments address an important question in relation to climate change impacts on the survival and lifehistory of fish species, specifically how the combined effects of temperature and oxygen concentration affect egg survival. While the literature contains a large number of experimental studies on these effects either singly or in combination, there lacks a physiological model of how the interaction between these variables can affect egg survival probability. The authors have in my view developed such a model and conducted several experiments with a salmon species to test it. The model successfully explains much of the vairation observed in the experiments.

I find the manuscript has several strengths. The main one is the mechanistic model of egg survival – it is intuitive, simple and elegant yet appears to represent the main processes affecting survival, as clearly supported by a large number of experimental studies. The model will be useful for understanding and predicting how eggs of other fish species will be affected by future combinations of temperature and oxygen concentrations, which opens mechanistic perspective to assessing how fish habitats and lifehistories could be influenced by those variables in future. The experimental studies themselves reveal the functional responses of how the eggs are affected by temperature and oxygen conditions throughout development and would be worthy of their own publication. The description of the model is supported by a detailed presentation in an appendix. Furthermore the manuscript is written and presently generally in a clear way and straightforward to follow with clear figures.

While there are many positive aspects of this work and ms, I am not too surprised that the survival-temperature relationship depends on another abiotic variable. At least for fish and crustacean eggs, there are quite a few studies showing for example how survival or development rate depends on a combination of and interaction between variables. E.g., survival or development time to hatch could be affected by salinity, pH, and other variables as well as temperature. So while I think the evidence that survival or development time is influenced both by temperature and oxygen concentration is for sure useful and gives new knowledge of how this species might be affected by these variables, it is not entirely novel or unique for egg development studies, when looking at different species. So some of the text about the discovery that response variables depend on the state of a 2nd variable are perhaps not so surprising and the wording could be changed accordingly.

I believe the manuscript is suitable for the journal after minor revisions.

Specific comments:

Lines 74-82 Are Methods, not results. Move text.
Lines 121-124. Seems to be some punctuation or words missing here. Does not read well.
Methods. The experimental setup is a bit difficult to visualize from the descrption provided. Could the authors provide schematics and/or photos of some of the containers and measuring instruments, potentially as appendix figures? This will help with possible replication by others in future.
Fig. 3 caption. The figure show model simulations and results of a lab experiment so neither shows results from nature. Please edit the caption.
Fig. 4 caption. Similar comment as for Fig.3.

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?
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.
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> Yes
>
> **Is it clear?**
> Yes
>
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Do you have any ethical concerns with this paper?
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The authors place their model into an environmental context in relation to flow of water in redds. This is an interesting and important next step to the work and is nicely done.

The only substantive point I have relates to the Discussion. I am disappointed the authors do not use the opportunity to be more expansive on their ideas and to use this paper as an opportunity to suggest how they may me tested further, or may explain other patterns in a wider range of animal groups. What might the next step in testing or applying these ideas be, in a broader sense? I would really like the authors to be more visionary and expansive in the Discussion. Given O2 supply is much more costly to increase for aquatic organisms, what might the implications be in terms of thermal tolerance and O2 supply and demand be in aquatic versus air-breathing organisms for example? What might the implications be in relation to increasing egg size (which leads to a reduced SA/volume ratio) and across different environment types?

As an aside, the authors may be interested in the paper:
Forster et al (2012) Warming-induced reductions in body size are greater in aquatic than terrestrial species. Proceedings of the National Academy of Sciences USA 109: 19310-19314. doi: 10.1073/pnas.1210460109
This explores other ways in which O2 supply and demand may be addressed in organisms, and specifically demonstrates that phenotypic plastic body size reductions with warming, which are especially pronounced in aquatic versus terrestrial species, may be related to O2 supply and demand issues that arise with warming.

Decision letter (RSPB-2020-1550.R0)

11-Sep-2020

Dear Dr Martin:

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.

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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.

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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.

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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.

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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 Gary Carvalho
mailto: proceedingsb@royalsociety.org

Associate Editor
Comments to Author:
We have now obtained reviews from two experts in the field and both were very positive, noting the combination of both modeling and empirical data as a major strength. I also found the study to be interesting and if modified as suggested by the reviewers, could be publishable in Proc B.

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Reviewer(s)' Comments to Author:
Referee: 1

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Fig. 4 caption. Similar comment as for Fig.3.

Referee: 2

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This explores other ways in which O2 supply and demand may be addressed in organisms, and specifically demonstrates that phenotypic plastic body size reductions with warming, which are especially pronounced in aquatic versus terrestrial species, may be related to O2 supply and demand issues that arise with warming.

Author's Response to Decision Letter for (RSPB-2020-1550.R0)

See Appendix A.

Decision letter (RSPB-2020-1550.R1)

28-Sep-2020

Dear Dr Martin

I am pleased to inform you that your manuscript entitled "The biophysical basis of thermal tolerance in fish eggs" has been accepted for publication in Proceedings B.

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

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

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

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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 Gary Carvalho
Editor, Proceedings B
mailto: proceedingsb@royalsociety.org

Associate Editor:
Comments to Author:
The revisions have addressed reviewer concerns, no further changes are requested.

Appendix A

Associate Editor
Comments to Author:
We have now obtained reviews from two experts in the field and both were very positive, noting the combination of both modeling and empirical data as a major strength. I also found the study to be interesting and if modified as suggested by the reviewers, could be publishable in Proc B.

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Response: Thank you for the feedback. I believe we were able to address both reviewers' concerns about the discussion by deemphasizing the novelty of variation in thermal tolerance across abiotic conditions, and instead to emphasize the consequences of oxygen limitation for climate change and the life-history of aquatic organisms.

In addition to the requested changes, we have made a subtle change to the introduction. While our paper was in review, a large meta-analysis of thermal tolerance in fishes was published in Science, showing that eggs, along with reproducing adults, have thermal tolerances 8C lower than other life stages (Dahlke et al. 2020). Thus, how species respond to climate change will to a large extent depend on the thermal tolerance of eggs. This finding further emphasizes the importance of our manuscript, which represents a significant advance in our understanding of the mechanistic basis of thermal tolerance for this sensitive life stage.

Dahlke, F. T., Wohlrab, S., Butzin, M., & Pörtner, H. O. (2020). Thermal bottlenecks in the life cycle define climate vulnerability of fish. *Science*, *369*(6499), 65-70.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)
General:

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Response: Thank you for this positive feedback.

While there are many positive aspects of this work and ms, I am not too surprised that the survival-temperature relationship depends on another abiotic variable. At least for fish and crustacean eggs, there are quite a few studies showing for example how survival or development rate depends on a combination of and interaction between variables. E.g., survival or development time to hatch could be affected by salinity, pH, and other variables as well as temperature. So while I think the evidence that survival or development time is influenced both by temperature and oxygen concentration is for sure useful and gives new knowledge of how this species might be affected by these variables, it is not entirely novel or unique for egg development studies, when looking at different species. So some of the text about the discovery that response variables depend on the state of a 2nd variable are perhaps not so surprising and the wording could be changed accordingly.

Response: We have revised the discussion to no longer belabor this point, and cite other studies demonstrating the interaction of temperature and other abiotic variables. We now instead emphasize the main contribution of this paper, a mechanic understanding of the underlying mechanisms of thermal stress in eggs. This also freed up space to emphasize the points brought up by reviewer 2.

I believe the manuscript is suitable for the journal after minor revisions.

Specific comments:

Lines 74-82 Are Methods, not results. Move text.

Response: We believe this short description in the results section is important for clarity and context. Our study consists of 3 different experiments, a mathematical model and a computational fluid dynamics simulation. Because the results from one component often motivated follow up experiments or models, we think our paper is presented best as results first, but with a little methodological overview in some places for context.

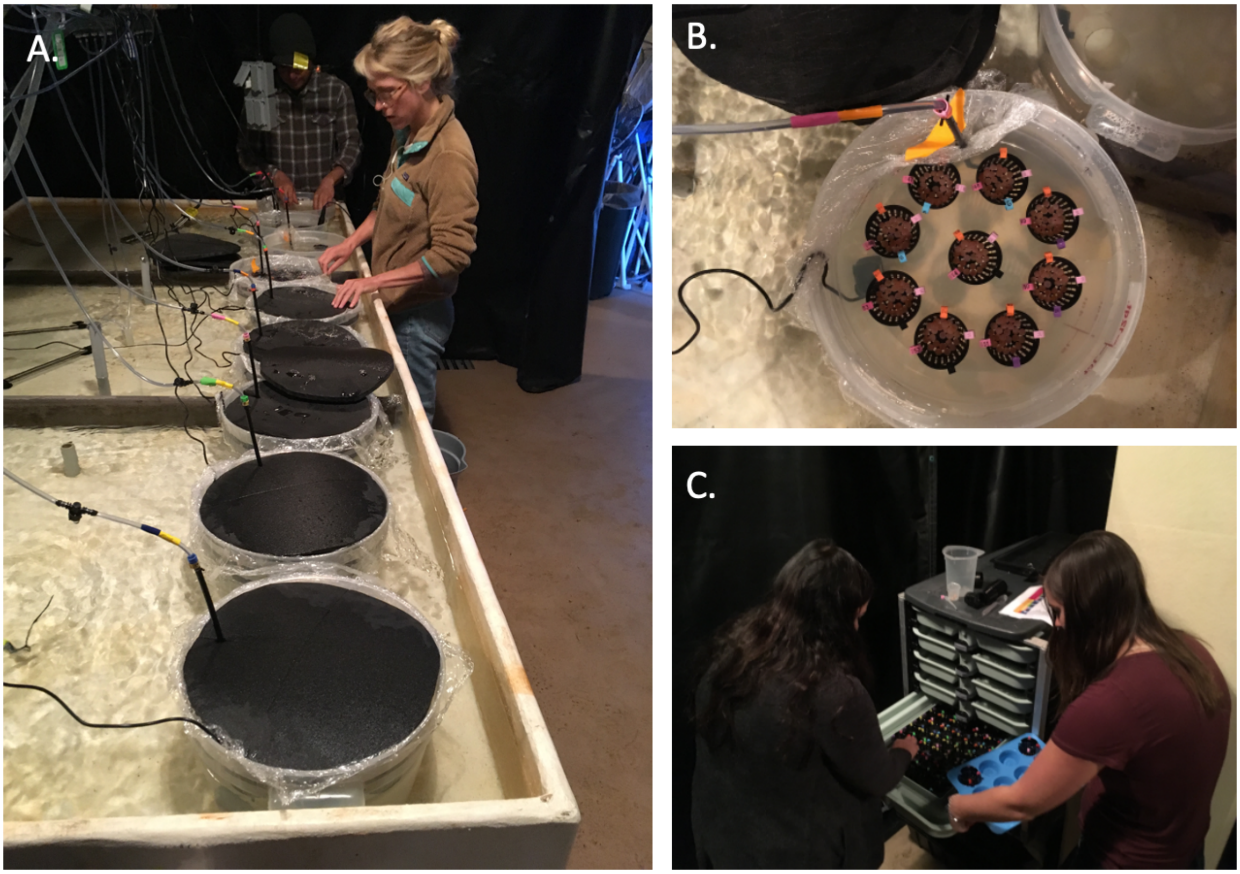
Lines 121-124. Seems to be some punctuation or words missing here. Does not read well.

Response: Thanks, we revised this section:

"In well mixed environments without O_2 depletion ($dC_s/dt = 0$), eq. 3 can be solved for the equilibrium C_i, which determines the realized metabolic rate, B (eq. 2). Using this relationship, we can predict the degree of O_2 limitation for any combination of O_2 supply and demand conditions, where the degree of O_2 limitation is defined as the fraction of intrinsic metabolic demands not met:"

Methods. The experimental setup is a bit difficult to visualize from the descrption provided. Could the authors provide schematics and/or photos of some of the containers and measuring instruments, potentially as appendix figures? This will help with possible replication by others in future.

Response: Pictures and schematics for the O2 distribution experiment were shown in Figure S3 (now S4). We additionally added photos of the oxygen supply demand experiment (Figure S3):



“Figure S3. Experimental setup for oxygen supply/demand survival experiments. **[A]** The 9 experimental aquaria are divided among 3 sections of a water bath. Aquaria in each of the three sections of the water bath were maintained at one of three temperatures (12, 14.5, 17 °C). Within each partition, each aquarium received water from one of 3, 500-l reservoirs (not pictured) maintained at three O_2 levels (saturation, 5.0 and 2.5 µg ml^{-1}). A layer of polyethylene wrap and a foam lid was placed on top of each aquarium to minimize gas exchange. **[B]** Top-down view of an experimental aquaria, with the 9 replicate experimental baskets originally containing 25 embryos each. The water inflow spigot (at 12 o’clock), and the recirculating pump (at 9 o’clock) can both be seen. **[C]** Before and after the experimental exposure period embryos were maintained in a Heath (Flex-a-lite Consolidated) recirculating incubation tray.”

Fig. 3 caption. The figure show model simulations and results of a lab experiment so neither shows results from nature. Please edit the caption.

We replaced “natural conditions” with “egg clusters”

Fig. 4 caption. Similar comment as for Fig.3.
We replaced “natural conditions” with “egg clusters”

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The authors place their model into an environmental context in relation to flow of water in redds. This is an interesting and important next step to the work and is nicely done.

Response: Thank you for this positive feedback.

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Response: We had longer discussion originally, but had to cut much of it due to space limitations. However, by addressing a comment by reviewer 1, we have cleared up space in the discussion to address some of these points. Notably these two additional paragraphs touch on the issues raised by reviewer 2 (lines 274-299):

"A longstanding goal of ecophysiology is understanding and predicting interspecific variation in thermal tolerance [21-23]. The biophysical model developed here provides a mechanistic framework for making such predictions. Across species, the metabolic rate of embryos is proportional to egg volume [8], but supply occurs by diffusion across the egg surface [6]. Thus, larger eggs should become O_2 limited at lower temperatures, and as a result our theory predicts that thermal tolerance should decrease with egg size. This prediction is supported by global patterns of fish egg sizes, where mean egg diameter is negatively correlated with sea-surface-temperature [24]. Moreover, for eggs of similar sizes, our theory makes testable predictions on how thermal tolerance should vary with species traits (e.g. variation in volume-specific metabolic rate, or surface-area-specific O_2 conductance) that can be measured form standard respirometry assays.

The differential scaling of O_2 supply and demand with egg volume also sets fundamental constraints on egg size in aquatic systems. The predominant trend in aquatic systems is that adults produce offspring in proportion to their size [25]. Specifically, in aquatic organisms ranging in mass over 17 orders of magnitude, adults of most species produce offspring that on the order of 1/100th their mass. Teleosts, however, are a striking exception to this pattern, as offspring size is independent of adult body size [25]. Our results suggest this pattern emerges because these eggs approach sizes where O_2-limitation constrains offspring size. Although elasmobranchs and marine mammals are as large or larger than teleosts and, produce large offspring in proportion to their body size, in both cases these taxa have evolved physiological adaptations to overcome the limitations of diffusion across an egg surface. For example, marine mammals supply O_2 to developing embryos through the placenta, and embryos of oviparous elasmobranchs are able to generate flow through slits that form in the egg case [26]. Because c have not evolved similar adaptions, they are constrained to produce more offspring instead of larger offspring. As a result, teleosts are among the most fecund animals on the planet, with profound implications for their life-history and demography [27]."

As an aside, the authors may be interested in the paper:
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This explores other ways in which O2 supply and demand may be addressed in organisms, and specifically demonstrates that phenotypic plastic body size reductions with warming, which are especially pronounced in aquatic versus terrestrial species, may be related to O2 supply and demand issues that arise with warming.

Response: Thanks, we are aware of the paper, and it is very interesting and compelling evidence for a more general role of oxygen limitation in other life stages. However, given that we are at the space limit for this paper, we don't have space for a discussion about oxygen limitation in other life stages.